ORIGINAL ARTICLE

# Study on γH2AX Expression of Lymphocytes as a Biomarker In Radiation Biodosimetry

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## ABSTRACT

Flow cytometry analysis was used to detect the changes of γH2AX protein expression in human peripheral blood lymphocytes. In the dose-effect study, the expression of γH2AX was detected 1 h after irradiation with $^{60}$Co γ-rays at doses of 0, 0.5, 1, 2, 4, and 6 Gy. Blood was cultivated for 0, 1, 2, 4, 6, 12, and 24 h after 4 Gy $^{60}$Co γ-rays irradiation for the time-effect study. At the same time, the blood was divided into four treatment groups (ultraviolet [UV] irradiation, $^{60}$Co γ-rays irradiation, UV plus $^{60}$Co γ-rays irradiation, and control group) to detect the changes of protein expression of γH2AX. The results showed that the γH2AX protein expression was in dose-effect and time-effect relationship with $^{60}$Co γ-rays. The peak expression of γH2AX was at 1 h after $^{60}$Co γ-ray irradiation and began to decrease quickly. Compared to irradiation with $^{60}$Co γ-rays alone, the expression of γH2AX was not significantly changed after irradiation with $^{60}$Co γ-rays plus UV. Dose rate did not significantly change the expression of γH2AX. The expression of γH2AX induced by $^{60}$Co γ-rays was basically consistent with the mice *in vivo* and *in vitro*. The results revealed that the detection of γH2AX protein expression changes in peripheral blood lymphocyte by flow cytometry analysis is reasonable and may be useful for biodosimetry.

**Key words:** $^{60}$Co γ-ray, γH2AX, biodosimetry, DNA damage, irradiation

## Introduction

Biodosimetry is used to estimate the absorbed dose in the exposed individuals and plays an important role in the triage and medical treatment and management of radiological casualties. The long-established dicentric assay is the gold standard for accurate biological dose estimation following a suspected radiation overexposure[1] but suffers from (a) long turn-around times; (b) low throughput;[2] (c) the reliance on highly skilled cytogeneticists for dicentric scoring, complicating the development of surge capacity. Recently, the phosphorylated H2A variant γH2AX has proved to play an important role in DNA repair, cell cycle checkpoints, genomic stability, and tumor suppression. The histone variant H2AX is phosphorylated in response to DNA double-strand breaks (DSB) induced by ionizing radiation.[3-5] Soon after the occurrence of a DNA DSB, the formation of γH2AX histone variants is expected. Because γH2AX is a reliable marker of DNA DSB, the γH2AX assay is very useful for detecting DNA DSB caused by ionizing radiation.[6-8] The detection of γH2AX protein expression changes in peripheral blood lymphocytes by flow cytometry analysis is reasonable and may be useful for biological dosimetry.

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## Materials and Methods

### Blood samples

Human blood samples were collected from healthy volunteers aged 30–45 years. Blood was taken with informed consent and the approval of the local ethics committee. Blood from the mouse was derived from the orbit of the BalB/C mouse with the approval of the local ethics committee. Blood was collected with strictly aseptic technique in sample tubes containing heparin and immediately stored on ice until further processing.

### Conditions of exposure

Whole blood was irradiated in heparinized tubes with $^{60}$Co γ-rays in the Laboratory of Quality Control for Medical Exposure Equipment (IAEA/WHO Second Standard Dosimetry



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Laboratory, National Institute of Radiation Protection). The blood was irradiated with ultraviolet (UV) light for UV irradiation.

### Lymphocyte separation

After exposure, samples were incubated at 37°C for various designated postexposure times before isolation. Lymphocytes were isolated from whole-blood samples by Ficoll-Paque density gradient centrifugation. Lymphocyte separation was performed according to the manufacturer's instructions. Blood samples diluted 1:1 with phosphate-buffered saline (PBS) were layered onto equal volume of Ficoll-Paque and centrifuged at 700 g for 25 min at 20°C. After centrifugation, the lymphocyte layers were washed three times with cold PBS.

### Flow cytometry analysis

Lymphocytes were fixed with 100% methanol (30 min, −20°C) and then washed in PBS containing 1% fetal calf serum (FCS) for 3 × 10 min at RT. Samples were incubated with a specific γH2AX-FITC (dilution 1:100) at RT for 30 min. Then, they were washed in PBS containing 1% FCS for 3 × 10 min at RT. The expression of γH2AX protein was analyzed by flow cytometry.

### Immunofluorescence analysis

Cells were resuspended in PBS and spotted onto coverslips for 6 min at RT followed by fixation in 100% methanol (30 min, −20°C). Lymphocytes were then washed in PBS containing 1% FCS for 3 × 10 min at RT. Samples were incubated with a specific γH2AX-FITC (dilution 1:100) at RT for 30 min. Then, they were washed in PBS containing 1% FCS for 3 × 10 min at RT. Samples were incubated with DAPI (1 μg/ml) for 10 min and washed with PBS containing 1% FCS for 3 × 10 min at RT. Immunofluorescence analysis was performed using microscope (Zeiss, Germany). γH2AX foci were detected and captured by machine with automatic imaging.

### Statistical analysis

For statistical analysis, the SPSS 15.0 (IBM Corporation, USA) software was used. Statistical analysis was performed using the Student's *t*-test for independent data. A difference with $P < 0.05$ was considered statistically significant.

### Ethical approval

The study was approved by the Institutional Research Ethics Committee and was performed in accordance with the ethical standards in the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

## Results

### The dose-effect curve of the γH2AX expression after 1 h

The results [Figure 1] showed that the γH2AX protein expression in human peripheral blood lymphocytes has a good dose-effect relationship with $^{60}$Co γ-ray radiation. Dose-effect curves showed a binomial relationship between the radiation dose and the expression of γH2AX protein. Binomial regression curve equation is as follows: $y = -2.32x^2 + 28.98x + 3.91$, $R^2 = 0.9997$. γH2AX expression increased with the increase of irradiation dose after 1 h. After 6 Gy or greater dose irradiation, the expression of γH2AX reached the peak.

### The foci number of γH2AX in nuclei

As shown in Figure 2, the foci number and size in nuclei of lymphocyte increased with irradiation dose. The results of fluorescence were consistent with the flow cytometry.

### The dose-effect curve of the γH2AX expression after 12 h

The results showed that the γH2AX protein expression was dose dependent. The dose-effect curve showed a linear quadratic relationship between the radiation dose and the expression of γH2AX protein. The linear quadratic regression curve equation is as follows: $y = 0.27x^2 + 2.78x + 4.79$, $R^2 = 0.9876$. The expression of γH2AX still had a dose effect with irradiation after 12 h, but the expression level was significantly decreased [Figure 3].

### The time-effect curve of the γH2AX expression after 4 Gy $^{60}$Co γ-rays irradiation

As shown in Figure 4, the expression of γH2AX peaked at 1 h after 4 Gy irradiation and began to decrease quickly. The level of γH2AX expression was close to background level after 24 h.

### The foci number of γH2AX in nuclei after 4 Gy $^{60}$Co γ-rays irradiation at different times

The results as shown in Figure 5 showed that the number and size of foci in nuclei of lymphocyte were peaked at 1 h after 4 Gy irradiation and began to decrease quickly.

### Influence of different dose rate of $^{60}$Co γ-rays on the effect of the expression of γH2AX protein.

The level of γH2AX protein expression had no significant difference after 4 Gy $^{60}$Co γ-rays of 0.25, 0.5, and 1 Gy/min dose rate γ-rays. The expression of γH2AX protein had no significant difference among three groups [Figure 6].

### Influence of ultraviolet on the effect of the expression of γH2AX protein induced by $^{60}$Co γ-rays

The expression of γH2AX in the UV irradiation group was increased compared with the sham-irradiation control group, and the peak expression of γH2AX was at 6 h after exposure, while compared with $^{60}$Co γ-ray group, γH2AX expression was unchanged

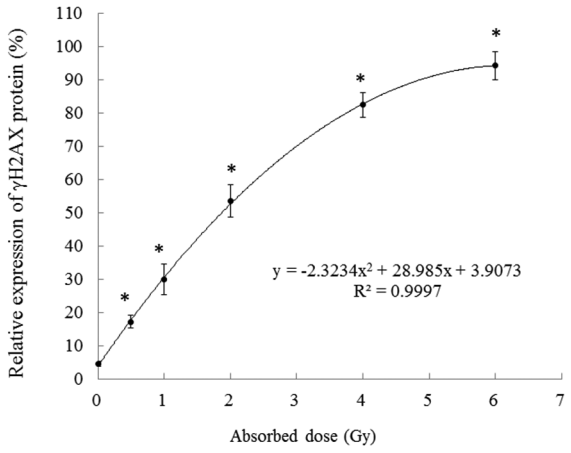


**Figure 1:** Dose-effect curve of γH2AX protein expression in peripheral blood lymphocyte by irradiation with 0–6 Gy $^{60}$Co γ-rays after 1 h. *$P < 0.01$ versus 0 Gy

in UV plus $^{60}$Co γ-ray group. UV irradiation had no effect on the expression of γH2AX expression induced by $^{60}$Co γ-rays [Figure 7].

## Dose-effect curve of the γH2AX expression of mouse *in vivo* and *in vitro*

The variation trends of γH2AX expression after $^{60}$Co γ-rays irradiation of mouse *in vivo* and *in vitro* are similar. The expression of γH2AX peaked at 97.9% and 96.5% after 6 Gy $^{60}$Co γ-rays irradiated. The variation trends of γH2AX expression after $^{60}$Co γ-rays irradiation of mouse *in vivo* and *in vitro* had no significant difference [Figure 8].

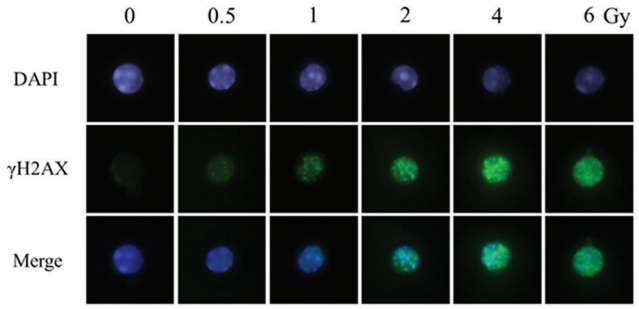


**Figure 2:** Images of γH2AX foci produced by irradiation with 0–6 Gy $^{60}$Co γ-rays after 1 h

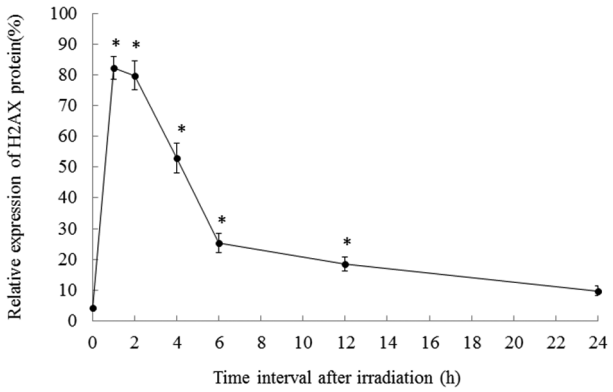


**Figure 4:** Time-effect curve of γH2AX protein expression in peripheral blood lymphocyte after irradiation with 4 Gy $^{60}$Co γ-rays *$P$ < 0.01 versus 0 h

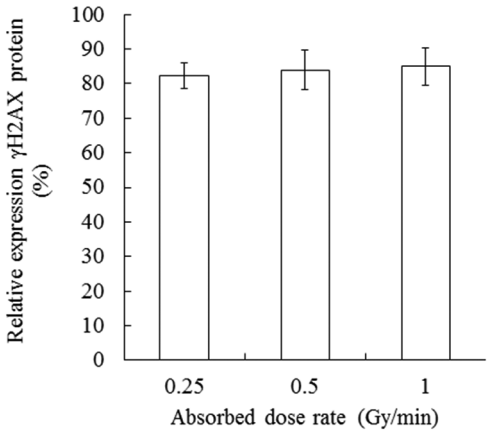


**Figure 6:** γH2AX protein expression in peripheral blood lymphocyte after irradiation with 4 Gy $^{60}$Co γ-rays of different dose rate

## The time-effect curves of the γH2AX expression of mouse *in vivo* and *in vitro* after 4 Gy $^{60}$Co γ-rays irradiation

The expression of γH2AX of mouse *in vivo* and *in vitro* peaked at 1 h after 4 Gy irradiation and began to decrease quickly. The

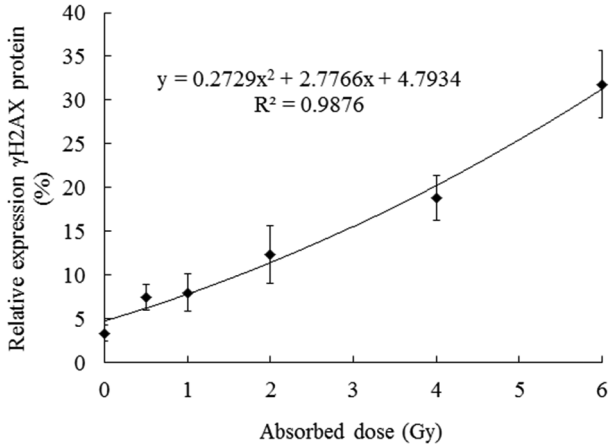


**Figure 3:** Dose-effect curve of γH2AX protein expression in peripheral blood lymphocyte by irradiation with 0–6 Gy $^{60}$Co γ-rays after 1 h

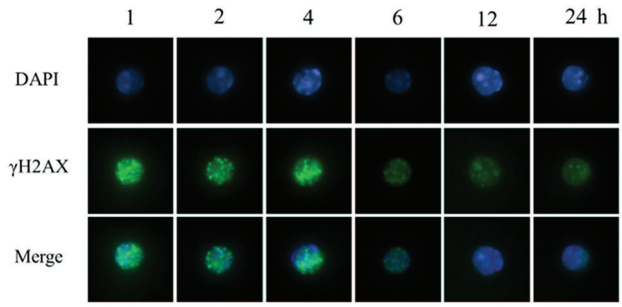


**Figure 5:** Images of γH2AX foci produced by irradiation with 4 Gy $^{60}$Co γ-rays after different time points

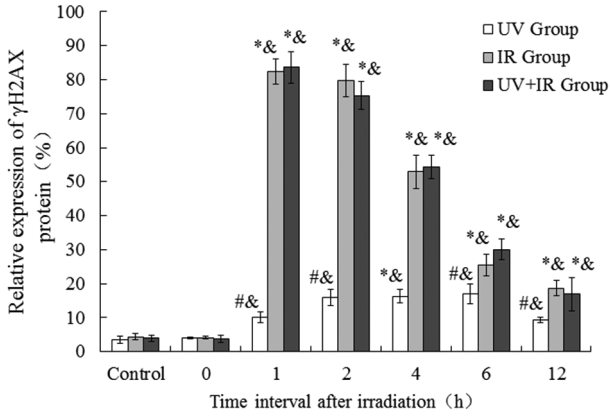


**Figure 7:** γH2AX protein expression in peripheral blood lymphocyte induced by ultraviolet irradiation and $^{60}$Co γ-rays. #$P$ < 0.05, *$P$ < 0.01 versus 0 h respective group, &$P$ < 0.01 versus respective control group

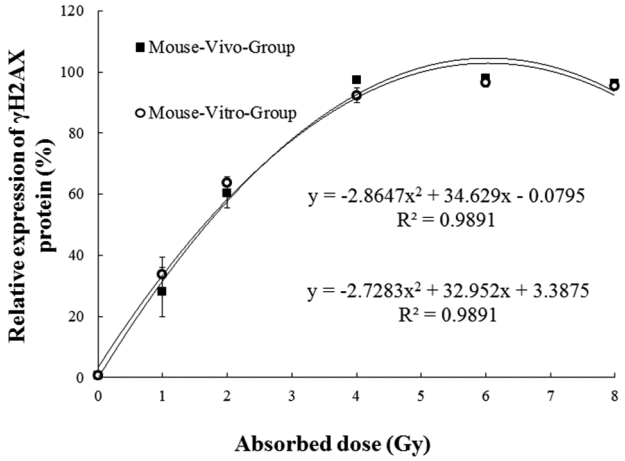


**Figure 8:** Dose-effect curve of the γH2AX expression of mouse *in vivo* and *in vitro* by irradiation with 0–8 Gy $^{60}$Co γ-rays after 1 h

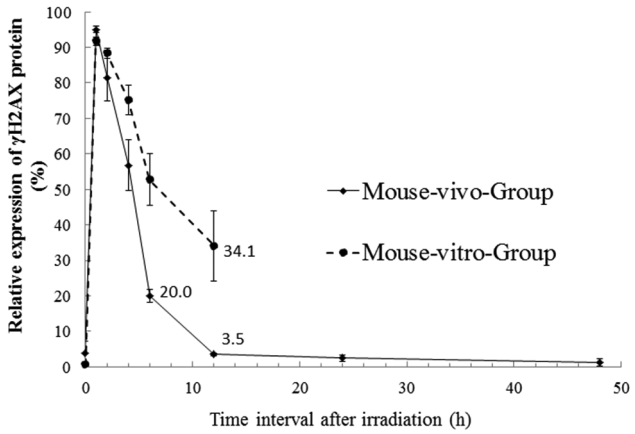


**Figure 9:** Time-effect curve of the γH2AX expression of mouse *in vivo* and *in vitro* after irradiation with 4 Gy $^{60}$Co γ-rays

expression of γH2AX of mouse *in vivo* decreased faster than that of *in vitro* [Figure 9].

# Discussion

After a radiation accident, approximate dose estimates need to be provided as soon as possible to support clinical decision-making and help manage concerns among the potentially exposed. In this study, we established a new method for measuring the level of γH2AX in irradiated human lymphocytes. This method could be used in initial triage and dose estimation for large-scale nuclear accidents. Detecting γH2AX protein expression in peripheral blood lymphocytes by flow cytometry can enable rapid screening for significant exposures at a much higher throughput than that achievable with other cytogenetic methods. Some laboratories have carried out the intercomparison on the γH2AX foci assay,[9,10] but this method was labor intensive. Detecting the γH2AX protein expression changes in peripheral blood lymphocytes by flow cytometry analysis is a simple, fast, and high-throughput assay. It could be used as a potential dosimeter for population triage and dose estimation during large-scale radiation emergency.

The dose-effect and time-effect relationships for the γH2AX protein expression after irradiation with $^{60}$Co γ-rays have also been established. UV and dose rate had no significant effect on γ-H2AX protein expression. The expression of γH2AX induced by $^{60}$Co γ-ray was consistent of that in mice lymphocytes irradiated *in vivo* and *in vitro*. The detection of γH2AX protein expression changes in peripheral blood lymphocyte by flow cytometry may be applicable for biological dosimetry.

Further work is also needed to fully characterize the γH2AX response after exposure to different radiation types such as X-rays and neutrons, drugs, and other chemical products.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

# References

1. International Atomic Energy Agency. Cytogenetic Dosimetry: Applications in Radiation Emergencies. A Manual. Vienna: International Atomic Energy Agency; 2011.
2. Maznyk NA, Wilkins RC, Carr Z, Lloyd DC. The capacity, capabilities and needs of the WHO BioDoseNet member laboratories. Radiat Prot Dosimetry 2012;151:611-20.
3. Fernandez-Capetillo O, Celeste A, Nussenzweig A. Focusing on foci: H2AX and the recruitment of DNA-damage response factors. Cell Cycle 2003;2:426-7.
4. Fillingham J, Keogh MC, Krogan NJ. GammaH2AX and its role in DNA double-strand break repair. Biochem Cell Biol 2006;84:568-77.
5. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 1998;273:5858-68.
6. Wang Z, Hu H, Hu M, Zhang X, Wang Q, Qiao Y, *et al.* Ratio of γ-H2AX level in lymphocytes to that in granulocytes detected using flow cytometry as a potential biodosimeter for radiation exposure. Radiat Environ Biophys 2014;53:283-90.
7. Depuydt J, Baert A, Vandersickel V, Thierens H, Vral A. Relative biological effectiveness of mammography X-rays at the level of DNA and chromosomes in lymphocytes. Int J Radiat Biol 2013;89:532-8.
8. Vandersickel V, Beukes P, Van Bockstaele B, Depuydt J, Vral A, Slabbert J. Induction and disappearance of γH2AX foci and formation of micronuclei after exposure of human lymphocytes to $^{60}$Co γ-rays and P(66)+ Be(40) neutrons. Int J Radiat Biol 2014;90:149-58.
9. Rothkamm K, Horn S, Scherthan H, Rössler U, De Amicis A, Barnard S, *et al.* Laboratory intercomparison on the γ-H2AX foci assay. Radiat Res 2013;180:149-55.
10. Rothkamm K, Beinke C, Romm H, Badie C, Balagurunathan Y, Barnard S, *et al.* Comparison of established and emerging biodosimetry assays. Radiat Res 2013;180:111-9.