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Micronucleus Frequencies in Mononucleated Cells of People Living in Takandeang Village – A High Level of Natural Radiation Area in Indonesia

Frekuensi Mikronukleus pada Sel Mononukleat Penduduk Desa Takandeang − Daerah Radiasi Latar Tinggi di Indonesia

Sofiati Purnami*, Masnelli Lubis, Suryadi, Siti Nurhayati, Dwi Ramadhani1, and M. Syaifudin

Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency
Jl. Lebak Bulus Raya No. 49, Jakarta 12440, Indonesia.
* E-mail : dhani02@batan.go.id

ABSTRACT

Micronucleus Frequencies in Mononucleated Cells of People Living in Takandeang Village – A High Level of Natural Radiation Area in Indonesia. The evaluation of micronucleus in mononucleated cells (MNMNC) could give a more comprehensive information about genomic instability in population exposed to high level of natural background radiation. Here in this study the evaluation of MNMNC in Takandeang Village, Mamuju inhabitants was performed to obtain more clearly information about genomic instability in population exposed to high level of natural background radiation. Twenty seven healthy adult subjects from Takandeang Village and normal background radiation area were included in this study. Results showed that mean MNMNC of Takandeang Village inhabitants were significantly higher compared to control samples [3.96 ± 0.488 vs 1.96 ± 1.675; p<0.05]. The age and gender factors did not affect the MNMNC in all samples (p>0.05). It is possible that the high background radiation exposure received by Takandeang Village inhabitants induced the aneugenic effect in lymphocytes. Overall our study showed that scoring of MNMNC can provide additional information in cytokinesis block micronucleus (CBMN) assay.

Keywords: Aneugenic, High Background Radiation, Micronucleus, Mononucleated, Takandeang

ABSTRAK

Frekuensi Mikronukleus pada Sel Mononukleat Penduduk Desa Takandeang − Daerah Radiasi Latar Tinggi di Indonesia. Evaluasi mikronukleus dalam sel mononukleat (MNMNC) dapat memberikan informasi yang lebih komprehensif mengenai ketidakstabilan genomik pada populasi yang terpapar radiasi latar tinggi. Pada penelitian ini dilakukan evaluasi MNMNC pada penduduk Desa Takandeang, Mamuju untuk mendapatkan informasi yang lebih jelas mengenai ketidakstabilan genomik pada populasi yang terpapar radiasi latar alam tinggi. Sebanyak dua puluh tujuh penduduk desa Takandeang dan penduduk daerah dengan radiasi latar normal disertakan dalam penelitian ini. Hasil penelitian menunjukkan bahwa rerata MNMNC penduduk desa Takandeang lebih tinggi secara signifikan dibandingkan dengan sampel kontrol [3,96 ± 0,488 *vs* 1,96 ± 1,675; *p*<0,05]. Faktor usia dan jenis kelamin tidak mempengaruhi jumlah MNMNC pada seluruh sampel penelitian (*p*>0,05). Terdapat kemungkinan bahwa paparan radiasi latar tinggi yang diterima oleh penduduk desa Takandeang menginduksi efek aneugenik pada sel limfosit darah tepi. Secara keseluruhan hasil penelitian menunjukkan bahwa evaluasi MNMNC dapat memberikan informasi tambahan dalam uji *cytokinesis block micronucleus* (CBMN).

Kata kunci: Aneugenik, Mikronukleus, Mononukleat, Radiasi Latar Tinggi, Takandeang

INTRODUCTION

The cytokinesis block micronucleus (CBMN) assay consider as the most promising technique to assess the genomic instability in human biomonitoring study. The CBMN assay is slowly replacing the chromosomal aberrations (CAs) assay in lymphocytes because easy to perform and the results can be obtained in a shorter time. Micronucleus (MN) defined as a

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small, round, DNA containing cytoplasmic bodies formed during cell division. Micronucleus (MN) can represented the acentric fragments or the whole chromosomes that lag behind at anaphase during cell cycle [1], [2]. Currently, it is possible to identify the MN originate from acentric fragment using fluorescence in situ hybridization (FISH) assay to labels centromeric DNA sequences and MN without centromere are termed C─, whilst MN contain centromere are termed C+ [3], [4].

The MN commonly assess in binucleated cells (BNC) that represented once dividing cells after blocking the cytokinesis using cytochalasin-B (Cyt-B) in mitotic cycle. Majority of the MN in BNC was formed during in vitro cell division, but pre-existing MN can also present *in vivo* before the start of blood culture [1], [5], [6]. For this reason it is recommended that to draw a more comprehensive assessment of DNA damage, the evaluation of MN in mononucleated cells (MNMNC) should be also included in human biomonitoring study [1]. Information about MNMNC can provide complementary information in biomonitoring study, thus making these cells interesting to analyze [3]. It also may give an estimation of the genome instability accumulated over many years in stem cells and circulating lymphocytes, while the MN in BNC additionally provide a measure of the lesions which have accumulated in the DNA since the cells last replicated in vivo [1].

Our previous study showed that the MN frequencies in BNC of Botteng Village inhabitants was not significantly different compared to normal samples [7]. The possible explanation for this result is the level of natural background radiation exposure in Botteng Village was not adequate to induce a significant higher of MN frequencies in Botteng Village inhabitants. Botteng Village was one of the three areas in Mamuju that used for settlement and have a high radiation dose rate which is defined as 700 nSv/h that refer to the annual dose rate 5 mSv/y [8]. Mamuju area in West Sulawesi Indonesia has been reported for the high radiation dose rate due to the natural uranium contents in soil surface [8], [9]. The average uranium concentration in Mamuju was 25 ppm eU, whilst the average in the Earth was only about 3 ppm eU [8]. In our previous study MN was analyzed only in BNC. Thus, to obtain more comprehensive information about genomic instability induced by high background radiation exposure the evaluation of MNMNC was performed in the peripheral blood lymphocytes (PBL) of Takandeang Village.

MATERIAL AND METHODS

Annual effective dose calculation

The annual effective dose calculation was performed as described in our previous study [10]. In detail the annual effective dose from indoor and outdoor background gamma dose rate was estimated using this equation.

$$E = (D_{out} \times OF_{out} + D_{in} \times OF_{in}) \times T \times CC$$

Where E (mSv/y) is annual effective dose, D_{out} and D_{in} (nSv/h) are average outdoor and indoor gamma dose rates, T (hr) is time to convert from year to hour (8760 hours), OF_{out} and OF_{in} are outdoor and indoor occupancy factors (30% and 70% for outdoor and indoor, respectively) and CC is conversion coefficient (0.7 for adults) reported by United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) to convert absorbed dose in air to the effective dose in human [11]. The occupancy factor for indoor and outdoor was calculated based on the observation of Takandeang Village inhabitants that spent almost 8 hours in outdoor and 16 hours in indoor.

Study design and blood sampling

A cross-sectional study was conducted in Takandeang Village, Mamuju. Purposive sampling method was used in this study. In total fifty four samples consisted of twenty seven healthy adult subjects from Takandeang Village as a high background radiation area (HBRA) and twenty seven healthy adult subjects from Keang Village as a normal background radiation area (NBRA) were included in this study. Peripheral blood samples were collected by venipuncture using heparinized vacutainer tubes. Detail about the ethical approvement, inclusion and exclusion criterias could be found in our previous publication [10].

Micronucleus assay

Micronucleus assay was conducted based on the protocol in our previous publication [10]. Three slides were made from each sample and one thousand mononucleated lymphocytes were scored minimally at the magnification of 400×.

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The slides were analyzed based on the International Atomic Energy Agency (IAEA) publication [4].

Statistical analysis

The statistical difference of categorical variables (gender) in Takandeang Village inhabitants and control samples using χ^2-test, whereas for the continue variable (ages) was using t-test analysis. The Saphiro-Wilk test was applied to find out the distribution of data. Unpaired t-test also used to compare the mean of MNMNC value in Takandeang Village inhabitants and control samples. The statistical significance of gender and age on MNMNC also the association between the numbers of MNMNC and MNBNC was determined using linier regression analysis. All tests were conducted using Statistical Package for the Social Sciences (SPSS) for Windows version 22.0.

RESULTS AND DISCUSSION

The average annual effective dose calculated from the average indoor and outdoor gamma dose rates was 2.52 mSv/y, with the range from 1.99 to 3.17 mSv/y. These values were 2.2 to 3.6 times higher than the value of effective environmental gamma dose rate due to cosmic rays and terrestrial gamma radiation estimated for the world average report by UNSCEAR which was 0.87 mSv/y [12]. The mean of MNMNC in Takandeang Village inhabitants were 3.96 ± 0.488. In control samples, the mean of MNMNC was 1.96 ± 1.675. Statistical analysis revealed that there were a significant difference of mean MNMNC of Takandeang Village inhabitants as compared to control samples (p<0.05; Figure 1). The MNMNC of Takandeang Village inhabitants were ranged from 0 to 10 ‰, with a median value of 4 ‰. The MNMNC in control samples were ranged from 0 to 6 ‰, with a median value of 1 ‰.

The range and median values of MNMNC in control samples found in this study were comparable with Elhajouji et al. study [13]. They found that the frequency of MNMNC from 240 healthy donors (230 men and 10 women) was ranged from 0 to 5.60 ‰, with a median value of 0.99 ‰. Interestingly the range and median values of MNMNC in Takandeang Village inhabitants were higher compared to both of Elhajouji et al. study and control samples in this study. A possible explanation for this finding is the high background radiation exposure received by Takandeang Village inhabitants induced the aneugenic effect in PBL. Until now several studies on the medical workers that occupationally exposed to the low doses of ionizing radiation showed a possibility of aneugenic effect induced by radiation. Thierens et al. study in 2000 found that the frequency of C+ MN in hospital workers was significantly higher than in control (14.7 *vs* 11.2 ‰) [14]. Another study by Sari-Minodier et al. in 2007 also revealed the higher total micronucleus frequency in hospital workers compared to control samples (14.9 *vs* 11.8 ‰) and about one-third of the total micronucleus number constituted of C+ MN [15].

The significant clastogenic and aneugenic effect of ionizing radiation also was demonstrated in a group of individuals dwelling in buildings with high ^{60}Co content in metallic constructions that exposed to chronic low dose gamma irradiation. A significant increase of the total MN frequencies (24.5 *vs* 9.8 ‰), as well as frequencies of separately C+ MN (10.4 *vs* 5.6 ‰) [16]. Vasilyev et al. (2009) stated that significant aneugenic effect of ionizing radiation on human cells in vivo was recorded in studies of groups exposed to relatively high radiation doses (up to 1.5 Sv and 7 Sv), whereas accumulated doses up to 200 mSv produced either slight or no aneugenic effect [17]. Since here in this study we found a slightly higher of MNMNC in Takandeang Village inhabitants, thus our study supported the Vasilyev et al. statement.

There are two different mechanisms of mutagenic induced the aneuploidy in somatic cells. First is chromosome nondisjunction and second is lagging. In nondisjunction event, both of sister chromatids in the chromosome move to the same pole in anaphase. On the other hand, lagging is a loss of the chromosome in the daughter cells, primarily caused by defects in the structure of spindle microtubules or impairment of the kinetochore [17]. Another study by Vasilyev (2010), showed that the main mechanism of aneuploidy in somatic cells of chemonuclear industry workers is chromosome nondisjunction [18]. Since both of chemonuclear industry workers and HBRA inhabitants were exposed to low dose radiation exposure chronically, thus it is possible that the main mechanism of aneugenic effect in Takandeang Village is also the chromosome nondisjunction.

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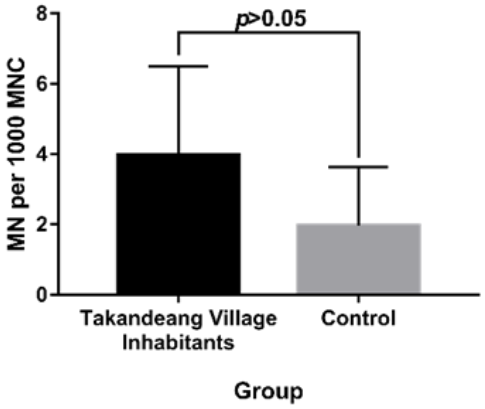

FIGURE 1. Mean MN numbers per 1000 MNC in Takandeang Village inhabitants and control samples.

In this study the correlation coefficient between the numbers of MNMNC and MNBNC was r = 0.148 (Figure 2). The correlation coefficient of MNMNC and MNBNC found in this study was lower compared to Elhajouji et al. study (r = 0.361). The higher of mean MNBNC found in Takandeang Village inhabitants (data not shown) could be the factor that caused the coefficient value in this study was lower than Elhajouji et al. study [13].

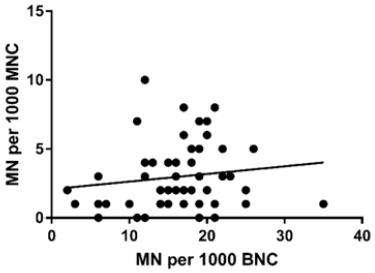

FIGURE 2. Correlation between MN per 1000 MNC and MN per 1000 BNC (r = 0.148) (p>0.05: Not significantly different)

Statistical analysis revealed that gender and age status between Takandeang Village inhabitants and control samples were not significantly different (p>0.05). The mean age of Takandeang Village inhabitants used in this study was 41.69 ± 13.82 with range 16 to 72 years. The ages in control samples were ranged from 16 to 75 years with a mean of 40.66 ± 14.64, respectively. The higher of MNMNC in female compared to male was found in this study (Figure 3). It is well known that the occurrence of X chromosome in micronuclei is significantly higher compared to other chromosomes and the rate of X chromosome inVol.ved in nondisjunction is higher than other chromosomes [18]. Since females have two copies of the X chromosome compared to only one in males, thus it can be predicted that the MNMNC in females will be higher compare to males.

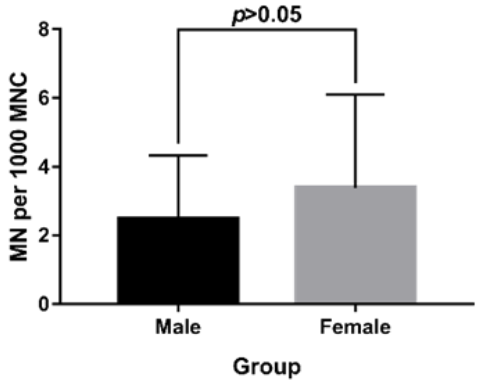

FIGURE 3. Mean MN numbers per 1000 MNC in males and females in all samples

In this study, a slightly increase of MNMNC linearly with age also found when all samples were pooled (Figure 4). The plausible explanation for this is similar to the cause of MN increase in BNC linearly with age. It has been reported that age and gender, either alone or in combination can significantly the MN frequency in BNC [19]–[22]. Several factors or the combination of each factor can increase the MN linearly with age likes the cumulative effect of acquired mutations in genes inVol.ved 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].

Here in this study the blood culture incubation was peformed for 72 hours. It is possible that the use of 24 hours blood culture incubation could give a higher number of MNMNC in Takandeang Village inhabitants. Kirsch-Vol.ders and Fenech stated that at this point MNMNC was resulted of in vivo rather than ex vivo division. Another advantage of this time point is the appropriate time to evaluate the apoptotic and necrotic cells [1].

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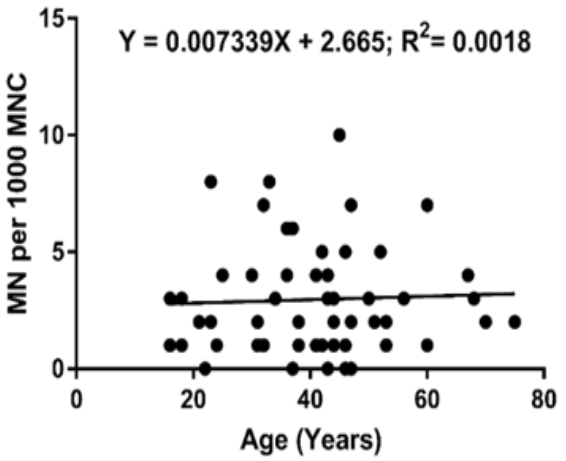

FIGURE 4. Linier regression between MN per 1000 MNC and age in all samples (p>0.05: Not significantly different).

CONCLUSION

This study showed that chronic low radiation dose exposure in Takandeang Village, Mamuju has significant effect on MNMNC. This study also showed that scoring of MNMNC could provides additional information on the chronic low radiation dose exposure effects in Takandeang Village inhabitants. Since scoring of MNMNC could distinguish clastogens from aneugens, thus this study results showed the possibility of aneugenic effect induced by chronic low radiation dose exposure in Takandeang Village inhabitants. Further study should be performed to validate this study results. An alternative procedure that would greatly facilitate the scoring of MNMNC in cells before enter mitosis at 24 hours post-PHA stimulation should be performed in further investigation.

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