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Preliminary Study of Expression of γ-H2AX and 53BP1 in Medical Radiation Workers

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Currently a huge number of professional and technical personnel in medicine, dentistry, and veterinary medicine are exposed to radiation while administering various radiologic procedures such as diagnostic, therapeutic, interventional, and nuclear medicine. The radiation workers in medical area have risks to be exposed to ionizing radiation that also potentially cause DNA damage. In this study, 43 blood samples were taken from medical radiation workers in three hospitals (Hasan Sadikin, Medistra, and Betsaida Hospitals) and administrative staff served as control. They were grouped according to gender (man and woman) and duration of working time (0 - 20 and > 20 years). The expression of γ-H2AX and 53BPI foci was detected by using antibody of γ-H2AX Ser-139 and 53BPI under fluorescence microscope observation. The mean γ-H2AX and 53BPI foci in medical radiation worker and control were 0.22 and 0.12 and 0.38 and 0.17 respectively ($P > 0.05$). There was correlation between γ-H2AX foci and 53BP1 ($P < 0.0001$) and no statistically significant of -H2AX and 53BP1 foci in behalf on gender and duration of working time of radiation workers ($P > 0.05$). It can be concluded that potency of DNA damaged that detected by γ-H2AX and 53BP1 foci between medical radiation worker and administrative staff and their duration of working time was not different.

Key words: γ-H2AX, 53BP1, DNA damage, medical radiation worker

1. Introduction

At present, a huge number of professional and technical personnel in medicine, dentistry, and veterinary medicine has a potent to be exposed to radiation while administering various radiologic procedures such as diagnostic, therapeutic, interventional, and nuclear medicine procedures[1)]. Diagnostic radiation workers are typically exposed to low doses at most areas of their body, which may poses cancer risk in organs and tissues. The cancer risk associated with radiation exposure has been widely studied and documented. However, health effects associated with occupational radiation exposure is not well known, because in general the data mostly obtained from the survivors of atomic bomb in Japan[2)].

An important effect of radiation exposure is the

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formation of DNA double-strand breaks (DSBs), considered to be one of the most damaging DNA lesions. High DSB levels can lead to cell death while low levels may lead to cellular senescence or genomic rearrangements that may induce cancer[3]. DSBs can be identified and quantified in situ by detecting the γ-H2AX foci formed at DNA break sites utilizing immunostaining techniques[4]. Curgently, calculating γ-H2AX foci is the most sensitive assay for irradiation-induced DSBs[3], with a ratio of DSBs to visible γ-H2AX foci close to 1:1[5, 6].

An important regulator of DSB signalling is p53-binding protein 1 (53BP1), which was first described as a binding partner of the tumour suppressor protein p53 almost two decades ago. The function of this protein is related with DSBs interactive protein. Another function of this protein also included in DSB repair pathway choice, checkpoint signaling, and synapsis of distal DNA ends, during NHEJ (non homolog end joining) one of the ned DNA repair process [7].

The phosphorylated histone H2A variant of γ-H2AX and p53 binding protein of 53BP1 are established immunocytochemical biomarkers of ionizing radiation-induced DSBs[7] and are emerging biomarkers of radiation exposure[8]. The γ-H2AX and 53BP1 foci are formed at the sites of DSBs and could be visualized within minutes of exposure[9]. Their potential for accurately estimating radiation dose has already been reported following ex vivo experimental in human[10], non-human primate in vivo[11], diagnostic[9] or therapeutic procedures[12], and human in vivo exposure and in the resident that living in natural background radiation[13].

There is a possibility that medical radiation worker in their activity have a risk to be exposed to x-ray or gamma ray in diagnostic or therapy procedures. This risk will be increased when some accidentally condition that result in overdose exposure. It also has potential to influence DNA damage both double strand break or single strand break. This preliminary study aimed to analyze the potential of DNA damage by observing γ-H2AX and 53BP1 in medical radiation workers (workers) and administratif staff (controls) by using an immunofluorescence method.

2. Materials And Methods

2.1. Subjects

This study was part of the research project named "Cytogenetic Effects in Community caused by Medical and Natural Radiation Exposures" of the Center. This study was approved by Ethical Committee of National Institute for Health Research and Development, Indonesian Ministry of Health with No. LB. 02.01/5.2.KE.05\2015. In this research, 43 blood samples were obtained from workers consist of 11 radiologist, 8 angiographers, 7 radiographer operators, 2 radiotherapists, 2 nurses and 1 radiation protection staff as exposed group and 6 administrative staffs as control group in Hasan Sadikin Hospital located in Bandung, and Betsaida and Medistra Hospitals located in Jakarta. The duration of their working time is also noted. Almost all volunteers is unsmokers. All volunteers were informed about nature, aims, and intention of the study and signed an informed consent form and questionnaire before providing blood samples. Any individuals suffering from an illness or taking medication were excluded.

2.2. Isolation of lymphocytes

A heparinized whole blood sample was collected from all volunteers in hospitals and transported to our laboratory. Histopague separation was used to isolate lymphocyte cells by layering 2.5 ml of whole blood mixed with an equal volume of phosphate buffered saline (PBS) pH 7.4 won to 2 volume of lymphocyte-separating medium (His opaque 1077, Sigma Aldrich, Cat# 10771, USA) and followed by centrifugation for 30 min at 1500 rpm (363 g) with brake 1 at 23℃ (Thermo Scientific, Heraeus, Biofuge Primo R, USA). The lymphocyte cells that appeared as a whitish/gray then carefully transferred to a new 15 ml centrifuge tube with 5 ml of PBS and centrifuged for 15 minutes at 1000 rpm (161 g). The lymphocytes were washed three times with PBS as in publication[14, 15] with some modifications.

2.3. γ-H2AX and 53BPI foci assay

The procedure for γ-H2AX foci assay was done according to previous papers with some modification[15]. Medium (RPMI) contained the isolated lymphocyte from the blood of volunteers was put on hydrophobic slides and left for 15 min (minutes). Cells were then fixed in 2% paraformaldehyde for 5 minutes, washed 3 times with PBS for 10 min each, permeabilized for 5 minutes on ice in 0.25% Triton X-100, and blocked in PBS with 1% BSA for 15 minutes at room temperature. After removing BSA the primary anti γ-H2AX antibody (anti-Phospho-Ser139 γ-H2AX Antibody, Thermo Fisher Scientific, Cat 250001, USA) and 53BP1 antibody (Thermo Fisher; Cat 16565, USA) also used as internal control staining were mixed at 1: 500 dilution in 1 % BSA (Bovine Serum Albumin Lyophilised, Cat P6154-100GR, Biowest, USA) in PBS. These antibodies were dropped on the slides and incubated in a dark moist chamber for 45 minutes at 30℃. To remove the first antibodies the slides were washed with 1% BSA for 3 x 15 minutes, the second antibodies (Goat Anti-mouse IgG Dylight 488mn Thermo Fisher Cat. PB 197295, USA) and antirabbit-Daylight 594 nm Thermo Fisher Cat. PB 198488, USA), diluted in 1% BSA and with DAPI (diluted 1:500) and incubated for 30 minutes at room temperature. After 2-3 washed with PBS each 15 minutes, slides were dried for 15 minutes with

Table 1. Expression of γ- H2AX and 53BP1 Foci in Workers and Controls

No	ID	Age(year)	Gender	Working Duration	Volunteer	γ- H2AX	53BP1
1	A	46	M	24	R	0.26	0.28
2	B	47	W	26	R	0.60	0.88
3	C	58	W	20	R	0.90	1.08
4	D	59	W	34	R	0.32	0.36
5	E	41	M	14	R	0.76	0.96
6	F	54	M	27	R	0.30	0.42
7	G	29	W	8	C	0.44	0.66
8	H	25	M	4	R	1.36	1.78
9	I	27	M	5	R	0.68	0.86
10	J	47	W	-	C	0.22	0.24
11	K	42	W	8	R	0.52	1.96
12	L	27	W	5	R	0.42	0.60
13	M	47	M	24	R	0.02	0.18
14	N	58	W	20	R	0.00	0.00
15	O	59	W	34	R	0.02	0.02
16	P	54	W	27	R	0.00	0.20
17	Q	25	M	4	R	0.02	0.06
18	R	47	M	-	C	0.04	0.12
19	S	42	W	22	R	0.04	0.04
20	T	27	W	5	R	0.06	0.16
21	U	34	M	13	R	0.02	0.06
22	V	51	M	17	R	0.18	0.48
23	W	50	W	23	R	0.12	0.16
24	X	50	M	19	R	0.02	0.02
25	Y	34	M	19	R	0.00	0.00
26	Z	29	W	3	R	0.00	0.00
27	AA	50	M	23	R	0.04	0.22
28	BB	71	M	48	R	0.10	0.32
29	CC	50	M	26	R	0.18	0.34
30	DD	40	M	22	R	0.03	0.14
31	EE	55	M	28	R	0.02	0.13
32	FF	28	M	2	R	0.10	0.22
33	GG	42	W	18	R	0.08	0.20
34	HH	51	M	21	R	0.02	0.02
35	II	39	M	20	R	0.02	0.14
36	JJ	25	W	7	R	0.29	0.64
37	KK	42	M	13	R	0.04	0.11
38	LL	22	W	3	R	0.14	0.28
39	MM	24	M	1	R	0.32	0.74
40	NN	26	M	28	R	0.04	0.10
41	RS1	40	W	-	C	0.00	0.00
42	RS2	40	W	-	C	0.02	0.02
43	RS3	35	W	-	C	0.00	0.00

R = workers C = controls

a fan. The mounting medium Entellan was dropped and mounting with coverslip and let 15 minutes in fridge. The observation was done by an experienced investigator (IK) using a fluorescence microscope (Nikon) equipped with red, green and blue fluorescence filters and a 100x lens under immersion oil. Generally, 50 cells per slide γ-H2AX foci were counted per individual[16]. The bright green foci was came from the result of binding of antibody γ-H2AX with daylight 488 secondary antibodies an the bright red as the result of binding antibody 53BP1 with daylight 594 secondary antibody.

2.4. Statistical analysis

First all datas were analyzed with Kolmogorov-Smirnov test to determine the normality of data distribution. The Correlation test was used to analyze the correlation ship between expression γ-H2AX and 53BP1 foci. Mann Whitney-tests is used to analyze data between γ-H2AX and 53BP1 foci in workers and controls. Kruskal-Wallis test was used to analyze the association between

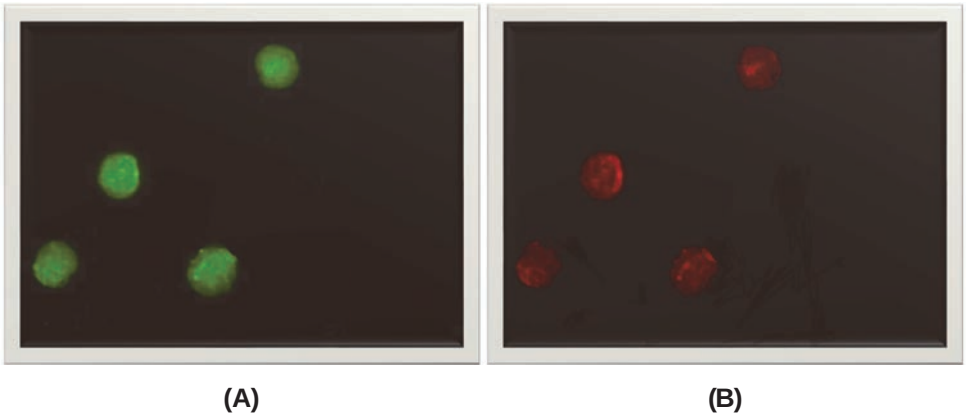

Fig. 1. Expression of γ-H2AX foci (A) and 53BP1 foci (B) in nucleus lymphocyte cell, and cell without γ-H2AX foci, originally magnification 10 x 100.

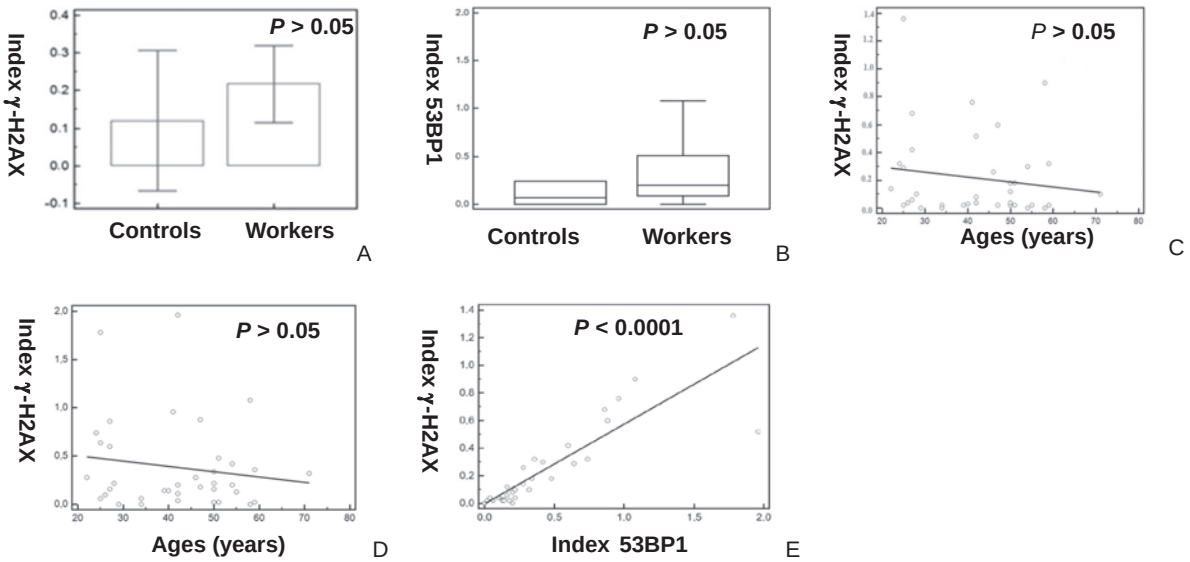

Fig. 2. Mean of γ- H2AX foci in controls and wotkers (A) 53BP1 in controls and workers (B), Corellation between Index γ-H2AX and ages (C), index 53BP1 and ages, (D) correlation between γ- H2AX and 53BP1 foci (E) in radiation worker.

expression γ-H2AX foci and 53BP1 with ages, working time and with the gender of workers, *P*-value of less than 0.05 was considered significant. All the data were analyzed with MedCalc Software 12.7.00.

3. Results

3.1. γ-H2AX and 53BP1 in workers and controls

This preliminary research had been done with 43 volunteers both workers and controls were presented in Table 1. The workers were in the radiology intervention as angiographer, medical doctor, nurse, and roentgen operator and consist of 22 men and 15 women, with ages of 22 - 71 years. Working time was from 1 - 48 years and divided into two groups (less than 20 years and more than 20 years). Where as administrative staff was used as control because they are almost never exposed to ionizing radiation. The detection and analysis of γ-H2AX and 53BP1 foci were conducted in blind, without knowing the volunteer primary data. All process was done in the laboratory with room temperature of 21℃. The basic reason of this study is to detect and confirm the possibility of occupational over exposure of radiation that may be received by workers. This over exposure may be caused by human error that occurred during 4 days before the blood sample collection. The expression of γ-H2AX foci was seen as originally green bright and 53BPI as red bright. Both their foci can be seen in the inside of cell nucleus in Figure 1. The mean of γ-H2AX foci of workers was 0.22 (0.00 - 1.36) and controls group was 0.12 (0.04 - 0.44) consecutively. Meanwhile the mean of 53BP1 foci were 0.38 (0 - 1.96) in radiation worker and

Table 2. The relationship between γ- H2AX and 53BP1 Foci with Gender and Working Duration of Workers

Biomarker	Gender	Working Duration
γ- H2AX	0.77ns	0.45ns
53 BP1	0.98ns	0.49ns

0.17 (0.06 - 0.66) in control groups. The highest mean index of γ-H2AX foci (1.36) and 53BP1 foci (1.96) were found in different volunteer but the same profession as radiographer . There were no statistical different (P > 0.05) between both index γ-H2AX and 53BP1 foci and between workers and controls (Figs. 2A and 2B). Two of of six (33%) administrative staff show no expression of γ-H2AX but only in four from thirty seven workers (10.8%).

3.2. γ-H2AX and 53BP1 in different gender of workers

Even though there was no statistical difference between DNA damage in workers and controls, in this current data also shown that potential of DNA damaged between man and woman in radiation worker also similar. As seen in Table 2, there was no statistical difference the mean γ-H2AX foci (P = 0.77 > 0.05) and 53BP1 foci (P = 0.98 > 0.05) between men and women, respecttively.

3.3. γ-H2AX and 53BP1 in working time and different age

To estimate the potential of DNA damage in workers, it was analyzed the mean of γ-H2AX and 53BP1 foci between workers who have worked for less and more than 20 years. In Table 2, there were no different in the mean of γ-H2AX (P = 0.45 > 0.05) and 53BP1 foci (P = 0.49 > 0.05) between the workers who have worked for more than 20 and less than 20 were a tendency of the mean γ-H2AX and 53BP1 foci to decline with an increase of age, even though it did not reach the statistically significance (P > 0.05), it can be seen in Figures 2C and 2D.

3.4. Correlation between γ-H2AX and 53BP1

It was known that all DNA damages should be repaired before the cell entered mitosis and continued dividing. The repairing process is in G1 or G2 of the cell cycle[17]. In this study, it was found a correlation between γ-H2AX foci as DNA DSB and 53BP1 as general DNA damage biomarker (P < 0.0001) as shown in Figure 2E.

4. Discussion

Some prime unexplained questions in radiation carcinogenesis revolve around the level of risk when exposure is received gradually over time. Radiation may induce cancer and human studies have provided a quantitative prediction of effects for over 100 years. But convincing and consistent evidence for effects arises at relatively high doses, more than 100–200 mSv, following brief exposures such as confirmed by the Japanese atomic-bomb survivors and patients treated for benign or malignant conditions, or among workers who have very high intakes of radionuclides, e.g. radium among dial painters and plutonium among Mayak plutonium workers. Animal studies are a moderately consistent show that is spreading dose over time (from radiations of low linear energy transfer) results in a lowering of the cancer risk, but human data are far less clear[18]. Radiologists and radiologic technologists are among the earliest occupational groups exposed to radiation. It was the observation of the earliest radiologists that led to the recognition of radiation-induced skin cancer, the first solid cancer linked to radiation in 1902. In the 1940s and 1950s, excess mortality from leukemia among radiologists was recognized[1, 2].

This current study was focused on the detection of expression γ-H2AX and 53BP1 foci as representation for DSB damage. In G1 cell arrest, p53 protein is activated to ensure the process of all damaged DNA be repaired before continuing cell cycle or induce apoptosis. As known that existence of DNA damage especially in DSBs will have potential to cause genome instability and can be continued to carcinogenesis process. Workers have daily potential exposed to higher radiation doses compared to administrative staff. There were no statistical difference of γ-H2AX and 53BP1 foci between workers and control. In this study, the highest γ-H2AX and 53BP1 foci was found in one radiographer. The proportion of lymphocyte cell with expression of γ-H2AX and 53BP1 which reflected of DNA damaged in workers were higher than controls. But it was no statistical different of DNA damage and process repair of DNA between workers and controls. It might also proved that there is no significantly DNA damage escpecially in DSBs damaged that was suspected caused by significant radiation accident exposure especially in workers. The factors which made high expression of γ-H2AX and 53BP1 spontaneously occurred or influenced of ionizing radiation exposure during their working have not been confirmed. Ambekar *et al.*[19] described that the rate of spontaneus DNA damage were 50000 single strand breaks and 10 double strand breaks per human cell per day.

There were also no adaptive response caused by low-dose ccupational exposure in workers as seen in resident of high natural radiation exposure and no high dose of x-ray significant exposure to the workers along 96 hours in their working time before blood samples were collected. Level of γ-H2AX foci remained significant until 96 hours in dose of 0.5 Gy exposure[20]. Logically workers always working with low dose irradiation exposure and also have the potential having an adaptive response as

in resident in high natural radiation exposure. In the previous publication[13], it was found that higher γ-H2AX foci were found in person living in high natural radiation area than control area was related to the adaptive response process. Perhaps a further study is needed to clarify the doses that can make an adaptive response only can be found in people that are living in natural radiation area and not man-made radiation source.

In this current data, it was not found the difference in the mean γ-H2AX and 53BP1 foci between men and women as seen in Table 2. The expression of γ-H2AX and 53BP1foci in worker that grouped into working duration for more than 20 years and less than 20 years also no difference. Increasing of the workers age shown slight tendency to decrease γ-H2AX and 53BP1 foci (Fig 2D and E).

Endogenous DSBs is higher in women than in men that may related with less efficient of DNA repair in women as reported by Meyer *et al.*[21]. Slyskova *et al.*[22] found no statistical difference in DNA repair between men and women. Garm *et al.*[23] reported that potential gender effect on DNA damage however it was exist in very old age. Probable reason of no difference of DNA damage between men and women in this study was the volunteer ages was not too old or beyond the limitation. The age and working duration of occupational exposure also related to an accumulation of DNA damage as shown in aging theory. Massudi *et al.*[24] reported increase of number γ-H2AX foci as increased of aging related with DSBs caused by oxidative stress. Schurman SH *et al.*[25] also reportend a significantly increase in γ-H2AX foci as increasing of age in Leukapheresis patient. Sedelnikova *et al.*[26] find that increase of γ-H2AX levels in younger adults (through age 50) and then decreasing there after. In this study the oldest age volunteer was above 70 years old and the youngest was 22 years old. This different is have not understand clearly. Perhaps any external factor may also affecting DNA damage accumulation as smoking that usually is the habit of adult men that does not seem in the workers.

The strong correlation between γ-H2AX foci and biomarker of DSBs damage and 53BP1 foci protein in this current study is also the same as some results from other research. But in this curent data shows that one of volunteer "K" in Table 2 express with not too high γ-H2AX foci but in 53BP1 in highest number, different with volunteer "H" expression of γ-H2AX also identic with 53BP1 expression. Maybe, DNA damage in general (single strand break, single base damage or other form DNA damage) is not mean directly with DSBs damage. The p53-binding protein 1 (53BP1) is a well-known DNA damage response (DDR) factor, which is recruited to nuclear structures at the site of DNA damage and forms readily visualized ionizing radiation (IR) induced foci. Depletion of 53BP1 can affected in cell cycle arrest in G2/M phase as well as genomic instability in human as well as mouse cells. Within the DNA damage response mechanism, 53BP1 is classified as an adapter/mediator, required for processing of the DNA damage response signal and as a platform for recruitment of other repair factors[27]. Other studis[28, 29] reported that 53BP1 function also related to accumulation of a DSB-modified chromatin protein. Once a DSBs formed, the cell initiates the DNA damage response, to which the ataxia telangiectasia–mutated (ATM) protein, ataxia-telangiectasia and rad3–related protein, (ATR), and DNA-dependent protein kinase (DNAPKs) are central. An immediate consequence of a DSBs is the phosphorylation of Ser139 of the minor histone H2 variant H2AX in megabase domains surrounding the DSB the phosphorylated molecule being termed γ-H2AX[7].

As conclusion, there were no different in potential of DSBs damage between workers and controls 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 of DNA damage, but to ensure of potential DSBs by γ-H2AX assay were reccommended. Expression of 53BP1 beside related with γ-H2AX but also connected with the process of DNA repair, cell cycle arrested and in NHEJ related DNA repair process[7]. This suggested further investigation to ensure the possibility of radiation response to ionizing radiation in man-made radiation source as in medical term or in the resident living around of nuclear installation and also supported by acceptance dose data of the of the volunteer that include in the investigation.

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Conflict of Interest

The authors declare no conflict of interest and competing.

References

1. Yoshinaga SK, Mabouchi AJ, Sigurdeson MM, Dood, Ron E.

Cancer risks among radiologists and radiologic technologists: review of epidemiologic studies. Radiology. 2004;233(2):313–21.

2. Scientific Committee on the Effects of Atomic Radiation, United Nations. Sources and effects of ionizing radiation: UNSCEAR 2000 report to the General Assembly, with scientific annexes. New York, NY: United Nations; 2000.
3. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA,Solier S, Pommier Y. Gamma H2AX and cancer. Nat Rev Cancer. 2008;8:957–67.
4. Redon CE, Nakamura AJ, Sordet O, Dickey JS, Gouliaeva K, Tabb B, Lawrence S, Kinders RJ, Bonner WM, Sedelnikova OA. Gamma-H2AX detection in peripheral blood lymphocytes, splenocytes, bone marrow, xenografts, and skin. Methods Mol Biol. 2011;682:249–70.
5. Rothkamm K, Lobrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low X-ray doses. Proc Natl Acad Sci USA. 2003;100:5057–62.
6. Sedelnikova OA, Rogakou EP, Panyutin IG, Bonner WM. Quantitative detection of (125)IdU-induced DNA double-strand breaks with gamma-H2AX antibody. Radiat Res. 2002;158:486–92.
7. Panier S, Boulton SJ. Double-strand break repair: 53BP1 comes into focus. Nat Rev Mol Cell Biol. 2014 Jan;15(1):7.
8. Rothkamm K, Horn S. Gamma-H2AX as protein biomarker for radiation exposure. Ann Ist Super Sanita. 2009;45:265–71.
9. Rothkamm K, Balroop S, Shekhdar J, Fernie P, Goh V. Leukocyte DNA damage after multi-detector row CT: a quantitative biomarker of low-level radiation exposure. Radiology. 2007;242:244–51.
10. Horn S, Rothkamm K. Candidate protein biomarkers as rapid indicators of radiation exposure. Radiat Meas. 2011;46(9):903–6.
11. Redon CE, Nakamura AJ, Gouliaeva K, Rahman A, Blakely WF, Bonner WM. The use of gamma-H2AX as a biodosimeter for total-body radiation exposure in non-human primates. PLoS One. 2010;5: e15544.
12. Sak A, Grehl S, Erichsen P, Engelhard M, Grannaß A, Levegrün S, Pöttgen C, Groneberg M, Stuschke M. gamma-H2AX foci formation in peripheral blood lymphocytes of tumor patients after local radiotherapy to different sites of the body: dependence on the dose-distribution, irradiated site and time from start of treatment. Int J Radiat Biol. 2007 Jan 1;83(10):639–52.
13. Hasan Basri IK, Yusuf D, Rahardjo T, Nurhayati S, Tetriana D, Ramadhani D, *et al.* Study of g-H2AX as DNA double strand break biomarker in resident living in high natural radiation area of Mamuju, West Sulawesi. J Enviroment Radioac. 2017;171:212–16.
14. Chua ML, Somaiah N, A'Hern R, Davies S, Gothard L,Yarnold J, *et al*. Residual DNA and chromosomal damage in ex vivo irradiated blood lymphocytes correlated with late normal tissue response to breast radiotherapy. Radiother Oncol. 2011;99 362–66.
15. Rothkamm K, Barnard S, Ainsbury EA, Al-Hafidh J, Barguinero JF, Moquet J, *et al*., Manual versus automated g-H2AX foci analysis across five European laboratories: can this assay be used for rapid biodosimetry in a large scale radiation accident? Mutat Res. 2013;756:170–3.
16. Eberlein U, Scherthan H, Bluemel C, Peper M, Lapa C, Buck AK, Port M, Lassmann M. DNA damage in peripheral blood lymphocytes of thyroid cancer patients after radioiodine therapy. J Nucl Med. 2016;57(2):173–9.
17. Houtgraaf JH, Versmissen J, van der Giessen WJ. A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovasc Revasc Med. 2006;7(3):165–72.
18. Boice Jr JD. The importance of radiation worker studies. J Radiol Protec. 2014;34(3):E7.
19. Shrinivas SA ,Shanta SH, Prajakta BB. DNA: Damage and Repair Mechanisms in Humans. Glob J Pharmaceu Sci. 2017; 3(2):555613.
20. Horn S, Barnard S, Rothkamm K. Gamma-H2AX-based dose estimation for whole and partial body radiation exposure. Plos One. 2011;6(9):1–8.
21. Mayer PJ, Lange CS, Bradley MO, Nichols WW. Gender differences in age related decline in DNA double-strand break damage and repair in lymphocytes. Ann Hum Biol. 1991;18:405–15.
22. Slyskova J, Naccarati A, Polakova V, Pardini B, Vodickova L, Stetina R, Schmuczerova J, Smerhovsky Z, Lipska L, Vodicka P. DNA damage and nucleotide excision repair capacity in healthy individuals. Environ Mol Mutagen. 2011;52(7):511–7.
23. Garm G, Villanueva MM, Burkle A, Petersen I, Bohr VA, Christensen K, *et al*. Age and gender effects on DNA strand break repair in peripheral blood mononuclear cells. Aging Cell. 2013;12:58–66.
24. Massudi H, Grant R, Braidy N, Guest J, Farns.worth B, Guillemin GJ. Age-associated changes in oxidative stress and NAD+ metabolism in human tissue. PloS one. 2012;7(7):e42357.
25. Schurman SH, Dunn CA, Greaves R, Yu B, Ferrucci L, Croteau DL, *et al*. Age-related disease association of endogenous γ-H2AX foci in mononuclear cells derived from leukapheresis. PLoS One. 2012;7(9):e45728.
26. Sedelnikova OA, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC. Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nat cell biol. 2004;6(2):168.
27. Gupta A, Hunt CR, Chakraborty S, Pandita RK, Yordy J, Ramnarain DB, *et al*. Role of 53BP1 in the Regulation of DNA Double-Strand Break Repair Pathway Choice. Radiat Res. 2014;181(1):1–8.
28. Huyen Y, Zgheib O, Di Tullio Jr RA, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature. 2004;432(7015):406.
29. Rothkamm K, Balroop S, Shekhdar J, Fernie P, Goh V. Leukocyte DNA damage after multi-detector row CT: a quantitative biomarker of low-level radiation exposure. Radiology. 2007; 242:244–51.