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Evaluation of Lymphocytes Proliferation in Botteng Village (A High Background Radiation Area) Inhabitants Using Binucleate Index

Evaluasi Proliferasi Sel Limfosit Penduduk Desa Botteng (Daerah Radiasi Latar Tinggi) dengan Indeks Binuklues

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ABSTRACT

Botteng Village in Mamuju, West Sulawesi was known for the high natural background radiation exposure. Botteng Village inhabitants exposed to high natural radiation in their daily life. Radiation exposure can inhibit the mitosis mechanism at various phases. Our previous study revealed that mitotic and nuclear division indexes in Botteng Village inhabitants were lower compared to control samples. To validate our previous study results here we evaluate the binucleate index in peripheral blood lymphocytes of Botteng Village inhabitants. Blood samples were collected from thirteen healthy adult subjects in Botteng Village and thirteen healthy adult subjects in normal background radiation area. Binucleate index was calculated as the proportion of binucleated cell (BNC) in 500 cells for each sample. The study result showed that the BI in Botteng Village was similar compared to control group (23.58 ± 9.60 *vs* 23.47 ± 6.24). It is possible that the small sample numbers used in this study were not adequate to represent the BI value in Botteng Village inhabitants. This study also showed that there was insignificant difference of BI in respect to gender and age for all samples. Further study using larger sample number should be conducted to verify whether the BI can be used as a marker of lymphocytes proliferation.

Keywords : Binucleated cells, Binucleate index, Botteng, Cell proliferation, Natural radiation

ABSTRAK

Desa Botteng di Mamuju, Sulawesi Barat diketahui memiliki paparan radiasi latar dari alam yang cukup tinggi. Penduduk desa Botteng menerima paparan radiasi alam tinggi dalam kehidupan sehari-harinya. Paparan radiasi dapat menghambat proses mitosis dalam berbagai tahapan. Studi yang telah dilakukan menunjukkan bahwa indeks mitosis dan indeks pembelahan inti pada penduduk desa Botteng lebih rendah dibandingkan dengan sampel kontrol. Untuk memvalidasi hasil studi tersebut dilakukan evaluasi terhadap indeks binukleus di limfosit darah tepi penduduk desa Botteng. Sampel darah diperoleh dari tiga belas penduduk dewasa yang sehat di desa Botteng Desa dan daerah dengan radiasi latar normal. Indeks binukleus dihitung sebagai proporsi sel binukleus dalam 500 total sel dari setiap sampel. Hasil studi memperlihatkan bahwa nilai indeks binukleus pada penduduk desa Botteng hampir sama bila dibandingkan dengan kelompok kontrol (23,58 ± 9,60 dan 23,47 ± 6,24). Terdapat kemungkinan bahwa jumlah sampel yang sedikit dalam penelitian yang dilakukan tidak dapat merepresentasikan nilai indeks binukleus di penduduk desa Botteng. Hasil studi memperlihatkan bahwa tidak terdapat perbedaan yang signifikan pada nilai indeks binukleus terkait dengan jenis kelamin dan umur pada semua sampel. Studi lebih lanjut dengan menggunakan jumlah sampel yang lebih besar harus dilakukan untuk memastikan apakah nilai indeks binukleus dapat digunakan sebagai penanda proses pembelahan sel limfosit.

Kata kunci : Desa Botteng, Indeks binukleus, Pembelahan sel, Radiasi alam, Sel binukleus

INTRODUCTION

Natural radioactivity can be found in the rocks, soil, oceans, and even in building materials and homes. Natural radioactivity appears at different levels in the soils of each region in the world and depends on the geological and geographical conditions [1]. Several areas in the

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world have higher levels of natural background radiation exposure (10–100× the normal levels respectively) and considered as a high natural background radiation areas (HBRA) like Ramsar in Iran and Kerala in India [2]. Mamuju area in West Sulawesi Indonesia also has been reported for the high radiation dose rate due to the Naturally Occurring Radioactive Material (NORM) especially the natural uranium contents. Uranium concentration in Mamuju was in range 0 to 1,529 with average 25 ppm eU, while the average abundance in the Earth's crust was about 3 ppm eU [3].

Several areas in Mamuju have an annual radiation dose rate more than 5 mSv y^{-1} [3,4]. It is well known that more than 50% of the average annual radiation dose is due to radon [5]. Radon 222 is a naturally occurring inert gas formed in the decay series of uranium 238. It can be found in trace amounts in many rocks and soils. As radon decays, it can emits significant levels of alpha radiation, along with lower levels of beta and gamma radiation [6]. Radon exposure can cause DNA damage and generate reactive oxygen species (ROS) resulting in cell cycle shortening [6]. A study conducted by Abo-Elmagd et al. in 2008 found that the mitotic activity of bone marrow cells in Swiss albino mice decrease with the increase of radon dose [7]. Mamuju inhabitants received radon exposure in their life, it is possible that the cell cycle activity in Mamuju inhabitants were lower compared to normal sample. Our previous study revealed that mitotic index (MI) and nuclear division index (NDI) in Botteng Village, Mamuju inhabitants were lower compared to control samples [8].

The MI usually used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression [9], while NDI can be used to measure the general cytotoxicity that represented the cell cycles progression of lymphocyte after mitogenic stimulation and how this has been affected by the exposure [10,11]. Another cell proliferation marker that can be used is the binucleate index. The binucleate index (BI) as the proportion of binucleated cells is useful parameters for comparing the mitogenic response of lymphocytes and cytostatic effects of agents examined in the assay [12]. In our previous study, we not determined the binucleate index in peripheral blood lymphocytes of Botteng Village inhabitants. Here in this study we evaluated the lymphocytes cycles progression of Botteng Village inhabitants using the binucleate index in cytokinesis block micronucleus (CBMN) assay. Aim of this study was to validate our previous study results that showed there was no significant effect on lymphocytes proliferation in Botteng Village inhabitants induced by high background radiation exposure. The binucleate index will compare to MI and NDI values from our previous study results [8] to verify whether the BI can be used as the additional parameter of proliferation cells marker.

MATERIAL AND METHODS

Blood sampling

Thirteen healthy adult subjects from Botteng Village and thirteen healthy adult subjects from normal background radiation area were included in this study. Peripheral blood samples were collected by venipuncture using heparinized vacutainer tubes. The study was approved by Ethics Committee of the National Institute of Health Research and Development, Indonesian Ministry of Health, number LB.02.01/5.2.KE.051/ 2015 date of January 29, 2015. All procedures performed in this study were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all donors. Informed consent was obtained from all donors. A detailed questionnaire was used to obtain information on age and occupation.

Micronucleus assay and binucleate index determination

Micronucleus (MN) assay was conducted based on the protocol in IAEA publication and our previous publication [13,14]. Binucleate index (BI) was calculated based on the proportion of binucleated cells (BNC) in a total number of cells analyzed, which were 500 cells contained mononucleated, binucleated, trinucleated and tetranucleated cells. Mono, bi and multi-nucleated cells should be viable cells with an intact cytoplasm and normal nucleus morphology containing one, two, three or more nuclei respectively.

$$BI = \frac{BNC}{1000} \times 100$$

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Identification of BNC was based on the Fenech (2007) publication [15]. Binucleated cells should have the following criteria.

a. The cells should be binucleated (BN).
b. The two nuclei in a BN cell should have intact nuclear membranes and be situated within the same cytoplasmic boundary.
c. The two nuclei in a BN cell should be approximately equal in size, staining pattern and staining intensity.
d. The two nuclei within a BN cell may be unconnected or may be attached by one or more fine nucleoplasmic bridges, which are no wider than 1/4th of the nuclear diameter.
e. The two main nuclei in a BN cell may touch but ideally should not overlap each other. A cell with two overlapping nuclei can be scored only if the nuclear boundaries of either nucleus are distinguishable.
f. The cytoplasmic boundary or membrane of a BN cell should be intact and clearly distinguishable

Mitotic and nuclear division indexes

Mitotic and nuclear division indexes data were obtained from our previous study [8]. Mitotic index was calculated based on the protocol in IAEA publication in 500 cells for each sample, while for NDI the proportion of mononucleated, binucleated, trinucleated and tetranucleated cells per 500 cells scored was assessed to calculated the NDI value.

Statistical analysis

Unpaired *t*-test was used to compare the BI values in Botteng Village inhabitants and control samples using SPSS 22.0 statistical software, if the data have a normal distribution. The Kolmogorov-Smirnov test was applied to know the distribution of data.

RESULTS AND DISCUSSION

In previous study, we found that the mitotic mechanism of Botteng Village inhabitants was lower compared to control samples [8]. This because the Botteng Village inhabitants were exposed to low radiation dose on their daily basis that can have the slower mitotic mechanism. Radiation exposure can stop the mitosis mechanism at various phases and causes cell death. Radiation can cause a delay in the cell cycle at G1, S and G2 phases of the interphase [16,17]. It is well known that DNA damaging agents can induce a cell cycle arrest and thus a longer time for repair is needed to protect the organism from the deleterious consequences of mutations. Deficient of cell cycle checkpoints could lead to significant accumulation of genetic mutations when the host is exposed to a carcinogen which was radon exposure in this study and consequently increasing the cancer risk. Based on this hypothesis the BI in Botteng Vilage inhabitants should be lower compared to control samples.

Interestingly this study revealed that the BI in Botteng Village inhabitants were similar compared to control samples and the different was not statistically significant (Table 1) (p=0.973). The higher of BI value in Botteng Village inhabitants compared to control samples was in contrast with MI and NDI data obtained from our previous study (Table 2). Our previous study showed that both of MI and NDI values in Botteng Village inhabitants were higher than to control samples [8]. A further study should be

Table 1. The mean BI value in Botteng Village Inhabitants and control groups.

Group	Number of sample	Total BNC	Total cells number	Mean BI ± SD (%)
Botteng	13	1533	6500	23.58 ± 9.60*
Control	13	1526	6500	23.47 ± 6.24

* Students t-test: p >0.05 (not different from control group)

Table 2. The mean MI, NDI and BI values in Botteng Village Inhabitants and control groups.

Group	Mean MI ± SD (%)	Mean NDI ± SD (%)	Mean BI ± SD (%)
Botteng	8.98 ± 2.50*	1.35 ± 0.15*	23.58 ± 9.60*
Control	9.09 ± 2.84	1.40 ± 0.15	23.47 ± 6.24

* Students t-test: p >0.05 (not different from control group)

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conducted in the future to validate this study result. It is possible that the small number of samples used in this study was not adequate to represent the BI value in Botteng Village inhabitants.

The BI is parameter that analyzes the number of cells that have correctly completed the cell cycle [18]. Binucleate index (BI) as well as MI and NDI are cell proliferation marker in blood cultures which is considered a measure of general cytotoxicity. In the blood lymphocyte cultures, a determination of the BI, MI and NDI inhibition has a practical meaning. The cell proliferation marker is interpreted in terms of the cell death or arrest of cells at any moment during the interphase [19]. Here in this study we used BI as the cell proliferation marker and compared it to MI and NDI. In this study, we failed to show that the BI can be used as cell proliferation marker, since the BI value in Botteng Village inhabitants was higher compared to control samples.

Our study also revealed that BI tend to decrease linieary with age when all groups were pooled, even though the correlation was not significant (p=0.973) (Figure 1). A possible explanation on this finding was the increase of MN in elderly people that we found in our previous study [14]. Several studies found that increase of MN frequencies with an increasing age was followed by a decrease in proliferating efficiencies of cells [20–22]. The increase of MN frequencies with age can be due to several factors likes the cumulative effect of acquired mutations in genes involved in DNA repair, the numerical and structural aberrations in chromosomes caused by exposure to endogenous genotoxins, inadequate nutrition, exposure to environmental or occupational genotoxins, as well as a wide range of unhealthy lifestyle factors [23].

Table 3. The mean BI values in female and male in all samples.

Gender	Mean BI ± SD (%)
Female	23.59 ± 6.80*
Male	23.45 ± 9.40

* Students t-test: p >0.05 (not statistically different)

In this study we also found a higher of BI in female compared to male in all samples (Table 3). The possible explanation for this phenomenon was the increase of MN frequency in female that also already found in our previous study [14]. Some studies suspect that the higher of MN frequency in female correlated with greater tendency of the inactive X-chromosome to be lost as an MN relative to other chromosomes [23,24].

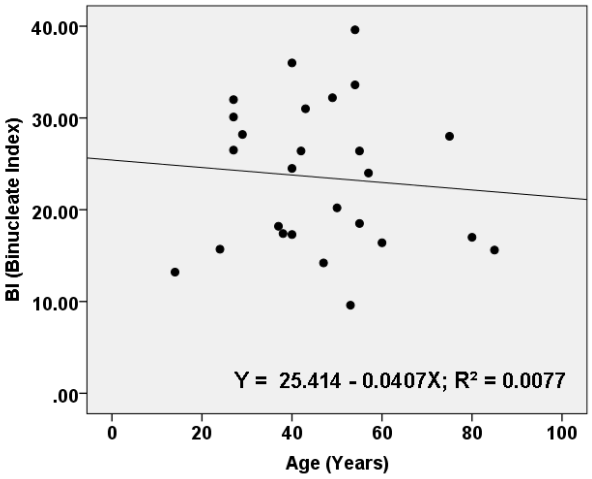

Figure 1. Regression analysis between age and BI in all samples.

CONCLUSION

In this study, we found that the mean BI value in Botteng Village was similar with control samples. A correlation between age and gender with BI also not found in this study. Overall it can be concluded that based on this study a further investigation should be conducted to verify whether the BI can be used as a cell proliferation marker in HBRA inhabitants.

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