Micronuclei and Gamma-H2AX Detection in Medical Radiation Workers

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Abstract. One of the most harmful DNA lesions brought on by exposure to ionizing radiation (IR) is DNA double-strand breaks. The working duration time may affect the dose accumulation of radiation workers routinely exposed to IR. The study presented here aims to determine the correlation between micronuclei (MN) frequency and γ-H2AX foci index in medical workers who are occupationally exposed to low-dose IR. Peripheral blood lymphocytes were collected from 30 radiation workers that grouped working less and more than 20 years, consisting of radiologists, nurses, cardiologists and radiology technicians. MN frequencies were calculated by analysing 1000 binucleated cells and γ-H2AX foci were calculated by analysing 50 cells. No significant difference was observed between the γ-H2AX foci frequencies and MN indexes when comparing the exposed group with working years ≥20 compared to those working <20 years. Chronic exposure to low-dose IR increases the DNA repair process and reduces micronuclei formation.

1 Introduction

The widespread use of ionizing radiation (IR) application in medical diagnostic and therapeutic tools represents the largest proportion of IR exposures in the general population [1, 2]. In particular, medical occupational workers represent a cohort of persons that are most consistently exposed to low doses of IR routinely. As a consequence of such chronic occupational low-dose IR exposure, the energy of the radiation could be absorbed by biomolecules, and it is potentially leading to altered cellular function [3].

The health consequences caused by chronic exposure to low-dose IR are nowadays great research interest to fully understand the health effects process [4]. Stochastic and deterministic effects are possible in the brain and head after long-term low-dose radiation exposure. Cancer is the main consequence identified at the regulatory and radioprotection level approach, and is comprised of stochastic or probabilistic effects [5].

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Humans have been shown to experience both acute and long-term health effects from high doses of infrared radiation; however, the possibility of negative effects from low doses of radiation is still up for debate and requires more research [3-5]. Genotoxic agents, including IR, can initiate mutations and cell transformations, especially by causing single-strand and double-strand DNA breakages, thereby leading to chromosome instability and potential carcinogenesis [5, 6].

It is believed that DNA double-strand breaks (DSB) occur before radiation-induced micronuclei form. Histone H2AX phosphorylation is one of the first indicators of DNA double-strand breaks. Shortly after a double-strand break, histone H2AX is phosphorylated at serine-139, producing γ-H2AX foci [6-8]. DSBs are a molecular event that can lead to chromosome aberrations that result in genomic instability or, when incorrectly or never repaired, decrease chromosome separation during mitosis and cause the loss of genetic information (8–10). Micronuclei (MN) are a sensitive indicator of radiation-induced or spontaneous chromosomal damage found in lymphocytes and proliferating cells [11]. It is possible to assess both aneuploid-genic induced events and clastogenic DSB events simultaneously using the MN analysis assay. Large-scale DNA damage, such as double-strand breaks (DSBs), can cause acentric chromatid/chromosome fragments, which can lead to asymmetrical chromosome exchanges and rearrangements if improperly repaired [12, 13].

It has been reported that an increase of MN formation after exposure to IR also increases γ-H2AX foci frequency detected in human astrocytoma SF268 cells following exposure to γ irradiation with a dose rate of 2.5 Gy/min. The results observed that IR could induce MN as a consequence of unrepaired DSBs, which contained broken chromosome ends marked with γ-H2AX foci [14]. The two biomarkers of genome damage used within this study, γ-H2AX foci and MN frequency in peripheral lymphocytes, were detected inflammation and to know the cytogenetic effects on the residents living in a high natural background radiation area and also found within obese children to describe the characterization of childhood obesity and [12,14-16]. Medical workers have a high potential to receive low-dose IR exposures as a consequence of their working activity. They may be accumulated in a long-term but low-dose chronic exposure. Our previous paper reported that the frequency of γH2AX had a positive correlation to annual occupational doses among medical radiation workers [15]. This present study focused on determining the association between MN and γ-H2AX foci frequencies as markers for expression of DNA DSB in individuals who are chronically exposed to low doses of IR occupationally and duration working-time of radiation workers.

2 Materials and methods

2.1 Subjects and sample collection

The sample group consisted of 30 medical staff who were radiation workers in the radiology and radiotherapy section in local hospitals. Exposed workers had a working duration of between 1 and 34 years, with a median of 20 years (with annual doses less than 5 mv per year). Venipuncture was used to obtain peripheral blood samples using heparinized vacutainer tubes (B.D. Vacutainer systems). All subjects gave their informed consent, and the National Institute of Health Research and Development, Indonesia’s local Ethics Committee, number LB.02.01/5.2.KE.051/2015, approved the study. Information about age, type of occupational radiation work, and occupation was gathered using a comprehensive questionnaire.
2.2. The cytokinesis-block micronucleus assay

Micronuclei were generated by cytokinesis-blocked cells using cytochalasin B (Cyt-B), and the technique created and published by Fenech et al. was followed with only slight modifications for analysis [12, 13].

2.3. The γ-H2AX assay

The procedure for the γ-H2AX assay was performed in accordance with previously published papers [18,19] with some modifications. A Nikon fluorescence microscope with a 100x lens submerged in oil and red, green, and blue fluorescence filters was used for the observation. Typically, each individual's 50 cells were used to count the γ-H2AX foci index [15-17].

2.4. Statistical analysis

The frequency of micronuclei and the γ-H2AX foci index were compared between exposed and control subjects using the unpaired Mann-Whitney test. The normality of the data distribution was ascertained using the Kolmogorov-Smirnov test prior to data analysis. Using MedCalc Software 12.7.00 2013, the relationship between the expression of γ-H2AX foci and micronuclei in working duration (those working for less or more than 20 years) was examined.

3 Result and discussion

In Table (1). MN frequencies in workers with a working duration time of exposure from 1 to 34 years with a median of 20 years showed that the number of MN was in the range of 1-29 (0.001-0.0029), with an average of 16.17 ± 8.19 (0.016). In nonexposed control subjects, a range of 2-24 with an average of 15.61 ± 6.02 (0.015) MN. Overall, the MN frequency in the exposed subjects compared to the nonexposed subjects is almost the same as the previous researcher’s published data (18-20). Regarding the analysis of the length of employment (working years), neither MN nor γ-H2AX foci indicated a statistically significant difference. Of interest is the significant negative correlation (p<0.05) found between the γ-H2AX foci index and MN index in workers with more than 20 years of occupation, and contrary to no significant correlation between γ-H2AX foci index and MN index within the workers of less than 20 years occupation (p>0.05) (Fig. 1A and B).

Table 1. The mean value of MN and γ-H2AX of workers more, less than or the same with 20 years

<table>
<thead>
<tr>
<th>Group</th>
<th>Σ of sample</th>
<th>Σ cells (BNC)</th>
<th>Range and Mean MN</th>
<th>Range and Mean γ-H2AX foci</th>
<th>MN and γ-H2AX &lt; 20 years</th>
<th>MN and γ-H2AX ≥ 20 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed group</td>
<td>30</td>
<td>30,000</td>
<td>1-29</td>
<td>0.0-0.9</td>
<td>19.92±7.76</td>
<td>14.82±8.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.17±8.19</td>
<td>0.15±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td>30</td>
<td>53,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Students t-test: p>0.05
Fig. 1. The correlation between γ-H2AX foci and micronuclei index in exposed subjects, duration working 19 years or less (A) and 20 years or more (B)

In practical terms, genotoxicity assays based on the CBMN assay offer useful and efficient information regarding the assessment of genetic material damage resulting from exposure to IR in the workplace and environment, as well as during the onset and progression of various pathogenic changes, such as cancer. In connection with this study, other authors have also found statistically significant differences between individuals who were not exposed and those who were, with very minor variations resulting from varying methodology, subject counts, and inter-individual variability in the scoring and evaluation of MN formation. According to a prior study, occupational radiation workers had a comparatively higher number of MNs than controls [21-23].

The relationship between the formation of micronuclei and the expression of γ-H2AX foci was investigated in the present investigation, as illustrated in Fig. 2. The outcome demonstrates that there is no discernible relationship between the frequencies of micronuclei and the expression of γ-H2AX foci. According to multiple studies, the connection between MN formation and DSB formation γ-H2AX foci is that MN formation is prevented from progressing to chromosomal fragments by the quick dephosphorylation of γ-H2AX foci, which is the signal for the proper recruitment of DNA repair proteins [18, 24, 25].

DNA damage is the cause of the DSBs found in CBMN, however, it is debatable when the DNA damage response should begin because MN does not have DNA repair machinery [26]. Micronuclei that result from unrepaired DSBs with broken chromosome ends identified by γ-H2AX foci may be induced by IR [14]. The presence of γ-H2AX foci in radiation-induced micronuclei suggests that these proteins aid in the process of repairing broken chromosomes during the subsequent cell cycle division [27].

In this current study, we have shown that a tendency of longer worker years of occupational exposure due to chronic low-dose IR increased the level of DNA DSB repair in the S phase and reduced micronuclei formation. Further scientific confirmation is needed to investigate whether there is some mechanism of adaptive DNA repair processes present, affecting workers with greater years of occupational exposure due to chronic low-dose IR as observed in residents living in natural high background radiation areas [28]. Increasing DNA repair activity was observed by 8-oxoguanine glycosylase 1 (OGG1) activity [29-31]. This enzyme was found in exposed groups, and only subjects exposed to IR dose had accumulation. The enzyme’s function is in the repair of free radical-induced DNA damage via the base excision repair (BER) pathway. Increased γ-H2AX foci and decreased micronuclei formation are related to function through BER [3]. One suggestion from this current study result is any potential for an adaptive response of radiation workers with long-term occupational exposure to low doses of IR.
4 Conclusion

Indication of low-dose chronic radiation exposure increases DNA repair and reduces micronuclei formation that related to increasing enzymatic reaction in the recovery process of DNA damage.

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