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Molecular Damage Evaluation and Chromosome Aberration in Blood Lymphocyte of Medical Radiation Workers

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***Abstract*—A preliminary evaluation on the cytogenetic effects and DNA damages was done in blood of twenty two person at the age between 30-56 years old from medical workers as expose group and their matched twenty from administration workers (control). Their bloods were collected with their full informed consent and culture set up for cytogenetic evaluation as well as nucleic acid damage observation with comet assay that were done according to the standard procedures. Lymphocytes were scored, either by using analysis of manual observation and automatically with metaphase finder, for the presence of chromosomal aberration and Mitotik Index. The result show that the Mitotik Index on expose group were no significant differences between exposure group and control. The frequencies of disentric chromosomes both in expose group and control were not found. Evaluation of DNA damage observation using Comet assay showed no any effects observed in lymphocytes for both study and control groups. Our data did not reveal any significant difference in expose group compared to control level in parameters by mean cytogenetics biomarkers studied which is supported by the Comet evaluation.**

Keywords: medical workers, dicentrics, MN, DNA damages, Comet assay

I. INTRODUCTION

The applications of radiation in the health sector such as diagnostic (CT Scan, interventional fluoroscopy), radiotherapy, and nuclear medicine, beside having considerable benefits, also has the potential for radiation hazards that need to be known , so that the use of this technology must have safety insight by making strict and carefully implemented regulations and supervision. Radiation workers have the potential to receive radiation exposure with an equivalent dose amount that exceeds or approaches the permissible dose limit value [1]. The effect of ionizing radiation on humans is the result of physical and chemical processes that occur immediately after exposure, then followed by biological processes in the body. The process includes a series of changes at the molecular, cellular and body tissues. In acute radiation exposure is relatively high, the effect that arises is the result of cell death which can cause disruption of the function of the tissues and organs of the body or known as deterministic effects which generally can be observed clinically [2].

The major effect in cells induced by ionizing radiation is DNA breaks, either a single strand or both strands, beside base lesions, sugar damage, and apurinic/apyrimidinic sites. Single strand lesions are the most abundant form of DNA damage whereas DSB is believed to be much more important biologically due to that it may lead to cancer cells formation and the determination of the extent of DSB induction may help to detect precancerous and cancer cells pronesess [3]. Study of chromosome aberration in peripheral blood lymphocytes is a sensitive assay for detecting exposure to natural radiation as indicator of cancer risk [4.5] Dicentric, a complex event that needs DSB in at least two different chromosomes in close proximity to each other, is considered as indicators of radiation-induced damage. Genotoksic examinations study can be carried out at the molecular level. In the last two decades, the single cell gel electrophoresis (SCGE) that also called Comet assay has been widely used for genotoxicity testing and in molecular epidemiology studies it was used to evaluate DNA damage as a biomarker of exposure [6,7,8,9]

In this paper a cytogenetic and molecular damge evaluation with alkaline Comet assay on lymmphocyte and chromosome aberration of medical radiation workers will be reported. The results will be used to provide an initial information regarding evaluation radiation medic workers as a consequence of work.

II. MATERIAL AND METHOD

A. *Ethics*.

The study procedures were explained to all respondences, who provided informed consent. The protocol was reviewed and approved by the Ethics Committee Review Board at the National Health Research Institute , The Indonesian Ministry of Health in Jakarta No. LB.02.01 / 5.2 / KE 171/2016

B. Subjects

Twenty two volunteers between 25 and 58 years old were Radiation medical workers of DR Sardjito Hospital as Expose group and 20 volunteer were administration workers as control. The questionnaire contained questions about their diet, and any food supplements or special drinks they were taking.

C. Sample preaparation and culture set up and harvest for aberration analyses

Ten ml of peripheral blood was drawn via venipuncture from each volunteer and placed into a heparin containing test tube (Becton Dickinson, N.J., USA) under sterile conditions for lymphocyte separation (DNA analysis) and cytogenetics preparation (culture). The analysis was done with a standard procedures given by IAEA with slight modifications.[10.11] Two milliliters of the whole blood samples were cultured in medium that consisted of 8.0 mL of RPMI-1640 supplemented with 10% heat inactivated feotal calf serum and 1% streptomycin/penicillin (Gibco). Into this solution, 3.0% mL of phytohemagglutinin (Gibco BRL, Grand Island, NY) was added to stimulate cell division. The culture was for 48 h and placed in incubator at 37°C with 5% CO_2. Colcemid (Gibco BRL) was added for the last 4 h of culture at a final concentration of 0.1 mg/mL to block the mitotic process of the cells at the metaphase stage. BudR was not added into the medium due to that it has been shown that the culture protocol used routinely in our laboratory results in <5% second division metaphases. The contents of the tube were then transferred into 15 mL centrifuge tube and centrifuged for ten minutes at 1500 rpm and the precipitate was re-suspend in 8 ml of 0.075 M KCl (pre-warmed to 37°C) for twenty minutes. After that, 2 ml of cold fresh Carnoys Fixative (3:1 = methanol : acetic acid glacial) was added into the tube, and this fixation step was repeated two times (until white sediment was obtained). The yield of metaphase cells was stored in freezer at least one night until the preparation of slide was made.

D. Scoring the metaphases and Mitotic Index Evaluation (MI)

Two-five slides were prepared for each sample, encoded, and then stained with 10% Giemsa (Merck) and mounted. Chromosome aberrations examination is used to evaluate the level of chromosomal abnormalities in human populations affected by genotoxic agents in various occupations and environments. Examination was carried out on the proliferation index based on Mitotic Index (MI) and disentric chromosomes. Mitotic Index then was calculated based on IAEA protocol report. Typically 500 cells were counted for a full Mitotic Index analysis for each sample. The number of aberrations (well spread cells and clear chromosomes) was observed under a microscope (Nikon Eclipse Japan) connected to Olympus CCD Camera System for taking picture. By using the 100x oil immersion objective, a cell was considered as aberrant if it had one or more dicentric chromosome from each culture. Scoring was done by a single scorer in complete metaphase with more than 46 centromers only as per the scoring criteria described in [11] IAEA. At least 250 first division metaphase cells were scored manually per sample. In the control samples 200-300 metaphase cells were analysed per donor.

E. Isolation of lymphocytes

Isolation procedure was done according to Panda [12]. Two and half milliliters of whole blood was mixed with the same volume of Phosphate Buffered Saline (PBS) pH 7.4 and then was carefully layered in a ratio of 1:2 onto the lymphocyte-separating medium (histopaque-1077) in a centrifugation tube. Then it was centrifuged for 30 min at 1500 rpm. The lymphocytes that appeared as a gray layer between the blood plasma and the lymphoprep were then carefully transferred to a new 15 mL centrifuge tube containing 5 mL of PBS (pH 7.4) and centrifuged for 15 min at 1000 rpm. The lymphocytes were washed three times and resuspended in PBS at a density of (5–6) × 10^4/mL. The cell viability was determined to be 98% by Trypan blue testing, and the lymphocyte suspension was stored in a refrigerator at 4°C. All chemicals were purchased from Sigma Chemicals (St. Louis, MO, USA).

F. The Comet assay

The Alkaline Comet assay was carried out according to procedure described in detail by Singh et al. [13]. Frosted slides were covered with 1% normal melting point agarose (Sigma). After solidification, the slides were then coated with 0.5% low melting point agarose (Sigma). When this layer had solidified, a second layer containing the whole blood sample mixed with 0.5% low melting point (LMP) agarose (Sigma) was placed on the slides. After 10 min solidification on 40C. Afterwards the slides were immersed for 1 h in 40C cold freshly prepared lysis solution [2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris–HCl, 1% Na-sarcosinate (Sigma), pH 10] with 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide(Sigma) added fresh to lyse cells and allow DNA unfolding. The slides were then placed in a horizontal gel electrophoresis tank, facing the anode. The unit was filled with fresh electrophoresis buffer (300 mM NaOH, 1 mM Na2EDTA, pH 13.0) and the slides were set in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkalilabile sites. Denaturation and electrophoresis were performed at 4°C under dim light. Electrophoresis was carried out for 20 min at 25 V (300 mA). After electrophoresis the slides were rinsed gently three times with a neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 μg/ml) and covered with a coverslip. Slides were stored at 4°C in sealed boxes until analysis(Tice RR, 2000).

G. Statistical Analysis

The statistical different of categorical variables (gender) in medical radiation workers and control samples using χ^2-test, while for the continue variable (ages) was using *t*-test analysis. Unpaired *t*-test also used to compare the mean of MI, and Comet value between expose group and control samples, if the data have a normal distribution. The Kolmogorov-Smirnov test was applied to know the distribution of data.

III. RESULTS AND DISCUSSION

A. *Cytogenetics evaluation*

The implementation of radiation workers sampling at the hospital was carried out after obtaining Ethical approvell no LB.02.01 / 5.2 / KE 171/2016. Blood samples from radiation workers obtained 22 people in the age range of 25 - 71 years

with years of duration works varying in the range of 3–32 years while the annual acceptance still below the NBD. The results of the evaluation of culturing process were carried out based on the value of the mitotic index (MI) which is a marker for estimating the general toxicity including exposure to ionizing radiation received as a consequence of expose group. Distribution of MI values were shown in Figure 1 and Table I. In the expose group the MI value varied in the range of 6.3 to 43% while for control group was in the range of 10.7 -45.3%. While the mean frequencies of MI for exposure groups are 20.54 ± 8.85 and control groups are 26.03 ± 9.95 and statistically not significantly different $p > 0.05$ (Figure 2). The previous study showed that MI radiation workers and Interventional individual no significant different [14]. The MI was used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression. There are two factors that can influence the MI. First is the proportion of the cell population that participates in the whole cycle of interphase leading to division, and second the relative lengths of interphase and recognizable mitotic stages [15].

TABLE I. The mean value of aberrant cells and Mitotic Indexs in Expose group and control

Group (n)	Age (Years) (Mean ±SD)	Dose receive	Mean Frequency of (Aberrant Cells)	Mean frequency of MI (Mean ±SD)
Expose group (22)	45,59±7.89	≤NBD	ND	20.54±8.85
Control (20)	48±9.21	≤NBD	ND	26.03 ±9.95

ND. Non Detectable

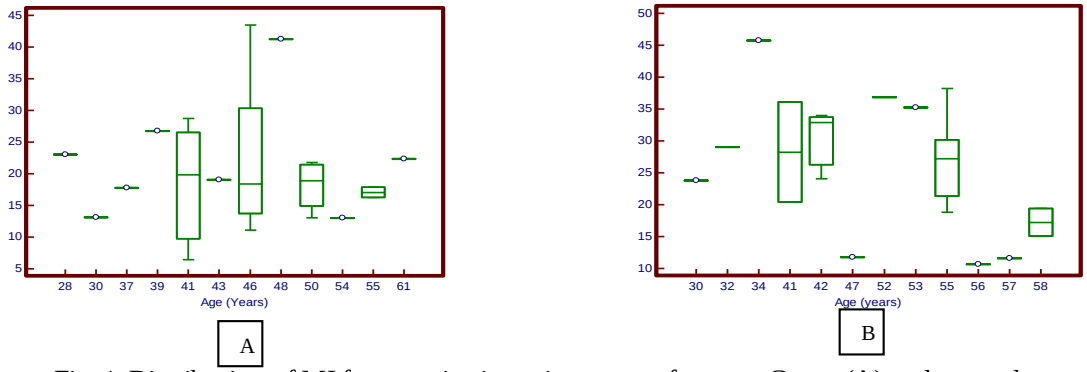

Fig. 1. Distribution of MI frequencies in various age of expose Group (A) and control group (B)

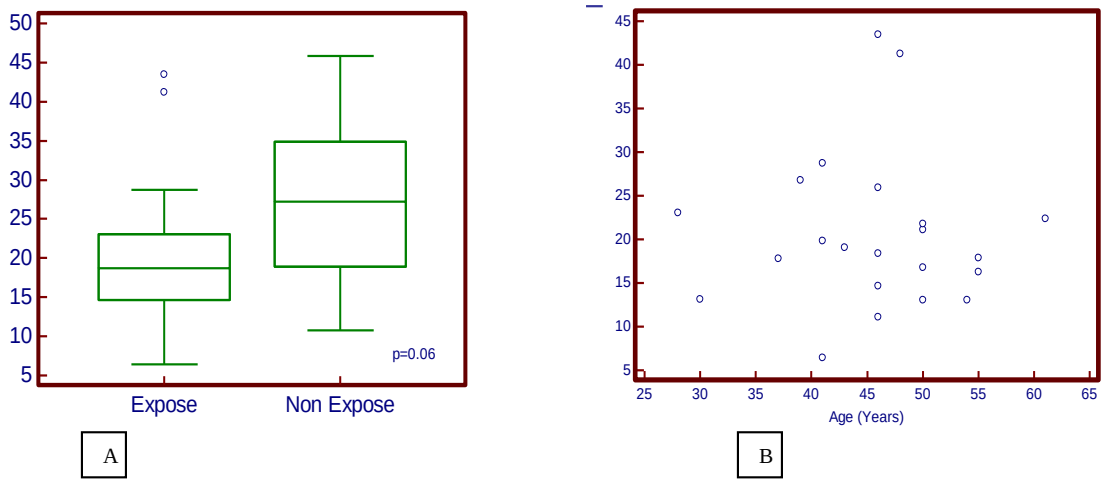

Fig. 2. The mean frequencies of % MI expose group and control (A), and corelation between age and % MI in expose group (B)

In the present study evaluation of chromosomal abnormalities especially dicentric chromosome as specific damage due to ionizing radiation has been carried out on 18000 metaphase cells from expose group and the results of these observations have not found any dicentric chromosomes in all exposure groups compare to control groups. Previous study also showed that the frequency of dicentric chromosomes both in exposed and control were not found [14]. Similar results had been reported in another research concerning with the induction of chromosome based on dicentric type, reporting the absence of centric ring and dicentric chromosome. In fact at this level of exposure a very high number of scored metaphases was required in order to detect the presence of dicentric [16,17] also reported that no significant increase in chromosomal abnormalities in their study population as there was a wide variation in the individual frequencies. According to Terzoudi & Pantelias [18] the cytogenetic response to ionizing radiation is intrinsic for each individual so that radiosensitivity between individuals may be an effect due to differences in DNA repair capacity, which can be explained by specific mutations or polymorphism in DNA repair genes or alternatively may be linked to cell cycle and feedback control mechanism. Thieren et al. [19] suggested that exposure to low radiation doses might induce inter-individual differences in susceptibility and the activation of DNA repair and the transcription of early response genes and stimulate DNA repair enzyme

B. Study of DNA damage evaluation with Comet Assay.

The results obtained of TL values from workers is ranging from 7.1 to 27.26 with an average value of 17.99, while in controls at the range is 10.14-22.34 with an average value. 13.65 ± 2.66 (Table 2). The principle of the comet test analysis is based on the size of denatured DNA fragments that migrate out of the cell nucleus during the electrophoresis process to form a comet's tail. Observations were made on 50 comets in each preparation. The visualization results of the comet test were calculated using the CaspLab program. The parameters used in this study were focused on comet tail length (TL).

TABLE II. The Mean value of comet tail length (TL) on expose group and control compare to another research

Subject	L Tail (µm)		Annotation
	Range	Mean	
Expose group	7.1 – 27.26	17.96 ± 6,16	This Study
Control	10.14 – 22.34	13.65 ± 2.66	
Expose group	15.12 - 0.21	17,49 ± 0,13	[9]
Control	11.92- 0.16	14.05 ± 0.13	

From Table II, it can be seen that the TL value of the expose group is slightly higher than the control group but not significantly different Figure 4 . T-test analysis showed that there were no significant differences between exposure group and control. Compare to another research Garaj-Vhovac [9] who reported that the TL measured in exposed group were in the range 15.12 - 0.21 to 22.56 - 0.47 µm, with a mean value of 17.49-0.23 µm. In control subjects the values of tail length were in the range 11.92- 0.16 to 15.51- 0.21 µm, with a mean TL of 14.05- 0.13 µm (Table II).

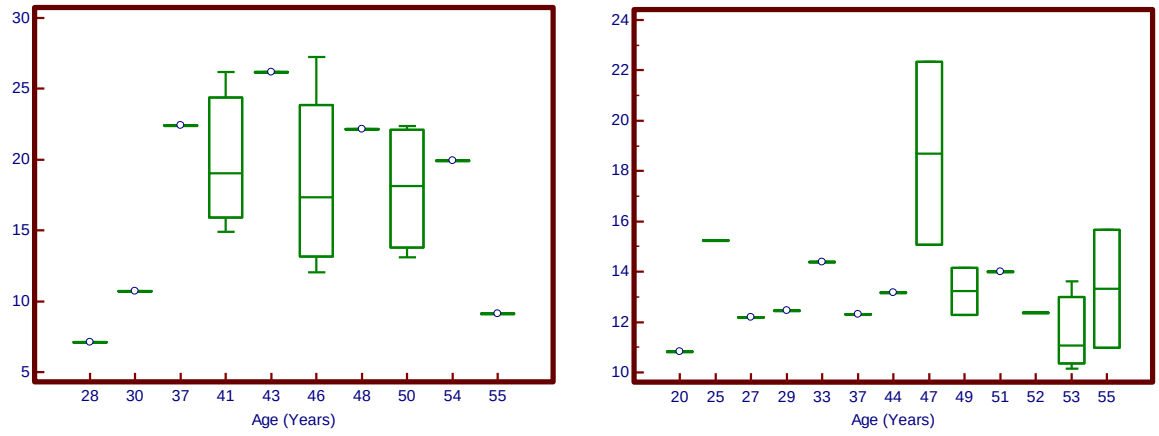

Fig. 3. Distribution of Tail length value on Expose group (A) and control group (B)

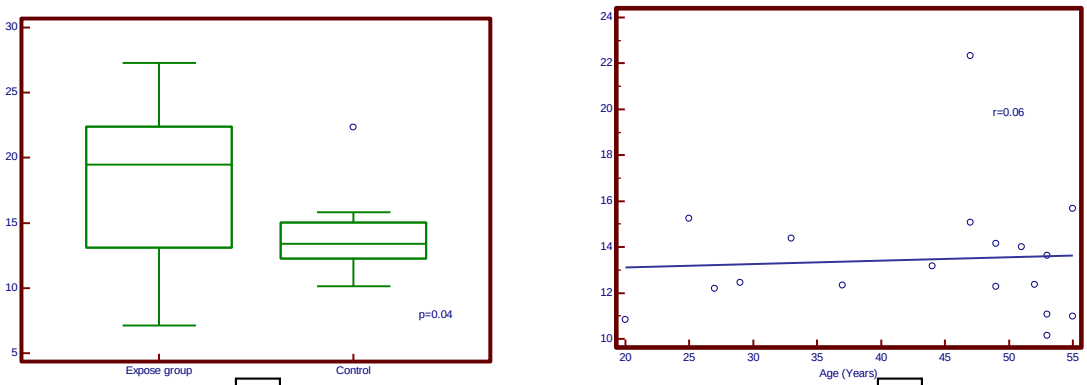

Fig. 4. The mean frequencies of % MI expose group and control (A), and corelation between age and % MI on expose group (B)

It also shows the distribution of TL value based on the age of varied workers in the range of 7.1 - 27.26 for the age range of 28-55 y / o in the exposed group while in the control it was in the range 10.14 - 22.34 for the age range of 20 -55 y / o (Figure 3) . In general, TL values tend to be relatively increased propotional to increasing age both in exposure groups and controls. Nevertheles from the statistical analysis there is no strong relationship between the age of individuals compare to TL values (Figure 4). This phenomenon also found in the research by Kumar et al. [20]. The increased of TL, simultaneously with age also found in our research (Fig. 4). It is logic that the cells of older people are more susceptible to be damaged by exogenous agents. The results from this assays need to be interpreted with caution as a number of confounding factors including age of the subject, smoking and diet have been reported.

IV. CONCLUSION

In conclusions, our study reported that lymphocytes proliferation by mean MI and comet assay on expose group were no significant differences between exposure group and control. Mean while the frequencies of disentric chromosomes both in expose group and control were not found. The MI and TL comet assay has no significantly positive correlation with age among radiation workers. In the future studies, it is necessary to examine the DNA repair genes polymorphism in populations with controlled non-genetic factors, such as lifestyles, environments, and exercises that affect the MN frequency as a biomarker of DNA damage.

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