EVALUATION OF TUMOUR CELLS DAMAGE FOLLOWING RADIOTHERAPY BY TC-99M PERTECHNETATE

Muhammad Afiq Bin Khairil Anuar[1], Siti Zanariah Ab Aziz[1], Raizulnasuha Abdul Rashid[1] , Safri Zainal Abidin[1], Norhayati Dollah[1] , Wan Nordiana A Abd Rahman[1]

[1] Medical Radiation Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.

Corresponding author: Dr Wan Nordiana W Abd Rahman (wnordiana@gmail.com)

ABSTRACT

Radiotherapy has become the most important modality in treating cancer with approximately 50% of cancer patient undergo the treatment. However, more improvement to the radiotherapy treatment efficacy is required to deprive cancer. Assessment of tumor progress during treatment is important to accommodate the changes that occur during the fractionation course. The objective of this study is to assess tumor cell damage after external beam radiotherapy by using technetium-99m pertechnetate ($^{99m}TcO_4^-$) as a tracer. In this study, HeLa cells were irradiated with 6 MV photon beam with different radiation dose ranging from 0.5 Gy to 10 Gy. The irradiated cells were recultured in 6-well plates and incubated for 10 days. After that, 2 mCi of $^{99m}TcO_4^-$ were prescribed to each cell colonies. The viable cells were separated from the rest and measured for $^{99m}TcO_4^-$ uptake using single head gamma camera with LEHR collimation. As results, the cells survival fractions clearly indicate diminishing effect to the cells at higher dose of irradiation. Good correlation were observed between $^{99m}TcO_4$ uptake and survival fraction for cells irradiated at lower dose and less significant correlation were indicated at higher dose. In conclusion, there is potential for the efficacy of external beam radiotherapy in treating cancer to be assessed by using radioisotope as a non-invasive tracer. In this case, technetium-99m pertechnetate ($^{99m}TcO_4$) could be attached to the specific antibody so that better correlation between the cells uptake and possible cell damages could be observed.

ABSTRAK

Radioterapi telah menjadi modaliti utama dalam merawat kaser, di mana lebih 50% pesakit kanser melalui kaedah rawatan ini. Namun yang demikian, masih banyak ruang penambahbaikan perlu dibuat untuk meningkatkan keberkesanan radioterapi dalam merawat kanser. Penilaian terhadap perubahan tumor adalah penting ketika membuat sebarang perubahan sepanjang prosedur rawatan berlangsung. Objektif kajian ini adalah untuk menilai kerosakan terhadap sel kanser akibat radioterapi dengan menggunakan technetium-99m pertechnetate ($^{99m}TcO_4^-$) sebagai penanda. Sel HeLa telah didedahkan dengan pancaran foton 6 MV, yang mempunyai dos radiasi antara 0.5 hingga 10 Gy. Sel HeLa tersebut kemudiannya dikultur semula dan diinkubasi selama 10 hari. Seterusnya sebanyak 2 mCi $^{99m}TcO_4^-$ telah dimasukkan kedalam setiap bekas sel. Sel yang hidup diasingkan untuk diukur kandungan $^{99m}TcO_4^-$ menggunakan kamera gamma berkolimasi LEHR. Hasil kajian jelas menunjukkan bahawa pecahan survival sel berkurangan apabila dos radiasi meningkat. Kolerasi antara survival sel dan penyerapan $^{99m}TcO_4^-$ adalah baik bagi dos rendah, namun kolerasi tersebut menurun apabila dos

meningkat. Konlusinya, radioisotop mempunyai potensi untuk digunakan sebagai penanda bagi melihat keberkesanan radioterapi secara tidak invasif. Dalam kes ini, technetium-99m pertechnetate ($^{99m}TcO_4^-$) boleh disambung dengan antibody yang spesifik bagi meningkatkan kolerasi antara penyerapan $^{99m}TcO_4^-$ ke dalam sel dan kerosakan sel akibat radioterapi.

Keywords: technetium-99m pertechnetate, molecular imaging, radiotherapy

INTRODUCTION

Cancers have become a main prominent cause of deaths among men and women around the world. According to GLOBOCAN (2012), an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths happened in 2012, compared with 12.7 million of cases and 7.6 million of death in 2008. Prevalence assessments for 2012 revealed that there were 32.6 million people (over the age of 15 years) alive who had a cancer diagnosed in the previous five years (WHO, 2014).

Debate continues about the best treatment to handle malignancy. Although recombination of treatment becomes most popular option to handle cancer, external beam radiotherapy is still a vital choice, especially if the tumour is spreading within small sizes. The patient undergoes radiotherapy require post therapy assessment to investigate the tumour's progress. General X-rays, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are commonly used for this purpose. But these modalities could assess response only after patient had completed the all fractions of treatments because anatomical changed may occur only after treatment. Efficacy of external beam radiotherapy could not be displayed with these diagnostic techniques during early of treatment.

If the radiotherapy efficacy can be assessed earlier by assessing the tumour cells damage via molecular imaging, treatment modification can be done and will possibly increase in the patient survival rate. The molecular marker such as cell death can potentially be used to predict tumour cell damage and its relationship with radiopharmaceutical uptake could be an indicator. Cell death was an essential biological processed for eliminating abundant and unwanted cells during embryonic development, growth, differentiation and maintenance of tissue homeostasis. There are few types of cell death, such as apoptosis, necrosis and mitotic (Verheij, 2008). Studies suggested that apoptosis is a major form of cell death following radiotherapy (Yang et al., 2012). To date, Annexin V-based tracers are the most frequently used agents for in vitro detection and quantification of apoptotic cells (Khoda et al., 2012). However, more applicable technique is required to assess tumour cell death in vivo and using nuclear medicine technique seems a good option.

The purpose of this study is to assess tumor cell damage after external beam therapy by using technetium-99m pertechnetate ($^{99m}TcO_4^-$) as a tracer. Correlation between irradiation dose and $^{99m}TcO_4^-$ uptake by HeLa cells were investigated.

MATERIALS AND METHODS

HeLa cell lines preparation.

HeLa (ATCC® CCL-2™) cell lines were prepared in Dulbecco's Modified Eagle's Medium (DMEM) Complete Media, which was supplemented with 10% FBS and a 100 unit/mL penicillin-streptomycin. All cells were

incubated at 37°C and 5% CO_2 humidified atmosphere. The cells were grown until confluence and harvested using 0.25% Trypsin-EDTA.

HeLa cells irradiation setup.

Solid water phantoms were organized with the thickness 13.5 cm on LINAC table couch. Cells samples were placed at the center of the beam on top of the solid water phantoms and then covered with 1.5 cm bolus. The samples were irradiated with different radiation dose (0.5 Gy to 10 Gy) using 6 MV photon beam at 100 cm SSD and 10 cm x 10 cm field size. The cell samples were counted immediately to see the viability right after irradiation and then recultured for 10 days for the cells to form colony (clonogenic assay). After 10 days, $^{99m}TcO_4^-$ uptake study was conducted on cell colony and the uptake were compared with the cells colony formed.

Cell viability measurement.

The viability of irradiated cell samples was measured using tryphan blue exclusion methods. The cells were stained using tryphan blue and counted on hemacytometer under microscope. The numbers of viable cells were counted. The counting of viable cells versus non-viable cells were made possible by using trypan as the non-viable cell cytoplasm will look darker compared to viable cell, which have clear cytoplasm after treated with this assay (Strober, 2001).

Clonogenic cell staining.

Cell samples that have been incubated for 10 days were rinsed off of their cell media using 0.5 ml of PBS. Cells were fixed using 0.5 ml ice cold methanol for 15 minutes. Crystal violet were used to stain the cells and after staining process for 30 minutes, the cells were rinsed gently using tap water, then let to dry completely. The visible cell colonies were counted using microscope and analyzed in form of cell survival fraction data using OriginPro 7.5 software.

$^{99m}TcO_4^-$ uptake measurement.

0.2 mCi of $^{99m}TcO_4^-$ in form of sodium pertechnetate were administered into each samples. The samples were incubated again for another 30 minutes to allow $^{99m}TcO_4^-$ uptake by cells. After 30 minutes, the cells were rinsed with 0.5 ml PBS. The cells were then make into suspension using Trypsin EDTA and were centrifuges at 1500 rpm for 5 minutes. The centrifuged cells were scanned using using gamma camera equipped with LEHR collimator. $^{99m}TcO_4^-$ uptake measurements were performed with the detector of gamma camera at 10 cm distance to the cell samples. The count reading was measured for 100 second using 20% window at 140 keV. The percentage $^{99m}TcO_4^-$ uptake was calculated and graph $^{99m}TcO_4^-$ percentage uptake versus irradiation dose was plotted.

RESULTS AND DISCUSSIONS

In this work, HeLa cells were used as an in-vitro model to identify tumor cell damage after radiotherapy. Figure 1 show the number of viable cells which were counted immediately after irradiation with different dose of 6 MV photon beam. Figure 1 clearly shows that the number of viable cells correlates inversely with radiation dose. The loss of reproductive capacity after radiation was associated with early cell death, which may represent the effectiveness of radiotherapy techniques used in the treatment. Joiner and Kogel (2009) pointed that the potential reason of the early cell death was resulting from activation of pathways in response to the initial cellular damaged caused by irradiation (Joiner et al., 2009).

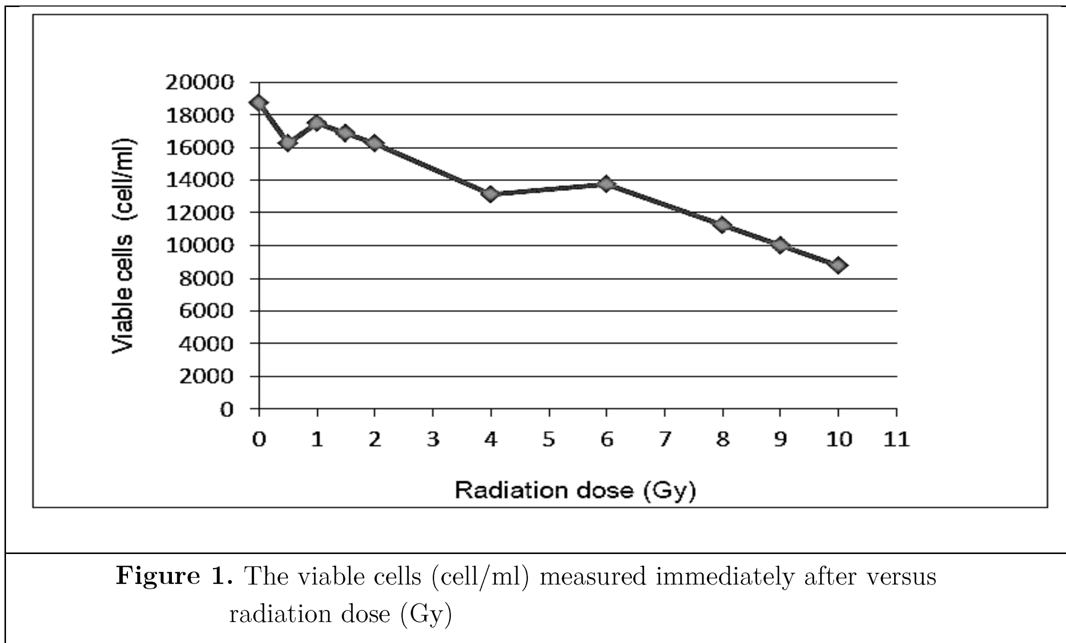

Figure 1. The viable cells (cell/ml) measured immediately after versus radiation dose (Gy)

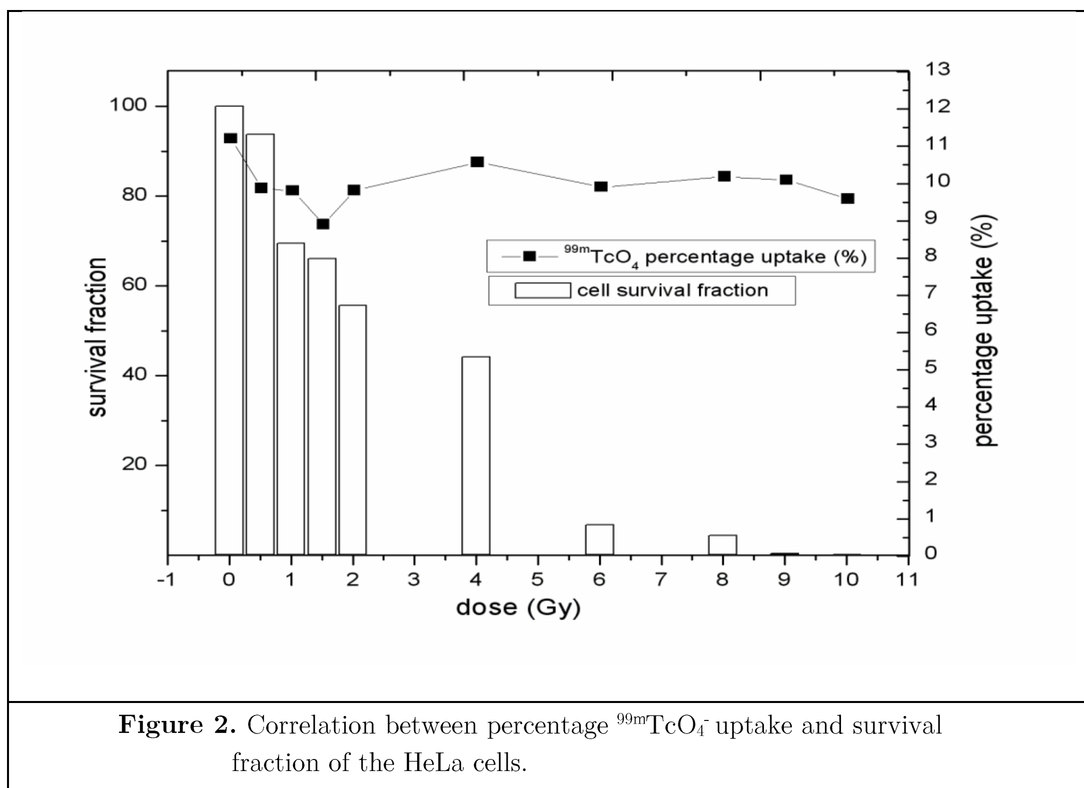

Figure 2. Correlation between percentage $^{99m}TcO_4^-$ uptake and survival fraction of the HeLa cells.

The cell damage also has been assessed using colony forming assay. The cells that form colony were tested for $^{99m}TcO_4^-$ uptake which was employed as indicator to assess cell damage in response to the radiation dose. Based on Figure 2, the cell survival fraction clearly shows decrement as the radiation dose increases. However, $^{99m}TcO_4^-$ uptake among the irradiated HeLa cells does not show any significant correlation with survival fraction. This result contradicted with another similar study conducted by Tabar et al. (2011) and Liang et al. (2008), which used radiopharmaceuticals in evaluating chemotherapy efficacy. Both of these studies shows inverse relation between their respective radiopharmaceutical uptake and cell's apoptotic response. But it is worth to point out that their study used the radiopharmaceuticals that are already tagged with carriers that can selectively be absorbed by their respective cell samples. So we assume that the reason for our contradicting results with these

studies is because of unsuitable pairing between radiopharmaceutical and target cells. Each radiopharmaceuticals have their own affinity with different type of cells and tissues. $^{99m}TcO_4^-$ are already well known to be used clinically for thyroid imaging, parathyroid imaging and Meckel's scan, so choosing the suitable pairing of radiopharmaceutical and targeted cells is crucial. In this case, $^{99m}TcO_4^-$ uptake by cells could be optimized with specific antibody and targeting agent.

CONCLUSION

In conclusion, there is potential for the efficacy of external beam radiotherapy in treating cancer to be assessed by using radioisotope as a non-invasive tracer. In this case, technetium-99m pertechnetate ($^{99m}TcO_4$) could be attached to the specific antibody so that better correlation between the cells uptake and possible cell damages could be observed. Further improvised study are advised so that we can understand more about the relation between cell damages due to radiotherapy, and its effect on intercellular uptake of radiopharmaceuticals.

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