Jurnal Sains Nuklear Malaysia, 2019, 31(2): 44 – 49
ISSN: 2232-0946

COMPARATIVE ANALYSIS OF HDR INTRACAVITARY BRACHYTHERAPY FRACTIONATION: IN-VITRO STUDY

WN Rahman[1], WNS Wan Mansor[1], R Ab Rashid[1] and R Abdullah[1,2]

[1]School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kota Bharu, Kelantan, Malaysia.
[2] Nuclear Medicine, Radiotherapy and Oncology Department, Hospital Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
Correspondence author: wandiana@usm.my

ABSTRACT

Cervical cancer is commonly treated with high dose rate (HDR) brachytherapy. However, the optimal fractionation and dependency on time interval between fractions in HDR intracavitary brachytherapy (ICBT) technique remain controversial. In this study, three types of fractionated irradiation technique were investigated using human cervical cancer (HeLa) cells to determine the most effective fractionation regime that would be implemented in ICBT treatment. HeLa cells were irradiated using HDR remote-afterloading brachytherapy machine with 3 types of fractionation technique; a single fraction, hyperfraction and hypofraction. The fractionated dose used was 9 Gy/fraction with 18 Gy of the total dose. Cell survival curves were obtained from standard clonogenic assay fitted with Pade Linear Quadratic (PLQ) model using OriginPro 9.2 software. Radiobiological analysis of irradiated HeLa cells was performed based on the cell survival curves. The cell survival curves for single fraction, hyperfraction and hypofraction technique indicate the time interval between fractions impacting the cells survival. The survival curve shoulder of single irradiation tends to be constant as the doses increase more than 12 Gy. For hyperfractionation, the shoulder region was wider which indicates the cells have higher survival. Hypofractionated irradiation had steeper shoulder as the dose increase. In this case, the cancer cells may not have chances to recover and have a lower survival fraction. The radiobiological analysis presented by PLQ model shows that hypofractionated irradiation with 9 Gy/fraction delivered in two fractions might be the most effective fractionation to be implemented in cervical cancer treatment using HDR-ICBT irradiation.

Keywords: Cervical cancer, dose, fractionation, HDR brachytherapy

INTRODUCTION

Cervical cancer is the most common cancer among women in Malaysia and worldwide for both incidence and mortality (Bray et al., 2018). Treatment of cervical cancer in locally advanced stages involved external beam radiotherapy (EBRT) in combination with concurrent chemotherapy (Ghosh et al., 2015). High dose rate (HDR) Intracavitary Brachytherapy (ICBT) is also the integral part of the treatment following EBRT and chemotherapy in order to maximize the radiotherapy therapeutic effects (Rao et al., 2017). HDR-ICBT has been widely accepted as the standard for radical treatment for cervical cancer replacing the use of low dose rate ICBT over many decades (Rao et al., 2017). However, the usage of high dose per fraction may induce unnecessary toxicity to healthy normal tissue.

The dose fractionation schedule for HDR-ICBT as recommended by American Brachytherapy Society (ABS) suggested that the individual fraction size should be less than 7.5 Gy per fraction with fractions number ranging from four to eight fractions (Rao et al., 2017). In contrast to ABS recommendation, some reports suggested that the higher dose per fraction might be safe and effective (Sood et al., 2002; Patel et al., 2005; Patel et al., 2011). A study by Sood et al. (2002) provides clinical experiences on using two fractions of HDR-ICBT with 9 Gy per fraction are practically safe with no increase in bladder and renal toxicity. Similar finding by other research suggests that HDR-ICBT with 9 Gy per fraction provide better survival rates with good local tumour control as well as minimal toxicity (Patel et al., 2005; Patel et al., 201). Therefore, optimization of the fractionation schedule of HDR-ICBT is crucial in the management of cervical cancer.

In the present study, comparison between fractionation regimes used in HDR-ICBT treatment such as hyperfraction, hypofraction and the single fraction was conducted *in-vitro* using cervical cancer cell lines (HeLa cells). The efficacy of each fractionation was assessed via clonogenic assay analysed with Pade Linear Quadratic (PLQ) model.

MATERIALS AND METHODS

Cell Culture Protocol

The HeLa cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Life Technologies, City, U.S.A.), 1% penicillin and streptomycin (Gibco, Life Technologies, City, U.S.A.). The cells were grown to confluence in a 75 cm^2 flask (Greiner Bio-One, Austria) and split in a ratio of 1:3. The cells incubated at 37^0C in a humidified environment of 5% CO_2 in air.

Cell Irradiation

The cells number are counted using automatic cells counter and approximately 5000 cells per well were seeded in 6 wells plates (SPL Life Sciences, Korea). The cells were irradiated in a single fraction of 18 Gy, hyperfraction and hypofraction of 9 Gy in 2 fractions with irradiation gap of 5 hours and 7 days respectively. The irradiation and fractionation schedule is depicted in Table 1. Irradiations were performed using Ir-192 source emitting gamma ray of energy 0.38 MeV from Microselectron HDR Brachytherapy V14.23 (Nucletron Corp, Columbia, Maryland). The cells culture plates were arranged on top of surface mould where the source catheter is placed and covered with bolus. Gafchromic EBT3 films were used for dose and uniformity validation across the cells samples. Figure 1 shows the irradiation setup.

Table 1: The difference in irradiation between 3 types of fractions and irradiation schedule

Details	Single Fraction	Hyperfraction	Hypofraction
Gap between fraction	No gap	5 hours	7 days
Dose (Gy)	Single irradiation (18 Gy)	1st fraction: 9 Gy 2nd fraction: 9 Gy	1st fraction: 9 Gy 2nd fraction: 9 Gy
Total dose (Gy)	18 Gy	18 Gy	18 Gy

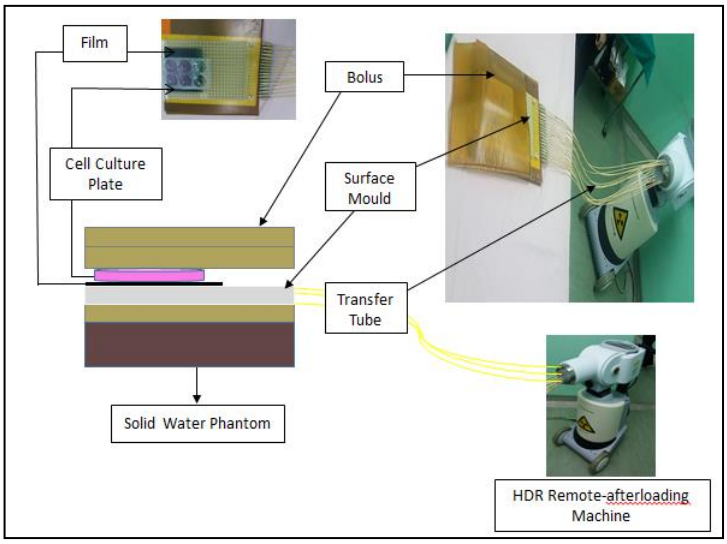

Figure 1: Cells irradiation setup with HDR Brachytherapy with Ir-192 source

Clonogenic Assay

The irradiated cells in the 6 well plates were left incubated for a week. After incubation, the cells were washed gently with 1 ml Phosphate Buffered Saline (PBS) and were fixed with 500 µl ice-cooled methanol. The fixed cells were then stained with crystal violet solution. After the cells were fixed and stained, the visible colonies were counted under a microscope. Survival fractions represented by the ratio of colony formation after exposure to radiation to those unexposed to radiation (control).

Radiobiological Model Analysis

The cell survivals were analyzed by calculating the survival fraction from the cell colonies according to the equation 1.

$$Survival\ fraction = \frac{Number\ of\ irradiated\ cell\ colonies}{Number\ of\ control\ cell\ colonies} \quad \text{.....equation 1}$$

The survival fraction were then plotted and fitted according to the Pade linear quadratic (PLQ) model using OriginPro 8.5 software. PLQ model can be described by equation 2:

$$S_F^{PLQ} = e^{-\frac{\alpha D+\beta D^2}{1+\gamma D}} \quad \text{.....equation 2}$$

Where,

S: surviving fraction for PLQ model,
D: the dose delivered in unit Gray (Gy),
A: the cell kill per Gy of initial linear component,
B: the cells kill per Gy^2 of the quadratic component,
γ: additional parameter given by βD_0

Statistical Analysis and Experimental Uncertainties

The experimental error and uncertainties were mainly originating from the nanoparticles preparation and cell culture techniques such as cells number and colonies count. The accuracy of radiation dose delivered to the cells was verified using Gafchromic EBT3 films and the errors were below 5%. The estimate overall uncertainties were below ±10%. The data are expressed as the mean ± standard deviation of three samples. Statistical analysis was performed with the OriginPro 9.2 software.

RESULTS AND DISCUSSION

The irradiated HeLa cells survival curves for three types of fractionation; a single fraction, hyperfraction and hypofraction fitted using PLQ Model is shown in Figure 2. The results clearly indicate the effects of fractionation on the cells survival. All survival curves for fractionated irradiation generate a wider initial shoulder at low radiation dose region and become steeper as the dose increases. Hypofraction irradiation present the fewer cells survived in comparison to single fraction and hyperfraction irradiation. At 9 Gy of dose, the cell survival irradiated with hypofraction dropped to 67 % compared to 81 % and 95 % for single fraction and hyperfraction, respectively. The irradiation at 18 Gy present reduction of cell survived up to 46 % for hypofraction, 77 % for single fraction and 89% for hyperfraction. The shape of the cell survival curve of hyperfractionation shows the widest shoulder which indicates the survival of cells in contrast to almost linear curve for hypofraction irradiation. It indicates that the irradiated cells had enough chances to repair themselves before the next fraction and the cells had higher survival fraction. In contrast, the cell survival curve for hypofractionated irradiation had a steeper shoulder as the dose increase. The cells do not have chances to recover and had lower survival fraction. This was proven in the results that show hypofraction are able to kill tumour cells effectively.

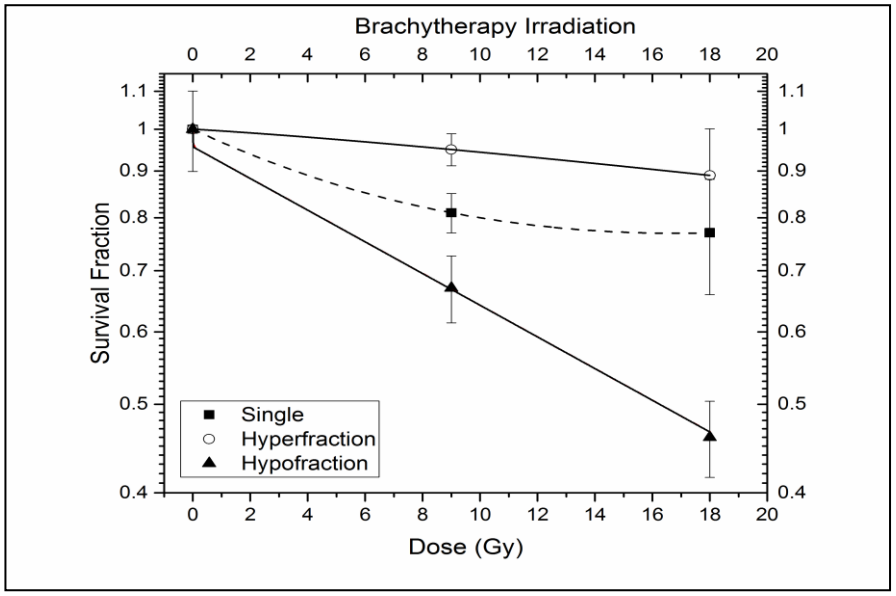

Figure 2: Survival curves of HeLa cell fitted by PLQ Model for single, hyperfraction and hypofraction irradiation

In this study, we have implemented the PLQ model for cell survival fitting. Basically, LQ model is widely used to describe the relationship between the radiation absorbed dose and the proportion of cells that survived. However, studies have shown that the LQ model is not suitable in the high-dose region where it underestimates the surviving fraction in the high dose range (Shuryak et al., 2015). Thus, Pade Linear Quadratic (PLQ) model is developed as a result of LQ model modification. The PLQ model generates an accurate prediction of experimentally measured survival curves in the ablative, high-dose range without losing the strength of the LQ model around the shoulder. In the PLQ model, the extrapolation numbers and final slopes of these models do not depend on the dose. It becomes dose-independent for large values of dose. Most experimental data for log-survival tend towards straight lines with some constant final slopes.

Table 2 present the parameters derived from PLQ models of survival curves. Four parameters were involved in PLQ model; α, β and γ. Here, the linear and quadratic component of the curve was described as α (Gy^{-1}) and β (Gy^{-2}). The additional parameter γ (Gy^{-1}) was given by βD_0, where the mean lethal dose D_0 can readily be extracted from the slope $k_0 = 1/D_0$ of the straight line in the cell survival curve. The α/(β/γ) ratio indicates the dose for which the number of acutely responding cell death was similar to the number of late-responding cell death (the dose at which the linear and quadratic components of cell death are equal). The R^2 parameter indicates the goodness of fit of a model. Higher R^2 value indicates that the data points are closer to the fitted values. The Pade Linear Quadratic (PLQ) model yields acceptable results for the experimental data sets. This model was a better choice for high-dose regions since their parameters were independent of dose and it can predict the survival more accurate.

Table 2: The radiobiological parameter derived from PLQ Model

Details	**Single fraction Irradiation**	**Hyperfractionated Irradiation**	**Hypofractionated Irradiation**
α Gy^{-1})	0.03	0.004	0.49 ± 0.54
β (Gy^{-2})	-9.21E-4	3.92E-4	0.41 ± 0.69
γ (Gy^{-1})	0.01	0.04	10.13 ± 0.58
β/γ	- 0.07	9.2E-3	0.04
α/(β/γ)	- 0.44	4.66	12.36
R^2	1	1	0.99

CONCLUSIONS

In conclusion, this study shows that hypofractionated irradiation with 9 Gy per fraction delivered in two fractions might be the most effective fractionation to be implemented in cervical cancer treatment using HDR-ICBT irradiation.

ACKNOWLEDGEMENTS

This work was funded by Research University Grant (RUI: 1001/PPSK/8012212). We also would like to spread our gratitude toward Hospital Universiti Sains Malaysia for providing necessary facilities for us to undergo this research.

REFERENCES

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A. (2018). Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J. for Clin*. 68: 394-424.

Ghosh, S., Rao, P.B. and Kotne, S. (2015). High dose rate brachytherapy in two 9 Gy fractions in the treatment of locally advanced cervival cancer – a South Indian institutional experience, *Asia Pasific J. Cancer Prevent.* 16: 7167-7170.

Patel, F.D., Kumar, P., Karunanidhi, G., Sharma, S.C. and Kapoor, R. (2011). Optimization of high dose rate intracavitary brachytherapy schedule in the treatment of carcinoma of the cervix, *Brachytherapy* 2:147-153.

Patel, F.D., Rai, B., Mallick, I. and Sharma, S.C. (2005). High dose rate brachytherapy in uterine cervical carcinoma, *Int. J. Radia.t Oncol. Biol. Phys*. 62: 125-130.

Rao, B.S., Das, P., Subramaniam, B.V., Jena, A., Rashmi, P., Konakalla, V.L.A. and Jayasree, K. (2017). A comparative analysis of two different dose fractionation regimens of high dose rate intracavitary brachytherapy in treatment of carcinoma of uterine cervix: A prospective randomized study, *J. Clinic. Diagnos. Res.* 115: 327-334.

Shuryak, I., Carlson, D., Brown, J. and Brenner, D. (2015). High dose and fractionation effects in stereotactic radiation therapy: analysis of tumour control data from 2965 patients, *Radiother. Oncol*. 115: 327-334.

Sood, B.M., Gorla, G., Gupta, S., Garg, M., Deore, S., Runowicz, C.D., Fields, A.L., Goldberg, G.L., Anderson, P.S. and Vikram, B. (2002). Two fractions of high dose rate brachytherapy in the management of cervix cancer: clinical experience with and without chemotherapy, *Int. J. Radiat. Oncol. Biol. Phys.* 53: 702-706.