Potential Use of Biomarker γ -H2AX on Peripheral Blood Patient Who Underwent Radioiodine Ablation Treatment in Indonesia

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ABSTRACT

Thyroid cancer is one of the most common endocrine malignancies. Although the 10-year survival rate of differentiated thyroid cancer (DTC) is about 90% after conventional treatments, a small proportion of patients still suffer from tumor recurrence or drug resistance. DNA double-strand breaks (DSBs) are important cellular lesions that can result from ionizing radiation exposure. The biomarker for DSB formation is the phosphorylated form of the histone H2 variant H2AX (γ -H2AX). We propose the use of γ -H2AX as a DNA DSB biomarker in thyroid cancer patients receiving radioiodine treatment as a possibility to detect the potential of instability genome after receiving the treatment. Evaluating DNA DSB damage with γ -H2AX biomarker might be important in managing thyroid cancer.

INTRODUCTION

Ablation that eliminates thyroid tissue remnants with radioiodine (I-131) is used to eliminate thyroid cancer/benign tissue remnants in patients after surgical treatment. Some studies reported that radioiodine treatment was the only best prognostic parameter on the free disease interval and improvement of survival rate of well-differentiated thyroid cancer patients [1,2].

Surgery is the main treatment of thyroid cancer, and all follicular and papillary carcinomas with a diameter of more than 1 cm - 1.5 cm are recommended to undergo total or near-total thyroidectomy [3]. Such an aggressive surgical approach can improve the capability of I-131 to ablate the remaining gland and to treat distant metastases. The remaining thyroid tissue will prevent detection of local and distant metastases on follow-up with I-131 scanning [4].

The therapeutic application of radioiodine-131(I-131) has been widely used in the therapy of well-differentiated thyroid cancer [5]. In this context, radioiodine therapy (thyroid ablation) consists of administering a large amount of radioiodine. Since momentous complications may occur, radioiodine treatment should be carried out

only when there is a reasonable expectation that will benefit the patient [6].

In ablation of remaining tissue, an activity of 3.7–7.4 GBq is usually administered, and this dose has commonly not been associated with severe complications. Anyhow, transient bone marrow abolishment occurs after therapy [7]. Therefore, the limiting factor is the radiation dose to the blood and bone marrow in most patients treated with a large amount of I-131 [8]. Dosimetric studies have calculated the radiation dose to the blood and bone marrow with a large amount of radioiodine [9,10]. Cytology radiation as shown in micronucleus assay and chromosomal damage to peripheral lymphocytes in vivo following radioiodine therapy has been known [11]. DNA damage-initiated nuclear foci in response to ionizing radiation represent complexes of signaling and repair that localize to sites of DNA strand breaks in the cell nucleus [12]. Recently, γ -H2AX foci immune detection has been reported as a useful biomarker of DNA double-strand breaks (DSBs) [13]. The γ -H2AX is the phosphorylation of the histone variant H2AX, one of the earliest stages in the cellular response to DSBs [14]. The γ -H2AX foci formed at the sites of DSBs are emerging biomarkers

of radiation exposure [15]. Their potential is accurately to estimate radiation doses reported in the following experimental studies in human in vivo, the non-human primate in vivo, and in vivo exposure in human diagnostic or therapeutic.

Especially in the use of γ -H2AX to detect of DSBs, we proposed the review paper to discuss the importance of DSB detection in thyroid patients after receiving thyroid ablation base on the update paper and result of γ -H2AX research in Indonesia. The monitoring of DNA DSB will be important to predict the potential of genome instability in patients after receiving thyroid ablation.

γ -H2AX Biomarker

The expression of γ -H2AX foci can be seen in **Figure 1**. Some studies have proved high sensitivity reach few milli gray, the ability of the γ -H2AX assay to identify a recent partial body exposure, and the existence of foci several days after exposure [17,18].

Figure 1. Expression of γ -H2AX as bright green foci in lymphocyte cell, and cell without γ -H2AX foci, originally magnification 10 x100 [16].

Figure 2. Schematic representation of the kinetics of γ -H2AX formation and loss in relation to DSB repair following ionizing irradiation. The number of γ -H2AX foci increases rapidly and reaches a maximum a few minutes after exposure [22].

Radiation exposure produces its main damage in the cell's nucleus. Clusters of ionization in the DNA result in the typical damage of ionizing radiation. The most important conditions are base damage, DNA single-strand breaks, and DNA double-strand breaks [19]. If DNA double-strand breaks are still unrepaired, it is not directly meant cell death that occurs but died after a limited number of cell divisions, also known as clonogenic death [20]. Double strand break is known as the most biologically significant lesion induced by ionizing radiation, and cells can repair this damage to some extent [21]. The DNA damage response consists of some pathways (programmed cell-cycle delays, DNA repair, cell death, or combinations of these). DNA repair can take place at several levels: base excision repair, single-strand break repair, and most importantly, double-strand break repair. Unrepaired DNA double-strand breaks can lead to cell death, and mis-repaired DNA damage can lead to chromosomal translocations, mutations, and subsequently carcinogenesis [19].

Within a few minutes after radiation exposure, phosphorylation of H2AX histone (γ -H2AX) starts. It can be detected using immunostaining as specific nuclear γ -H2AX foci as shown in **Figure 1**. After a rapid increase, with maximum values usually seen after about 1 hour, DNA repair results in a decrease in these γ -H2AX foci, with maximum repair at 24–48 hours as shown in **Figure 2**.

Unrepaired foci at that time point are considered residual damages and might be the most important for killing tumor cells. These processes are dose-dependent, assessment of γ -H2AX foci as a useful tool for quantification of radiation-induced DNA damage. Thus, the number of γ -H2AX foci is considered as a biomarker of exposure, and the remaining foci are a measure of repair capacity and are considered a biomarker of susceptibility [23–25]. However, direct quantification of exposure has a limited value because of the fast kinetics of decline and the wide variation in foci numbers between individuals, as clearly observed in the study by Denoyer et al. [26]. Koch et al. [27] showed that residual γ -H2AX foci were more likely to predict local tumor control after radiotherapy than initial damage or the kinetics of repair.

One main public health problem in Indonesia is cancer. Based on Basic Health Research 2007, cancer is the 7th cause of death among all causes (5.7%). The national prevalence of cancer is 4.3 per 1,000 population [28]. Based on the Indonesian fact sheet of cancer in Indonesia, thyroid cancer ranked 28th on the number of cases in 2018 [29].

In normal conditions, thyroidal iodine has a role in metabolism involving absorption oxidation and organification of iodide ions (I^-) forming the thyroid hormones triiodothyronine (T3) and thyroxine (T4) [30]. The specified avidity of thyroid tissue to absorb iodide offers a highly targeted means of treating thyroid disease.

In the benign thyroid disease, I-131 is showed in hyperthyroidism due to Graves' disease (GD) or toxic goiter from single toxic adenomas or a multi-nodular goiter [31]. It can also be used in subclinical hyperthyroidism [32] and euthyroid goiter [33]. In malignant thyroid disease, I-131 ablation or therapy is recommended for most patients who have undergone total or near-total thyroidectomy for a tumor >1 cm in diameter. I-131 may also be offered to patients with distant metastases or sites not amenable to surgical resection which are iodine avid. I-131 is not indicated for I-131 refractive thyroid cancer [9,34]. I-131 is a systemically administered radioactive nuclide that decays to xenon 131 by emitting both beta and gamma particles. The principal beta particles (89.3%) exhibit energy of 606 keV and can be used for radiotherapy. In contrast, the principal gamma particles (81.2%) exhibit a 364 keV photopeak and are used for clinical imaging. Beta-irradiation has a maximal tissue penetration range of 2.2 mm, achieving cytotoxicity by direct and indirect DNA damage of thyroid cells which subsequently triggers cell death [35].

From the radioiodine therapy on thyroid cancer, Doai et al. [36] report the number of foci per lymphocyte nucleus by 0.41 ± 0.51 before, which increases to 6.19 ± 1.80 after 3.7 GBq I-131. The frequencies of lymphocyte γ -H2AX foci in cells exposed by I-131 were significantly higher than those before therapy as a control, and γ -H2AX foci can be detected until 4 days after treatment. Since there were at least 6 months between the observation time and the last radioiodine therapy, no significant effect on the frequency of lymphocyte γ -H2AX foci in cells by previous exposure of lymphocytes to I-131 has been detected. Cytological DNA damage to lymphocytes induced by I-131 may be repaired by 6-month post-therapy [36].

Nuclear medicine treatments involve systemic internal irradiation as compared with external irradiation (e.g., in radiotherapy). After radionuclide incorporation, the cells are not only irradiated by intra- and extracellular I-131 for seconds or minutes but also continuously irradiated over a longer period with a permanently changing dose rate. This could alter the effect of radiation damage compared with a fractionated high-dose-rate partial-body irradiation in radiotherapy. In a report of peptide receptor radionuclide therapy (PRRT) study by ^{177}Lu , Eberlein et al. [37], the first linear relationship between the number of γ -H2AX foci/cells and the absorbed dose to the blood only occurs over the first 4–5 hours after therapy starts [16]. After this period, the disappearance of the DSB foci is reflected by the decay of the γ -H2AX foci/cell numbers [37,38].

Medical radiation workers, including doctors, nurses, and other medical staff, are exposed to low doses of ionizing radiation from a variety of sources, including diagnostic x-rays and other medical devices [39], and constitute the largest occupational group exposed to

man-made sources of radiation [40]. There is a possibility that medical radiation workers, Cath lab, and nuclear medicine employees, have a risk to be exposed to x-ray or gamma-ray in diagnostic or therapeutic procedures in their activity. This risk will be increased when some accidental conditions result in overdose exposure. It also has the potential to influence DNA damage, both double-strand break (DNA DSB) and single-strand break. The effect of ionizing radiation is not only high but also low chronic doses that are known as mutagenic and carcinogenic agents in mammals, including humans. Medical staff using radiation for diagnostic and therapeutic purposes are potentially at risk of overexposure [41]. Fortunately, due to the application of principles of radiological protection, the levels of exposure of medical staff to ionizing radiation have been decreased until below the limit of 20 mSv/year [42].

For example, from the study in nuclear medicine workers, the ionizing radiation occupational exposure, the level of DNA damage, depends on the kind of work. Dobrzyńska et al. [41], leads to enhanced levels of reversible DNA damage in leukocytes of nuclear medicine employees. The kind of work in this study includes 86 samples that consist of 46 medical workers and 40 controls [41]. The data from our previous study found that no statistical different potential of DNA DSB damage between medical radiation workers who are mostly radiologists and radiography operators with administrative staff as a control. The DNA damage was detected by γ -H2AX assay. However, in this study, medical radiation workers have not been grouped as clinical radiologists (medical doctors, nurses, and radiography operators), and nuclear medicine employees. Based on our previous study with 33 volunteers consisting of 9 administrative staff as the control, 6 nuclear medicine workers (medical doctor and nurses), and 18 Cath lab workers, there were no differences in the mean γ -H2AX foci between control and radiation workers. Both γ -H2AX foci worker and non-worker were still in the range of normal value [42].

In another study using 53BP1 and γ -H2AX foci in medical radiation workers, there were no differences in the potential of DSBs damage between medical radiation workers and administrative staff and no indication of radiation response to low ionizing radiation exposure in medical radiation worker. Both γ -H2AX and 53BP1 foci assay could be used to detect DNA damage, but to ensure the potential, DSBs by γ -H2AX assay were recommended. Expression of 53BP1 is not only related to γ -H2AX expression but also connected with the process of DNA repair, cell cycle arrested, and in non-homologous end joining related DNA repair process [43].

Indonesia has a region with high dose natural ionizing radiation. Mamuju, a city in the suburb of West Sulawesi in the Sulawesi Sea, has background radiation around 13 times higher than normal. This radiation is the result of natural uranium content (Radium-226 and Radon gas,

both of which are highly water-soluble) in rock and soil. The major concern is due to its location which is near to the inhabitant settlement area.

This place has a higher average dose rate compared to other regions in Sulawesi island and even Indonesia, which can reach 2.800 nSv/h [44,45]. The hunt to estimate the genetic consequences of ionizing radiation on humans has been one of the most outstanding issues of human genetics in the past 60 years [46]. Some previous experimental studies have not entirely explained radiation-induced germline mutation in humans as a topic that remains controversial. All current estimations of genetic hazard of radiation exposure for humans are largely derived by extrapolation of animal experimental data that has its limitation [47].

From our studies that grouped the exposed area (dose of 7 mSv/year) and the control area (dose of 2 mSv/year), even though it did not reach statistical difference, the average expression of γ -H2AX foci in the exposed area was higher than the control area ($p > 0.05$). In this condition, in Mamuju, West Sulawesi, high background radiation in the exposed area effectively induces an excess of DNA double-strand breaks more than in residents living in the control area. The higher expression of γ -H2AX foci still can be detected on lymphocyte cells more than 6 hours after the blood sample is collected. It means that the foci are proof of the process of DNA repair after exposure by background irradiation. After finishing the DNA repair process, the foci will disappear [16]. By simulation of radiation emergency setting, Rothkamm and Horn [22] explain that by increasing post-exposure time, the number of γ -H2AX foci decreases rapidly to about 50% of the initial level within one hour. This initial fast decline is followed by a slower loss of the ~30% residual foci presence a few hours after exposure. Twenty-four-hour post-exposure, residual foci levels are still distinctly higher than background levels, at least for doses of several hundred mGy or more. Based on these characteristics, the minimum dose required for the reliable detection of radiation exposure can be expected to increase sharply to tens of mGy within the first couple of hours and then more slowly as more and more foci are lost post-exposure [48].

The age and sex factors did not show any difference in DSB damages in both control and exposed areas. Age and different habits for women and men that are not related to the duration of exposure are not enough to make significant DSBs repair shown in the existence of γ -H2AX foci as reported from our study [16]. In a population study with 246 volunteers, Garm et al. [49] conclude that gender has no association with DNA repair capability for both single-strand and double-strand break.

CONCLUSION

Limited references are explaining the relationship between ionizing radiation exposure in nuclear medicine treatment, especially on thyroid ablation with I-131 with risk of a secondary tumor. Evaluating DNA DSB damage with γ -H2AX biomarker might be important in the management of thyroid cancer.

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