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Effect of low dose X-ray on membrane fluidity of thalassemic red blood cells

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► ***Original article***

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ABSTRACT

Background: Chest X-ray is one of the examinations required for an annual health checkup. The interaction of radiation to the medium produces free radicals, which consequently causes biological changes either structural or properties of the cells. Whether the radiation from Chest X-ray upright technique affects the plasma membrane fluidity of thalassemic red blood cells (RBCs) is still unclear. ***Materials and Methods:*** Whole blood samples of non-thalassemia group (G1), non-clinically significant thalassemia group (G2) and clinically significant thalassemia group (G3) were irradiated with the Chest X-ray upright technique with a dose of 6.57 µSv (90 kVp). One-tube osmotic fragility (OF) test, dichlorophenol-indophenol (DCIP) test for hemoglobin E (HbE) screening and glucose-6-phosphate dehydrogenase (G6PD) activity analysis were performed on those samples. ***Results:*** The % OF values of G1 and G3, between irradiated and non-irradiated RBCs, were comparable, while those of G2 were significantly increased. However, the irradiation did not affect HbE screening test by DCIP technique in G2. In addition, it did not alter the G6PD activity in both thalassemia and non-thalassemia samples. ***Conclusion:*** Chest X-ray upright technique with a dose of 6.57 µSv (90 kVp) did not alter the membrane fluidity and the G6PD activity of thalassemic and non-thalassemic RBCs. Thus, it did not affect the thalassemia screening by OF and DCIP tests. However, increasing % OF value in HbE trait might be used as one of the screening test for HbE trait.

Keywords: *X-ray, radiation, thalassemia, osmotic fragility, red blood cell*

INTRODUCTION

Thalassemia and hemoglobinopathy are inherited hemoglobin (Hb) disorders that result in a production of structurally abnormal Hb variants or a reduction in the synthesis of structurally normal Hb, consequently, to red cell pathology. These diseases are generally spread especially in Southeast Asia, Southern China, Africa, Middle East and Mediterranean. In Thailand, a-thalassemia, β-thalassemia and HbE (β26 (B8) Glu→Lys, GAG>AAG) are prevalent. The incidences are 20-30% for α-thalassemia, 3-9% for β-thalassemia and up to 54% for HbE [1]. In order to provide a rapid method for identifying thalassemia in a region with massive population and limited resources, the preliminary screening of α- and β-thalassemia is performed using one-tube osmotic fragility (OF) test [2] and/or mean corpuscular volume (MCV) [3, 4] whereas the screening of HbE is performed using dichlorophenol-indophenol (DCIP) test [5].

The Chest X-ray upright is one of the annual health checkup methods to assess lung function. The lung function test is included in not only for healthy persons, but also in ongoing medical

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care of patients. Other than Chest X-ray, the dual -energy X-ray absorptiometry (DXA) is required for osteoporosis at eight years of age and annually thereafter, as necessary [6-8]. The effective doses for Chest X-ray technique (7.00-50.00 μSv) and DXA technique (9.00-27.00 μSv) can be classified as low dose level [9, 10]. A previous study showing that the medical diagnostic X-ray at 50, 70, and 100 kV did not influence hemolysis, osmotic fragility and fluorescence anisotropy values of irradiated red blood cells (RBCs) from normal individuals [11]. However, there has been no report about the effect of diagnostic X-ray on thalassemic RBCs. The aim of this study was to analyze the effect of radiation used for Chest X-ray on the RBC fragility of thalassemia subjects.

MATERIALS AND METHODS

Blood samples and thalassemia diagnosis

Ethylenediaminetetraacetic acid (EDTA) blood samples were obtained from the Thalassemia Laboratory, Associated Medical Sciences-Clinical Service Center (AMS-CSC), Chiang Mai University, Chiang Mai, Thailand, where red cell parameters including, RBC counts, Hb, packed cell volume (PCV), MCV and mean corpuscular Hb (MCH) were analyzed by the ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostic, Deerfield, IL, USA) while quantification of HbA_2 (for detection of β-thalassemia) and identification of hemoglobinopathies including HbE were performed by using capillary electrophoresis (Capillarys™ 2 Flex Piercing; Sebia, Norcross, GA, USA). In addition, the molecular diagnosis for α^0-thalassemia (SEA and Thai type deletions) was detected by real-time PCR with SYBR Green1 and high resolution melting (HRM) analysis as previously described [12, 13]. Ethical procedures for using the samples that are outside the primary purpose were reviewed and approved by the Ethics Committee of the Faculty of AMS, Chiang Mai University, Chiang Mai, Thailand (ethical approval number AMSEC-61EX -048).

Radiation exposure

The radiation output was quantified using an ion chamber detector (Radcal, 10X6-6, USA). On the same day of blood collection, 100 μL EDTA whole blood samples of thalassemia and non-thalassemia subjects were aliquoted into 1.5 ml micro-tubes and irradiated by using an X-ray machine (Shimadzu, Collimator type R-20J, Japan) utilizing Chest X-ray upright technique (90 kVp 100 mA 0.071 second, Field size 14" ×17", Source to Sample Distance 72").

Determination of the RBC osmotic fragility

The OF test was performed to determine the degree of RBC hemolysis immediately after the blood sample was irradiated. The 20 μL of irradiated and non-irradiated RBCs were suspended into 5 mL of 0.36% buffered saline solution (BSS) and 5 mL of distilled water (DW). The solutions were then placed at room temperature for 5 minutes, and centrifuged at 3000 rpm, for 5 minutes. The optical density (OD) of released hemoglobin in saline solution and distilled water was determined by spectrophotometer at 540 nm [14, 15]. The percentages of the hemolysis (% OF) were calculated as follow: $\% OF = (OD_{BSS}/OD_{DW})\times100$ and those with cut-off values of less than 85% were used for the thalassemia screening [14]. In addition, the delta-osmotic fragility (ΔOF) was also calculated as follow: $\Delta OF = OF_X - OF_{NX}$. Where, the OF_X and OF_{NX} were percentages of the OF values of irradiated and non-irradiated RBCs, respectively.

Assessment the effect of radiation on HbE screening and G6PD activity

We hypothesized that radiation might produce reactive oxygen species (ROS) that affects the precipitation of unstable Hb, especially HbE. Furthermore, we hypothesized that the G6PD activity might be affected by ROS produced since G6PD is one part of a ROS elimination pathway. Thus, irradiated and non-irradiated RBCs from five non-thalassemia subjects and five HbE traits were randomly selected and tested with the DCIP kit (Thalcon®-DCIP, Surathin International Co., Ltd., Samutprakarn, Thailand) according to

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manufacturers' instructions. The optical density (OD) of precipitated HbA and HbE in DCIP solution were measured by spectrophotometer at a wavelength range of 800 nm as described previously [16]. In addition, the G6PD activity was also analyzed by using the CareStart™ G6PD Biosensor (AccessBio, NJ, USA) according to manufacturers' instructions.

Statistical analysis

Descriptive data were informed as of the mean ± standard deviation (SD). The statistical analysis was performed by Origin Pro8 Software. All obtained parameters were analyzed within group (irradiated RBCs and non-irradiated RBCs) by using paired sample t-test and between groups by using One-way ANOVA (Tukey procedure). The p <0.05 was considered statistically significant.

RESULTS

The radiation output of this X-ray machine was determined for 10 times. The result indicating the dose rate of X-ray machine that used for Chest X-ray technique at the energy of 90 kVp 0.071 second was 1.32 ± 0.02 mGy/s. The effective dose for each sample was about 6.57 μSv. Based on thalassemia diagnostic results, the 108 studied samples could be divided into three groups: G1) non-thalassemia group (n = 50), G2) non-clinically significant thalassemia group (n = 27) and G3) clinically significant thalassemia group (n = 31). The characteristics, thalassemia types and red cell parameters of each group are shown in table 1. The lowest mean levels of Hb, packed cell volume (PCV), MCV and mean corpuscular Hb (MCH), and the highest mean level of RBCs were observed in G3. Mean ± SD of the % OF value for non-irradiated RBCs of G1 was 93.84 ± 4.84%, which was higher than the cut-off value (85%) for thalassemia screening while those of G2 and G3 were lower (82.74 ± 15.09% and 41.10 ± 20.58%, respectively). However, the % OF values between non-irradiated and irradiated RBCs of G1 and G3 were comparable while in G2, the % OF values of irradiated RBCs were significantly increased than those of the non-irradiated (figure 1). In addition, the ΔOF mean of the G2 were significantly higher than those of the G1 but not of the G3 (figure 2).

The HbE trait samples had higher OD values of precipitated HbA and HbE in the DCIP solutions than those of the non-thalassemia samples. However, the OD values between irradiated and non-irradiated RBCs in each group of HbE trait samples and non-thalassemia samples were not significantly different (figure 3). Moreover, the values of G6PD activity between irradiated and non-irradiated RBCs in each subject group of non-thalassemia and thalassemia samples were also comparable (figure 4).

Table 1. The characteristics, thalassemia types and red cell parameters of each sample group.

Subject Groups	α- and β-thalassemias	Age (Years)	Male/ Female	Hb (g/dL)	PCV (L/L)	RBCs (x 10^{12}/L)	MCV (fL)	MCH (pg)
G1: Non-thalassemia (n = 50)	Normal individual ($\alpha\alpha/\alpha\alpha;\beta^A/\beta^A$)	33.63±14.95 (16.0-78.0)	20/30	13.20±1.91 (8.3-17.0)	0.40±0.05 (0.27-0.52)	4.77±0.67 (3.0-6.1)	85.93±6.18 (80-112)	27.69±1.31 (25.1-30.3)
G2: Non-clinically significant thalassemia (n = 27)	HbE trait ($\alpha\alpha/\alpha\alpha;\beta^E/\beta^A$)	17.38±6.44 (3.0-25.0)	13/14	12.14±1.89 (7.2-16.9)	0.36±0.05 (0.22-0.47)	4.84±0.64 (3.7-6.0)	74.64±7.51 (52-86)	25.1±2.56 (18.3-29.5)
G3: Clinically significant thalassemia (n = 31)	α-thal trait ($--/\alpha\alpha;\beta^A/\beta^A$; n = 6) HbH disease ($--/-\alpha;\beta^A/\beta^A$; n = 5) β-thal trait ($\alpha\alpha/\alpha\alpha;\beta^T/\beta^A$; n = 11) α- and β-thal traits ($--/\alpha\alpha;\beta^T/\beta^A$; n = 3) α-thal and HbE traits ($--/\alpha\alpha;\beta^E/\beta^A$; n = 1) Homozygous HbE ($\alpha\alpha/\alpha\alpha;\beta^E/\beta^E$; n = 3) α-thal trait and homozygous HbE ($--/\alpha\alpha;\beta^E/\beta^E$; n = 2)	36.17±18.69 (1.0-68.0)	16/15	10.38±2.46 (4.7-15.7)	0.33±0.07 (0.16-0.48)	5.14±1.24 (2.1-7.7)	64.90±9.90 (47-82)	20.48±2.83 (15.5-26.7)

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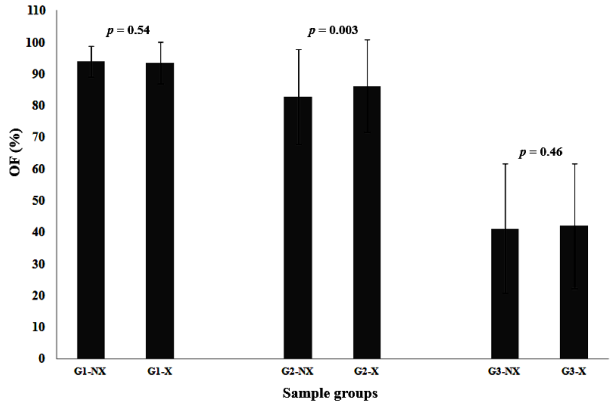

Figure 1. The osmotic fragility of non-irradiated and irradiated RBCs among the three groups of samples. Data are presented as mean and standard deviation (SD). G1, G2 and G3 are non-thalassemia group (n = 50), non-clinically significant thalassemia group (n = 27) and clinically significant thalassemia group (n = 31), respectively while X and NX are X-ray and Non X-ray, respectively.

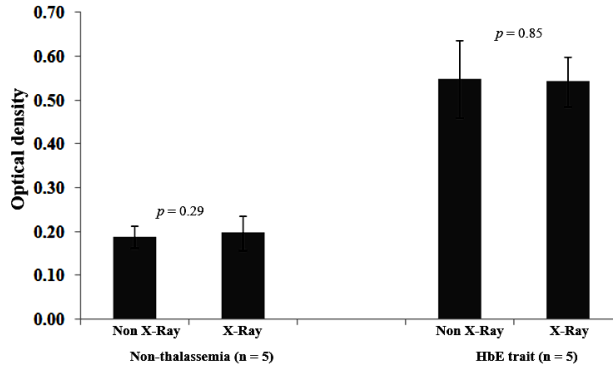

Figure 3. The optical density (OD) of precipitated HbA and HbE in DCIP solution between irradiated and non-irradiated RBCs among two groups of non-thalassemia and HbE trait subjects. Data are presented as mean and standard deviation (SD).

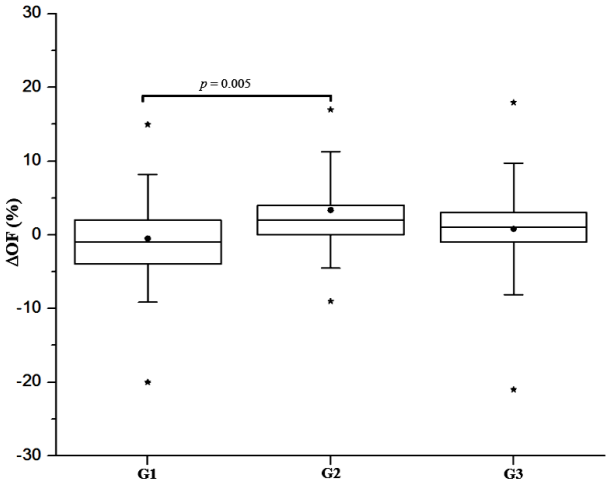

Figure 2. The delta-osmotic fragility (ΔOF) of plasma membrane of red blood cells among the three groups of samples. Data are presented as median (horizontal line) and mean (black circle) while the box represents percentile 25 and 75, the upper and lower whiskers represent standard deviation (SD), and the maximum and minimum values are shown as star (*). G1, G2 and G3 are non-thalassemia group (n = 50), non-clinically significant thalassemia group (n = 27) and clinically significant thalassemia group (n = 31), respectively.

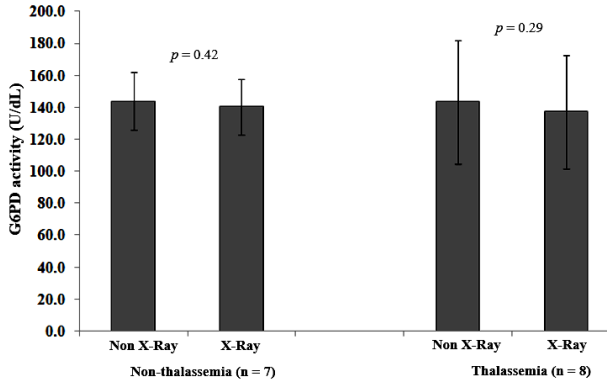

Figure 4. The G6PD activity between irradiated and non-irradiated RBCs among two groups of non-thalassemia and thalassemia subjects. Data are presented as mean and standard deviation (SD).

DISCUSSION

X-ray is high-energy electromagnetic radiation causes matter ionization during the interaction. The matter ionization induces a free radical formation, in which produces a variety of changes in cell membrane. Normally, in a whole blood system, an anti-oxidant capacity decreases after the X-ray irradiation, in which it will lead to cause an abundance of polyunsaturated lipids on red cell membranes and initiate lipid peroxidation by free radicals [17, 18]. In the current study, red blood cells of non-thalassemia and thalassemia subjects were directly exposed to the X-ray at the radiation dose of about 6.57 µSv (90 kVp). Red blood cells from non-thalassemia subjects showed similarity of diagnostic X-ray results at energy of 50 to 100 kVp, which did not induce any changes in osmotic fragility after irradiation for 30 min [11].

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Moreover, in the clinically significant thalassemia group (G3), the osmotic fragility did not change after irradiation. These results exhibited that the red blood cell membrane fluidity of non-thalassemia and clinically significant thalassemia samples did not respond to low dose radiation. Even though the low dose irradiation did not show significant effect on cell membrane, the alteration of cell membrane however was observed in human peripheral blood mononucleated cells [19]. Surprisingly, the red blood cells from non-clinically significant thalassemia subjects responded on the irradiation by increasing their osmotic fragility. This indicates that the radiographic examination such as chest X-ray or DXA prior blood sample collection might change the red blood cell osmotic fragility in this subject group. All subjects in the group of non-clinically significant thalassemia were HbE trait, thus, the RBC irradiation procedure can be used as one of the screening tests for HbE trait. However, the appropriated cut-off values of increasing % OF and ΔOF are needed to be further studied in a larger population.

Generally, the screening of HbE is performed by using DCIP test whereas the OF test is not recommended for HbE screening [20, 21]. Therefore, the irradiated and non-irradiated RBCs from non-thalassemia individuals and HbE traits were randomly selected and tested with the DCIP kit (Thalcon®-DCIP, Surathin International Co., Ltd., Samutprakarn, Thailand). The study showed that the OD value of precipitated HbA and HbE in DCIP solution between irradiated and non-irradiated RBCs in each subject group were not significantly different (figure 3). In addition, the G6PD activity, which plays a role in protecting cells from potentially harmful molecules (the ROS) between irradiated and non-irradiated RBCs in each subject group of non-thalassemia and thalassemia subjects were also comparable (figure 4). These results correspond with the previous study by Smith et al who found that low dose ionizing radiation produces too little ROS to directly affect antioxidant concentration in cells [22]. Therefore, taken together, the present study demonstrated that the Chest X-ray upright technique with a dose of 6.57 μSv (90 kVp) did not alter the membrane fluidity of thalassemic RBCs and also did not affect the thalassemia screening test results. The increasing values of % OF and ΔOF in irradiated RBCs of HbE trait could be used as one of screening test for HbE trait.

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***Conflicts of interest*: Declared none.**

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