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# APPLICABILITY OF LINEAR QUADRATIC AND MULTI-TARGET MODEL TO SINGLE AND FRACTIONATED RADIOTHERAPY IRRADIATION

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## *ABSTRACT*

*The effect of fractionation in radiotherapy had been experimentally proven to increase the surviving fraction of the cells instead of single irradiation. Cell survival described by radiobiological models could be used to predict the radiosensitivity and sublethal damage repair of cells to radiation. In this study, the linear quadratic (LQ) and multi-target (MT) cell survival curve models were used to describe the cell survival curves with single and fractionated irradiation. HeLa cells were irradiated using 6 MV photon beam with 10×10 cm$^2$ field size, 100 cm SSD at different doses. Standard clonogenic assay was performed to determine cell survival. The experimental data were fitted to the LQ and MT model using OriginPro 9.2 software. Radiobiological parameters were evaluated from the fitting curves generated from these models. Results show that the survival curves fitted with LQ and MT model for single irradiation displayed steep initial slope and small shoulder. The sublethal damage repair was better for fractionated irradiation with a wider shoulder. The parameters of LQ model showed a larger α and α/β ratio whereas for MT model showed a smaller n, $D_o$ and $D_q$. Analysis of the LQ and MT model parameter shows that single irradiation induced more cell death. MT model seems to be more accurate in describing the radiosensitivity of the cells especially at a high dose for both single and fractionated irradiations. The most commonly used model, LQ seems to provide unsatisfactory fitting at a high dose.*

**Keywords:** Dose, fractionation, linear quadratic model, multi-target model, radiotherapy

## INTRODUCTION

The cell survival curve presents the relationship between radiation dose and the effects on surviving cells. Puck et al. (1957) published in vitro mammalian cells irradiated with X-rays and demonstrated the connection between the dose delivered and the killing of the cell i.e: cell survival. This curve has been proved as a guideline for many models to determine the effects of radiation. The radiobiological models implemented to describe the survival curve might be used to compare various clinical situations in radiotherapy treatment planning such as different dose distributions and fractionation schedules (Jones et al., 2001).

Radiobiological models were used to describe the radiation effect at the subcellular level by modeling the biophysical events using a mathematical approach with parameters that indicate the events. A radiobiological model typically converts a physical quantity (absorbed dose) to a biological quantity (cell survival fraction) (Wang, 2010). The linear quadratic (LQ) model was the most commonly used model for quantitative predictions of dose or fractionation dependencies in

radiotherapy. Now, the linear quadratic model was globally used in calculating radiotherapeutic isoeffect doses for various fractionation or protraction schemes (Brenner, 2008). As been demonstrated by Brenner (2008), the LQ model had the following useful properties to determine the isoeffect doses. Firstly, the linear quadratic model was a mechanistic and biologically-based model. Second, it had sufficiently few parameters to be practical. Third, various mechanistic model of cell killing predicted the same fractionation dependencies as LQ model. Fourth, it had well documented predictive properties for fractionation and dose rate effects in the laboratory. Fifth, it was reasonably well validated, theoretically and experimentally up to about 10 Gy per fraction and would be reasonable for use up to about 18 Gy per fraction. Until now, there was no evidence of problems when LQ model had been clinically applied.

According to Jones et al. (2001), there were a few uses of the LQ model in terms of fractionation regimes. One of the main benefits of LQ model was associated with fractionation effects and able to compare various fractionation schedules. Besides, LQ model was able to simply assess a fractionation schedule which was not necessarily distributed evenly in fraction size or delivery time throughout the entire treatment. Addition to that, the LQ model and its modifications can also be used to consider an inhomogeneous dose distribution that was resulting in fraction sizes which were not consistent through the target volume.

However, the LQ model was not applicable to high dose rate radiotherapy (Kirkpatrick et al., 2008). In his study, Kirkpatrick et al. (2008) highlighted some reasons why the linear quadratic model was not applicable at high dose rate irradiation. The LQ model might be inappropriate for high doses per fraction practiced in radiosurgery because it did not explain accurately the observed (in vivo) clinical data. Second, it was derived largely from in vitro, rather than in vivo observations and therefore did not consider the impact of ionizing radiation on the supporting tissues. Next, the LQ model did not consider the impact of the subpopulation of radioresistant clonogens. LQ model also could create a "false belief" that this simplified model to represents absolute truth.

Based on the study conducted by Iwata et al. (2013), LQ model was capable for fractional doses of 5 Gy or less. According to another study by Shibamoto et al. (2012), the LQ model was not being applicable with high doses per fraction. This happened because dose-survival curves for cultured cells cannot be well fitted by the LQ model in high-dose ranges. The LQ model showed the cell survival curve continues to bend downwards at high dose did not seem to fit the actual experimental data.

Target theory became one of the crucial concepts for understanding radiobiology other than LQ model. Multi-target single-hit model was most commonly used and had the assumption that the cells had several targets, each requiring one hit for inactivation (Spring and Holmberg, 1968). The research was conducted on the effects of radiation on bacteria and viruses demonstrated that the survival of irradiated organisms decreased exponentially with increased in radiation dose (Lea and Coulson, 1949; Lea et al., 1941; Lea, 1946; Nomiya, 2013). The multi-target model could be applicable in explaining high dose irradiation and fractionation.

In this study, the linear quadratic (LQ) and multi-target (MT) cell survival curve models were used to describe the cell survival irradiated with single and fractionated radiotherapy irradiation. The applicability of both models in fractionated radiotherapy was investigated.

## MATERIALS AND METHODS

### Cell Culture

The HeLa 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 37ºC in a humidified environment of 5% $CO_2$ in the air.

### Cells Irradiation

The irradiations were performed using 6 MV photon beam generated by Siemen Primus linear accelerator (LINAC) at Nuclear Medicine, Radiotherapy and Oncology Department, Hospital USM. Irradiations of cells were conducted in single delivery and fractionation of 2, 4, 6 and 8 Gy of total doses. Fractionation was performed in 2 dose delivery of half of the doses in the first exposure and completed the total doses in second exposure in the next 24 hours.

### Cell Survival Analysis

After irradiation, the effects of single and fractionated dose were determined by analyzing cell survival. Cell survivals were obtained by performing the colony assay and after 10 days the cells were fixed with 50 % methanol and stained with 0.5% crystal violet. Cells colonies were counted manually and the survival fraction was calculated.

### Radiobiological Analysis Using Linear Quadratic and Multitarget Model

In this study, linear quadratic and multitarget models were used to analyze the cell survival curves for single and fractionated irradiation. Each model was generated according to its equation and fit the experimental survival fraction using OriginPro 9.0 software (OriginLab Corporation, Northampton, MA, USA). Analysis of the parameter values from the survival curves generated by each model was also obtained. Equation and parameters for LQ and MT are indicated in equation 1 and 2.

The LQ describes the relationship between cell survival and irradiation dose, based on:

$$S = \exp^{-(\alpha D + \beta D2)} \quad \text{.....equation 1}$$

Where α and β are dependents, and D is the absorbed dose. In other words, the LQ model assumes that S is made of two terms: a linear term αD and a quadratic term, $\beta D^2$.

The second radiobiological model used is a multi-target model. Data were fitted according to the model equation expression that also describes the relationship between cell survival and irradiation dose:

$$S = 1 - ( 1 - \exp^{(-D/D_o)})^n \quad \text{.....equation 2}$$

D is the dose in Gray, $D_o$ is a parameter that determines the final slope of the survival curve, and n is the y-intercept of the asymptote.

**Statistical Analysis**

Data uncertainties were calculated as the standard error of the mean (SEM). One way analysis of variance (ANOVA) was used to determine the significance of the difference between control and experimental group.

**RESULTS**

The percentage of survival fraction for single and fractionated irradiation was presented in Table 1. From the data, it was apparent that the percentage survival for fractionated irradiation was higher compared to single irradiation. At 0 Gy dose, survival is assumed to be 100%. Irradiations with 2 Gy of dose indicate percentage survivals for single and fractionated irradiation are 60% and 85% respectively. Single irradiation with 4 Gy dose shows 27.5% survival while slightly higher survival of 37.5% was obtained for fractionated irradiation. Results are similar at 6 Gy where single irradiations have fewer cells survived with 12.5 % survival meanwhile fractionated irradiation have 25 % cell survival. On the other hand, only 5% of cells survived at 8 Gy, the highest dose applied for both single and fractionated irradiations.

Table 1: Survival fraction for single and fractionated irradiation

| Dose (Gy) | Percentage Survival for Single Irradiation (%) | Percentage Survival for Fractionated Irradiation (%) |
|---|---|---|
| 0 | 100 | 100 |
| 2 | 60 | 85 |
| 4 | 27.5 | 37.5 |
| 6 | 12.5 | 25 |
| 8 | 5 | 5 |

Figure 1 shows the HeLa cell survival curves fitted by LQ model for single and fractionated irradiation with 6 MV photon beams. The curves demonstrate the relative surviving fraction decreased almost linearly with an increased dose for single irradiation. Cell survival curve generated by LQ model showed a good fitting at low and high doses and a bit deviated at a very high dose (8 Gy) for single irradiation. When the irradiation was delivered in fractionation schedules, the surviving fraction tended to decrease gradually with an increase in the radiation dose. Cell survival curve obtained by LQ model demonstrated a good fitting at low doses and deviated at high doses (4 and 6 Gy) for fractionated irradiation. The survival curve of fractionated regimes had a wider shoulder compared to single irradiation, reflecting the ability of the cells to repair damage better. Thus, the number of cells survive was higher for fractionated irradiation instead of single irradiation. The relative surviving fractions significantly increased with fractionation.

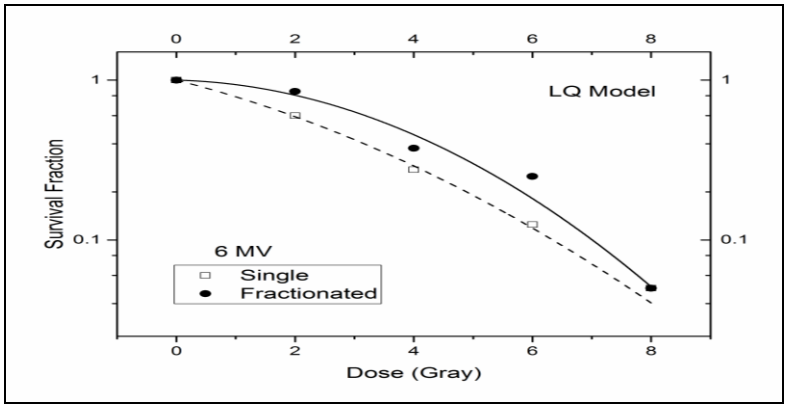


Figure 1: Survival curves of HeLa cell for single and fractionated irradiation with 6 MV photon beam fitted by LQ model

Figure 2 shows the results of the HeLa cell survival curves fitted by MT model for single and fractionated irradiation. Cell survival curves for single irradiation described by MT model indicate good fitting for both at low and high doses. The survival curve obtained for fractionated irradiation show MT model is fitted at low doses (0 and 2 Gy) but start to deviate at high doses. Survival curve fitted by MT model for fractionated regimes also had a wider shoulder compared to single irradiation, reflecting the ability of the cells to repair before the next fraction. Single irradiation had a narrow shoulder and automatically had a lower surviving fraction. Biological effects will be different from the fractionation schedule although the total dose is the same.

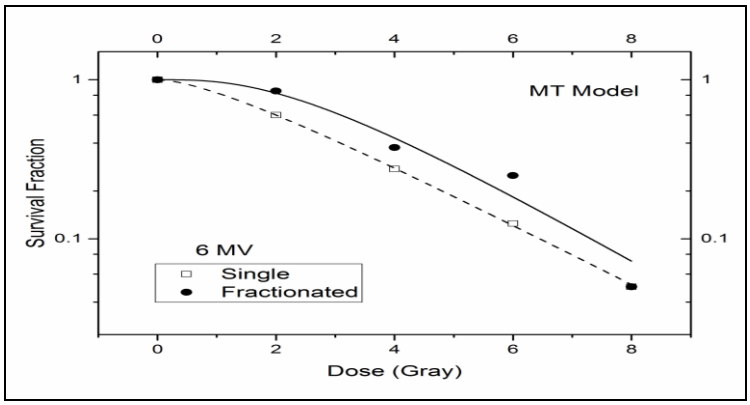


Figure 2: Survival curves of HeLa cell for single and fractionated irradiation with 6 MV photon beam fitted by MT model

Radiobiological analysis from the LQ models' parameter is tabulated in Table 2**.** Comparison of the α values revealed differences in initial slope between irradiation with a single and fractionated irradiation. The fractionated irradiation present value of α is 0.021 ± 0.06 and the value of β is 0.044 ± 0.01. For single irradiation, the value of α is 0.217 ± 0.02 and the value of β is 0.023 ± 0.004. The α/β values for fractionated and single irradiation are 0.48 and 9.42 respectively.

Table 2: Radiobiological parameters from linear quadratic model for single and fractionated irradiation

| Energy | α ($Gy^{-1}$) (SE) | β ($Gy^{-2}$) (SE) | α/β value |
|---|---|---|---|
| Single | 0.217 (± 0.02) | 0.023 (± 0.004) | 9.42 |
| Fractionated | 0.021 (± 0.06) | 0.044 (± 0.01) | 0.48 |

Radiobiological analysis using multi-target model is shown in Table 3. The model was represented by three parameters which are $D_o$, $D_q$ and n. A lower value of $D_o$ and $D_q$ were obtained for the irradiation of cells in a single dose. Smaller 'n' was also observed for single irradiation that indicated more radiosensitisation effect compared to fractionated irradiation. The value of $D_o$ for the fractionated irradiation was 0.487 ± 0.09 and n was valued 3.65 ± 1.283. Meanwhile, for single irradiation, the value of $D_o$ was 0.434 ± 0.006 and the value of n was 1.68 ± 0.034. The values of $D_q$ for single and fractionated irradiation were 0.098 and 0.274 respectively. Obviously, $D_o$, $D_q$ and n value for fractionated irradiation were higher. The findings showed that the cells irradiated in fractionation were less radiosensitive.

Table 3: Radiobiological parameters from multi-target model for single and fractionated irradiation

| Energy | $D_o$ (SE) | $D_q$ | n (SE) |
|---|---|---|---|
| Single | 0.434 (± 0.006) | 0.098 | 1.68 (± 0.034) |
| Fractionated | 0.487 (± 0.09) | 0.274 | 3.65 (± 1.283) |

## DISCUSSION

Numerous radiobiological modeling had been established to determine the radiobiological response of the cells by plotting the survival curve. Joiner and Kogel (2009) stated that the simple LQ formula provided a better explanation of radiation response in the low-dose region ranging from 0 - 3 Gy. Basically, LQ survival curves were continuously bending with no straight portion either at low or high radiation doses. Joiner and Bentzen (2009) found that extrapolations by the LQ model beyond 5 - 6 Gy per fraction were likely lacking in clinically useful precision. As mentioned by Iwata et al, (2013) the LQ model may be only applicable to fractional doses of 5 Gy or less whereas MT models seemed to be more valid for 6 Gy or higher doses (Rashid et al., 2018).

For the extended target idea, just one hit by radiation on each sensitive target in the cell will cause the cell death. Multi-target survival curves had been proved to be useful in describing the radiation response of mammalian cells at high doses. They did not describe the survival response well at lower clinically relevant doses. An obvious result of the multi-target model was that it assumed a response that is flat for very low radiation doses. According to Iwata et al. (2013), MT model is still valuable because it fits the empirical data well, especially in the high-dose range.

In this study, all cell survival curves for fractionated irradiation exhibited a large initial shoulder at the low radiation dose. The cells eventually had enough chances to repair themselves before the next fraction. Thus, the cells had higher survival fraction. In contrast, the cell survival curve for

single irradiation showed steeper and smaller initial shoulders reflecting lower survival fraction. For single irradiation, a large α value at initial slope valued 0.217 and for α/β ratio the value is 9.42. The HeLa cell irradiated in a single dose, showed a larger α and α/β ratio indicating an increased in the radiosensitivity. Conversely, the α and α/β values for fractionated irradiation are 0.021 and 0.48 respectively. The α value was smaller for fractionated irradiation because the dose was delivered in fractionation. So, the total dose required to cause a given degree of mortality was greater when the irradiation was given in two fractions than as a single dose. So, we can conclude that when the cells were irradiated with a fractionated dose, more cells were survived.

An indication of radiosensitivity from LQ parameter has been evaluated by Fertil et al. (1985). In this study, α value predicts the intrinsic radiosensitivity of human cell lines in which the larger α were observed in radiosensitive cell lines such as fibroblasts and ataxia-telangiectasia (AT) fibroblast. On the other hand, smaller α were observed in radioresistant cells such as glioblastomas. Duchesne et al. (1986) explained the condition for higher α/β ratio in which when the linear component α is predominant, the sensitivity toward low radiation doses increase. Meanwhile, the low α/β ratio indicating domination of the quadratic component of the radiation response implying fewer cells kills when low doses of radiation used. Beyzadeoglu et al. (2010) also stated that sublethal damage repair and the value of the β parameter in the cell survival curve decreases as the dose rate decreases.

According to the analysis using MT model as shown in Table 3, the parameter of $D_o$ that represents the dose that causes a mean of one-hit per cell (mean lethal dose) showed the smaller value which is 0.434 for single irradiation instead of fractionated irradiation. The same goes to n value for single irradiation which showed the smaller value of 1.68 compared to fractionated schedules and 'n' represents the number of targets (required number of hits for cell death). Meanwhile, the $D_o$ and n value for fractionated irradiation are 0.487 and 3.65, respectively. The values of $D_q$ for both single and fractionated irradiations are 0.098 and 0.278 respectively. As mentioned in Alpen (1997), one of the important parameters of the multi-target model is quasi-threshold dose ($D_q$). If interception happened when the linear portion of the logarithm of surviving fraction versus dose was extrapolated back to the point where it crossed the 1.0 surviving fraction ordinate, that intercept is the dose that is called quasi-threshold dose. $D_q$ was excellent in estimating the parameters of the relationship from laboratory data.

Analysis of survival fraction for single and fractionated irradiation show the survival fraction for fractionated irradiation was higher compared to single irradiation indicate that the number of cells survived was higher in fractionated irradiation compared to single irradiation. This could be due to the cells repair in between the fraction in comparison to the single exposure. The wider shoulder indicated a substantial amount of sublethal damage repair (SLDR) which means the cells can be repaired within hours unless additional sublethal damage (SLD) occurs (such as a second dose of radiation) with which it can interact to form lethal damage. The sublethal damage can be defined as the damage of the cells can be repaired within hours under normal conditions unless an additional radiation dose is given (inducing further SLD). This generally happened due to the indirect effect of radiation and formed single strand breakage DNA. This kind of damage usually observed in low-LET radiation. Elkind and Sutton (1959) concluded that, at least by 18 h after a conditioning dose of 5 Gy, surviving cells fully repaired their sublethal damage, since survival following the second exposure was the same as for cells that had received no prior irradiation.

The rate of repair of sublethal damage was determined by the surviving fraction as a function of the interval between the two doses (Tubiana et al., 1990). When the cells were irradiated in fractionated schedules, SLDR was demonstrated better. The irradiation was conducted in several fractions thus;

the sublethal damage was repaired during the interval separating the fractions. The resulting survival curve is an exponential passing through the origin, whose slope is reduced as the individual fractions become smaller.

## CONCLUSIONS

Analysis of the LQ and MT model parameter shows that single irradiation induced more cell death. MT model seems to be more accurate in describing the radiosensitivity of the cells especially at a high dose for both single and fractionated irradiations because the curve plotted fitted closely to the experimental data. The most commonly used model, LQ seems to provide unsatisfactory fitting at a high dose. In conclusion, MT model is found to be more accurate in describing the radiosensitivity of the cells especially at a high dose for both single and fractionated irradiations.

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