Biological Dosimetry Method: a Probable way for Measuring Percent Depth Dose

Mohammad Mehdi Abtahi (1)
Mahmood Reza Aghamiri (2)
Masoumeh Yadolahi (3)
Aziz Mahmoodzade (4)

(1) Physics Department, Imam Khomeini International University, Qazvin, Iran
(2) Radiation Medicine Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran
(3) Radiology Department, School of allied medical sciences, Semnan University of Medical Sciences, Semnan, Iran
(4) Department of Bioscience and Biotechnology, Malek-Ashtar University of Technology, Tehran, Iran

Corresponding author:
Masoumeh Yadolahi,
Radiology Department, School of allied medical sciences,
Semnan University of Medical Sciences,
Semnan, Iran

Abstract

Aim: Since genetic materials are the critical targets of cell irradiations, obtaining the depth dose distribution by cytogenetic methods is supposed as an accurate and realistic method for dosimetry in the field of treatment.

Material and Method: In this study, five vials containing blood were positioned in a tissue equivalent phantom. The space between test vials was 12 mm and they were positioned at the maximum depth dose point.

Results: The results indicated that the calculated dose obtained by micronuclei assay method has a significant similarity to the dose calculated by the Ion Chamber. However, the limitation of the minimum needed blood which limits the minimum size of test vials, could destroy the spatial resolution.

Discussion: Authors recommend more research to make the biological dosimeters as a precise method for radiation therapy.

Key words: Biological Dosimetry; Percentage Depth Dose; Micronuclei; Phantom; Linear Accelerator

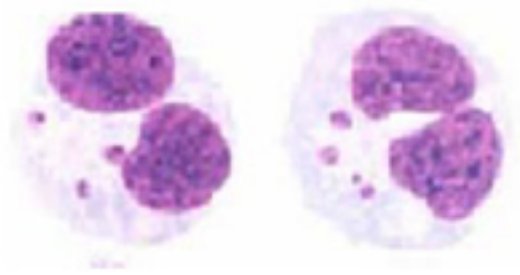
Please cite this article as: Yadolahi M. et al. Biological Dosimetry Method: a Probable way for Measuring Percent Depth Dose. World Family Medicine. 2018;16(1):193-197
DOI: 10.5742/MEWFM.2018.93218

Introduction

In 1964, the investigation of chromosome aberrations (cytogenetic methods) was suggested as a biologic method for estimation of radiation dose [1]. Today, it's been years that the investigation of chromosome aberrations in blood lymphocytes has been used for the dosimetry of ionizing radiation [2]. The most important cytogenetic methods that are used for radiation dosimetry are metaphase analysis, G-banding, the premature chromosome condensation [PCC], the fluorescence in situ hybridization [FISH] and micronuclei assay using cytochalasin B (CBMN).

The Cytokinesis-block micronucleus (CBMN) was developed by "Marley" and "Fenech" in 1985[3]. Micronuclei (MN) is a small nucleus in addition to the main nucleus which results from chromosome breaks or the whole chromosomes lagging behind, during anaphase. By adding cytochalasin B to the lymphocyte cultures, cytokinesis will be blocked without inhibiting the nuclear division. The cytokinesis-blocked cells accumulate in the first division cycle and can be easily identified from their binucleate appearance (Figure 1). Therefore, the micronuclei (MN) can be easily, specifically and efficiently counted in these binuclei cells while excluding the non-dividing mononuclear cells [3].

Figure 1: BN Cells with MN



MN can arise from exposure to various clastogenic agents in the form of acentric chromosome fragments, as well as to aneugenic agents as whole chromosomes, to the extent that the MN index in human cells, is one of the standard cytogenetic tests of genetic toxicology tests. However, because ionizing radiation is a strong clastogenic agent, and thus a potent inducer of MN, the CBMN assay has proven to be a very reliable, thoroughly validated and standardized technique in the field of radiation biology to evaluate in vivo radiation exposure of occupational, medical and accidentally exposed individuals [4]. For instance, in a study which was conducted to investigate chromosome damage in individuals who occupationally had exposures to the low-level ionizing radiation, it has been indicated that the frequency of MN in the workers was higher in relation to the control group [5].

Many studies have shown that the number of radiation induced MN is strongly dependent on radiation dose as well as radiation quality [6-8]. For low LET radiation, linear quadratic dose–response relation has been reported. While a linear dependence is observed for high LET radiation, therefore it causes the radiations with more LET to be more effective in generating MN at the same dose levels.

Since one of the important goals in radiotherapy is to deliver the maximum amount of dose to the tumoral cells with the minimum damage to the cells of the healthy tissues, determination of dose distribution in the tissue or tissue equivalent phantom is of significant importance. Determination of this important parameter has a vast application and one of its most important applications is the confirmation of treatment planning. Today the depth dose distribution is determined by the use of physical dosimeters. Among the dosimeters used for PDD determination, the ionization chamber, TLD and types of silicon diodes could be referred to. The ionization chamber functions by measuring the charges produced by direct ionization in the gas [9,10]. Thermoluminescence Dosimeters are the crystal materials which function according to the thermoluminiscence phenomenon [10]. The diode dosimeter is also a crystal of silicon which is mixed with some impurities. The irradiation causes an inductive electronic current in the diode; this current is measured by the use of an amplifier which is embedded on the diode [10].

Since the genetic materials are the critical targets of cell irradiation, obtaining the depth dose distribution by cytogenetic methods is suggested as an accurate and realistic method for dosimetry in the field of treatment. The main aim of this study was using CBMN method in order to obtain the depth dose distribution and comparing the obtained dose-response curve with a common dosimeter to investigate the capability of this method for obtaining the spatial dose distribution. The next goal was to make a tissue equivalent liquid material which becomes gelatinized at room temperature to hold the cells in stable positions. Hence, we approached a structure which was similar to body tissue.

Materials and Methods

5 ml blood samples were collected in heparinized tubes from a healthy 29 year-old woman, then under sterile circumstances and under the laminar hood, each 1 mL of blood was poured in a vial with 1 cc volume and placed at different depths in a cylindrical container. The space between test vials was 12 mm and they were positioned at the maximum depth dose point (Figure 2). Then the liquid phantoms were added to the container containing the vials. Consequently by addition of a crosslinker, the phantom was gelatinized (Figure 3). After the irradiation, each 1.00 mL of blood, was added to 9.00 mL of RPMI 1640 culture medium (Gibco) containing 10% fetal calf serum, 20 µL/mL phytohemagglutinin (Gibco), 50U/mL penicillin, 50 µg/mL streptomycin, and 2mM glutamine (sigma). All cultures were incubated at 37±10C in a humidified atmosphere of 5% CO2/95% air (Figure 4). Cytochalasin B (Fluka; final concentration: 6 µL/mL) was added after 44 hours of culture. At the end of 72 hours of incubation, the cells were collected by centrifugation and re-suspended in 0.075M cold potassium chloride for 8 minutes, at 1000 rpm; they were immediately fixed in a fixative solution (methanol:acetic acid, 6:1) three times. Fixed cells were dropped onto clean microscopic slides, air dried, and stained with a Giemsa solution. All slides were coded and scoring was done at 40× magnification for the frequency of micronuclei in cytokinesis-blocked binucleated cells with well-preserved cytoplasm. To be counted as micronuclei, candidates had to have a diameter between 1/16th and 1/3rd of main nuclei, be non-refractile, and not be linked to, or overlap with, the main nuclei[11]. Frequency of MN per 1000 BN cells was counted.

A. Irradiation

Irradiation was performed using a linear accelerator (Campact Elektra AU051) with 6 Mev energy and a dose rate of 35o MU/min. The field-size was 20 cm×20 cm and the Source Surface Distance (SSD) was 100 cm. The dose at dose-maximum amounted to 4 Gy.

B. Phantom Structure

In order to build a phantom to be malleable and capable of being molded like the container's shape, the best way is to use hydrogels that at the beginning are liquid and then it is possible for them to be gelatinized. One of the most important hydrogels is gelatin. But unfortunately the gelatin hydrogels have a weak structure and melt at a temperature around 30 degrees centigrade which makes working with this phantom difficult.

One of the most important reasons for the melting of the gelatin networks is the existence of the weak hydrogen bonds in the gelatin. Therefore, in order to avoid it melting it is necessary for this phantom to be made of a material which instead of hydrogen bonds, have stronger bonds like covalence bonds. After conducting different investigations, the Poly Vinyl Alcohol (PVA) (Merck Germany) (10% w/w) together with a cross linker was found suitable.

In order to build the phantom, first we dissolved PVA in the water at 90 degrees centigrade. When the PVA becomes completely dissolved, we turn off the heater to let

Figure 2: The container containing the vials

Figure 3: The container containing vials with gelatin phantoms

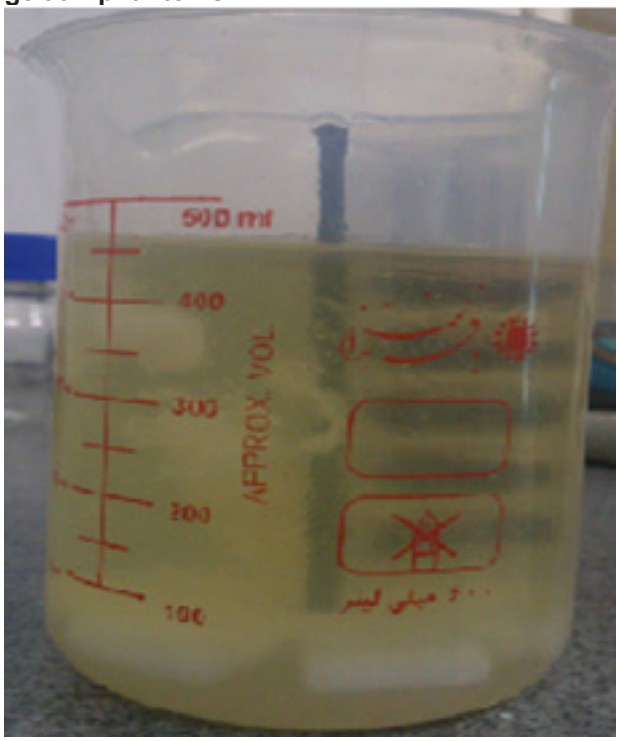


Figure 4. The cultures in incubator

the stirring continue without heating. Simultaneously, we prepare the cross linker on a stirrer in another container. This compound included 5cc of water and glutaraldehyde. The required materials for making the phantom are mentioned in Table 1. Then the crosslinker solution was added to the PVA solution at room temperature and evenly homogenized by stirring and the compound was moved to the container. This compound could be preserved at room temperature.

Table 1: Required materials to make the phantom

Amount (%)	Material
89.754	Water
10	PVA
0.246	Glutaraldehyde

The number of MN per 1000 BN as a function of depth in the phantom is demonstrated in Figure 5.

The statistical calculations have shown that the p-value concerning the relation of the depths 0.5 Cm and 1.7 Cm and also 1.7 Cm and 5.3 Cm is less than 0.001 and the p-value concerning depth 1.7 Cm in relation to the depth 4.1 Cm is less than 0.01. The depth 2.9 Cm in relation to the depth 4.1 Cm and also 2.9 Cm in relation to the depth 5.3 Cm has a p-value <0.05. Generally, the comparison of the cell groups located in five depths indicate a meaningful difference ($P<0.05$) in frequency of micronuclei.

In another study, the frequency of micronuclei was obtained in lymphocytes of 5 healthy humans after irradiation with doses 0.3 to 5 Gy X-rays (Figure 6) [12], therefore, in our work, the PDD curve according to the depth has been drawn according to this study.

The PDD curve according to the depth has also been reached by ionizing chamber and finally the obtained PDD curve using MN method was compared with the obtained curve based on ionizing chamber (Figure 7). As has been shown in Figure 7 the results obtained using both of the methods have an acceptable similarity to each other. It should be mentioned that the study based on which we have drawn the calibration curve was performed in 250 Kev energy whereas the energy used in our work is 6 Mev and it is possible that a percentage of the difference between the responses obtained using the two methods of MN and ionizing chamber is caused by that. The influence of energy on the Frequency of micronuclei is now being investigated by the current group and in future studies, the corrections concerning this parameter would be implemented, if necessary.

As was already expected, there is an increase in the amount of MN up to the “build-up” region and then the MNs were decreased with a moderate slope. The ascending and descending procedure of the MN with depth were compared with the dose measurement using the ion chamber. The results indicated that the calculated dose obtained by MN measurement method has a significant similarity to the dose calculated by the ion chamber. However, the limitation of the minimum needed blood which limits the minimum size of test vials, and could destroy the spatial resolution. Therefore, authors recommend more research to make the biological dosimeters a precise method for radiation therapy.

Figure 5: The number of MN per 1000 BN as a function of depth in the phantom (the standard deviation has been shown in the figure as "error bar")

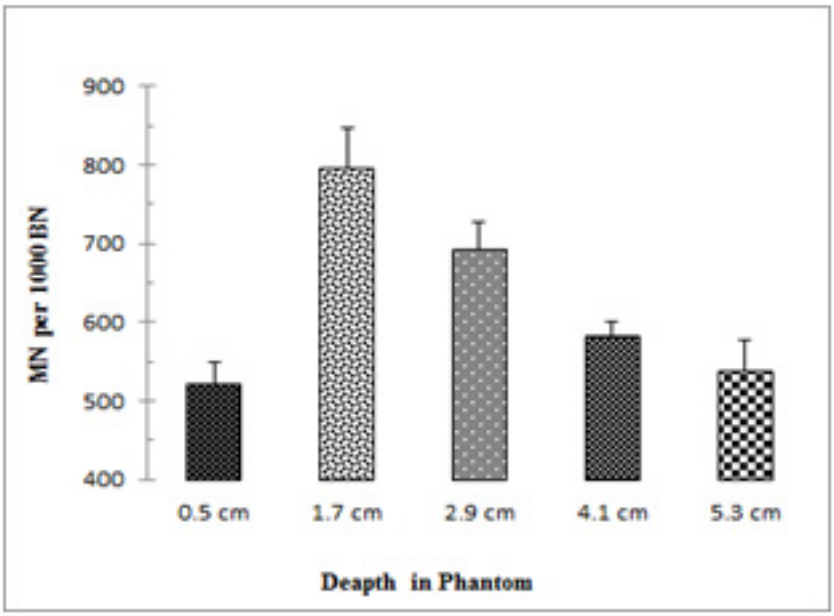
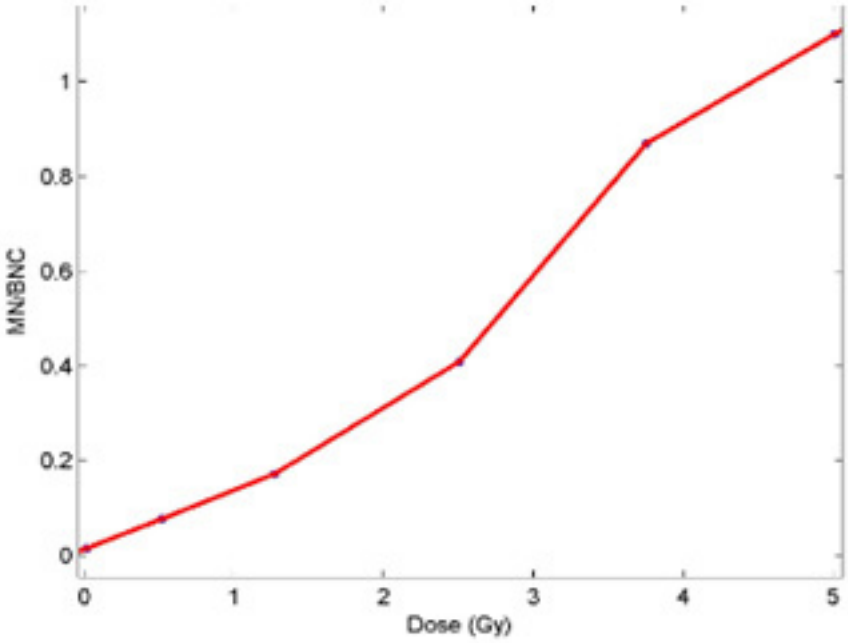


Figure 6. MN/BNC in human lymphocytes after x-irradiation



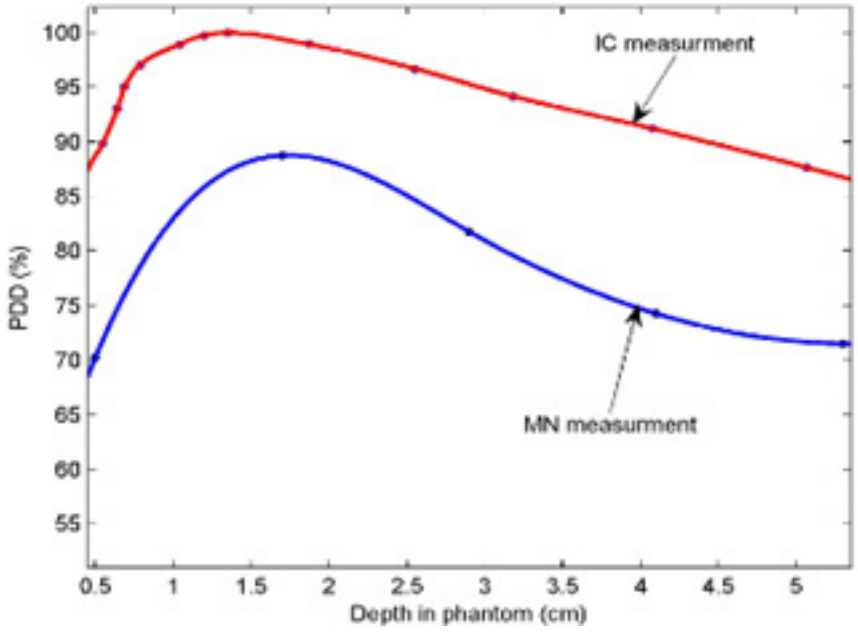
Discussion

In this research, the application of biological dosimetry for the measurement of PDD, has been investigated. The findings indicated a good correspondence to the PDD was resulted from an ion chamber. According to the achieved results in this research, it could be concluded that the biological dosimetry is potentially suitable for PDD measurement in radiation therapy. MN assay was used to assess individual radiation sensitivity between different patients undergoing radiotherapy [13]. The response of cancer patients to radiotherapy varies in severity due to differences in intrinsic cellular radio sensitivity.

Conclusion

The identification of radiosensitive patients, through the MN assay, will allow the individualization of patient's treatment; therefore this method considers individual differences in response to the radiation. Furthermore, this tool, due to its substantial traits, has more relevance to the natural tissues by view of the response to the radiation but in the physical dosimeters category, these advantages do not exist. Nevertheless, more research in the direction of improvement of spatial resolution and dose resolution of this method is recommended for future research.

Figure 7: PDD obtained using two methods of MN assay and ion chamber



References

[1] Bender MA. Chromosome aberrations in irradiated human subjects. Annals of the New York Academy of Sciences. 1964;114(1):249-51.
[2] Bender MA, Awa AA, Brooks AL, Evans HJ, Groer PG, Littlefield LG, et al. Current status of cytogenetic procedures to detect and quantify previous exposures to radiation. Mutation Research/Reviews in Genetic Toxicology. 1988;196(2):103-59.
[3] Fenech M, Morley AA Measurement of micronuclei in lymphocytes. Mutation Research/Environmental Mutagenesis and Related Subjects. 1985;147(1):29-36
[4] Vral A, Fenech M, Thierens H. The micronucleus assay as a biological dosimeter of in vivo ionising radiation exposure. Mutagenesis. 2011;26(1):11-7.
[5] Maffei F, Angelini S, Forti GC, Lodi V, Violante FS, Mattioli S, et al. Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors. Mutagenesis. 2002;17(5):405-9
[6] Verhaegen F, Vral A. Sensitivity of micronucleus induction in human lymphocytes to low-LET radiation qualities: RBE and correlation of RBE and LET. Radiation research. 1994;139(2):208-13.
[7] Wuttke K, Müller W-U, Streffer C. The sensitivity of the in vitro cytokinesis-blocked micronucleus assay in lymphocytes for different and combined radiation qualities. Strahlentherapie und Onkologie. 1998;174(5):262
[8] Gersey B, Sodolak J, Hada M, Saganti P, Wilkins R, Cucinotta F, etal. Micronuclei induction in human fibroblasts exposed invitro to Los Alamos high-energy neutrons. Advances in Space Research. 2007;40(11):1754-7.
[9] Tsoulfanidis, N., Measurement And Detection Of Radiation, T. Francis, Editor. 1995.
[10] Khan FM, The physics of radiation therapy. third ed. Philadephia: Lippincott Williams & Wilkins. 2003.
[11] Fenech M. The in vitro micronucleus technique. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2000;455(1):81-95
[12] Streffer C, Müller W-U, Kryscio A, Böcker W. Micronuclei-biological indicator for retrospective dosimetry after exposure to ionizing radiation. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1998;404(1):101-5.
[13] Jones L, Scott D, Cowan R, Roberts SA. Abnormal radiosensitivity of lymphocytes from breast cancer patients with excessive normal tissue damage after radiotherapy: chromosome aberrations after low dose-rate irradiation. International journal of radiation biology. 1995;67(5):519-28.