INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
DOI:10.22192/ijcrcps Coden: IJCROO(USA) Volume 4, Issue 5 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2017.04.05.005

Micronucleus Assay as Genotoxicity Method to Determine the Human Health Risk

Velickova, N.*, Milev, M.
Faculty of Medical Sciences, Krste Misirkov,
10A, University "Goce Delcev" – Stip, R.Macedonia

*Corresponding Author: **Nevenka Velickova PhD**
E-mail: nevenka.velickova@ugd.edu.mk
Misko Milev Mrs
E-mail: misko.milev@ugd.edu.mk

Abstract

Medical personnel are often occupationally exposed to chronic ionizing radiation (IR). It is known that IR may have damaging effects on chromosomes and genes. The aim of this study was to evaluate the genotoxicity of IR on medical personnel using the micronucleus assay (cytokinesis-block MN) as genotoxicity method and to determine the human health risk. In this study we observed that medical personnel (with long time of exposer of IR) have a increasing the incidence of micronuclei (MNi) (average 16 MN per 1000 binuclear lymphocites). According to our results we concluded that the micronucleus assay determined that all of them have a high risk of professional disease and cancer. This study has a practical importance because it indicates the necessity of introducing a permanent genotoxicological and other type of monitoring of medical personnel exposed to IR and suggests the need of application of different cytogenetic methods and genotoxicological test (like MN assay) that can confirm the chromosome rearrangements.

Keywords: Cancer, Cytokinesis-block MN, Ionizing radiation, Lymphocites, Medical personnel

Introduction

Ionizing radiation (IR) is a type of energy released by atoms that travel in the form of electromagnetic waves (gamma or X-rays) or particles (neutrons, beta or alpha) and that carries enough energy to ionize or remove electrons from an atom. One form of IR is the excess energy that is emitted in the spontaneous disintegration of atoms, process known as radioactivity. There is an established WHO program for protection of patients and medical workers against radiation. IR and different physical and chemical agents can cause major alteration to the genetic material and other types of chromosomal instability and damage, or from the genetic point of view, they have chromosomal mutagenic effect. The micronucleus assay, as basic genotoxic test in molecular epidemiology and cytogenetics, is applied as a diagnostic tool and procedure for measuring micronucleus (MN) frequency in peripheral blood lymphocytes (PBL). This assay can be used for determination of the harmful influence of many of the damaging agents, as a method to biomonitor human exposure to genotoxic agents and it can be also used for detection of a susceptible genetic profile, like presence and extent of chromosomal damage in human populations (Fenech et al, 1999). The MN assay in the last few years is increasingly applied in cultured lymphocytes, because the possibility of blocking cytokinesis has made it possible to identify culture cells that finished one division (second cycle interfase cells). After the lymphocytes are exposed to a genotoxin, most of the MNi are forming from previous DNA damaged

areas, when the the cells are in vitro stimulated to divide. MNi originate from acentric fragments, whole chromatids or chromosomes and they are a result of chromosome breaks or spindle disruption (Kirch-Volders et al, 1997; 2001; 2003; Fenech, 2000; Fenech, 2005). Studies have shown that the genotoxic effects can be reversible when the exposure to IR is interrupted and also most of the previous studies concluded that MN frequency were significantly higher in the exposed subjects compared to the controls (Sahin, et al., 2009). Comet assay results showed a significant increase of tail length in workers exposed to IR. The exposed medical personnel need to carefully apply radiation protection procedures and minimize, as low as possible, IR exposure to avoid possible genotoxic effects (Sakly et al.,2012).

Materials and Methods

The study was approved by the Ethical Committee of the Faculty of medical sciences in "Goce Delcev" University in Stip, R. of Macedonia, and all subjects provided their written consents, according to the Declaration of Helsinki (2013).

For each individual 1000 binucleated cells were scored. The number of MN was recorded by criteria proposed by IAEA (International Atomic Energy Agency) and Fenech, 2007 The MN score (frequency) varied from 2 to 36 per 1000 binucleated cells. The cytokinesis-block MN was used to examine peripheral blood lymphocytes (PBL) in medical personnel as radiologist and radiotechnicians, exposed to IR. They have a different period of exposer (work experince), age, gender and life style. All subjects were informed about the aim of the study and they completed a comprehensive questionnaire about their life style. Venous blood sample (3 ml) was collected in heparinized tubes by trained nurses from each individuals. Fresh blood was collected by venipuncture and transferred into sterile, heparinized vacutainers. 0.5-ml of blood sample was added to the culture tubes containing 4.5 ml of RPMI 1640 media enriched with 20% fetal bovine serum, L-glutamine and 0.2 ml of phytohemagglutinin 1 % and eachsupplemented with 100 units/mL penicillin and 100 µg/mL streptomycin. The lymphocytes were cultured in 15 mL sterile plastic tubes with conical bottom (Nunc), the tubes were mixed gently by invertingfor a few minutes and incubated (CO_2 incubator for 24 hours) for 44h at 37 °C in a slant position. Cytochalasin B was then added to each culture at a concentration of 3 µg/ml to block cell cytokinesis and cultures were reincubated at 37 °C for further 28 h. Cells were then harvested by centrifugation at 1,000 rpm for 10 min. Supernatant was discarded by pipetting the media, leaving as little medium as possible over the cell pellet. Cell pellet was resuspended in the supernatant remains and 10 ml of 0.56% KCl warm hypotonic solution was added gently to each tube. Fixation was carried out during 2x15 minutes by fixative (glacial acetic acid: methanol = 1:3). Fixation steps were repeated for three more times. After that, the fixed lymphocyte cells were dropped from about 30 cm height using a Pasteur pipette into microscopic slides. The slides were stained by 2% alkaline Giemsa for 8 minutes, then washed in distilled water and examined by light microscope Leica DM4500 P (×40 and×100). MNi are defined as small, round nuclei (one (Fig.1) or two (Fig.2)) clearly separated from the main cell nucleus. Only cells with clearly visible cytoplasm and approximately similar-sized nuclei were analyzed.

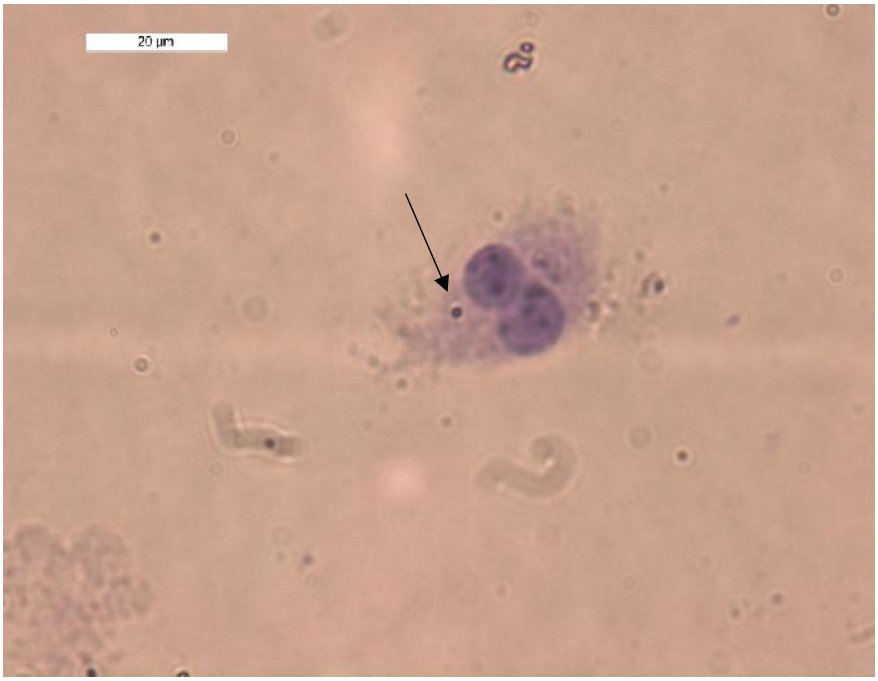

Fig.1 (binuclear lymphocyte with one micronucleus)

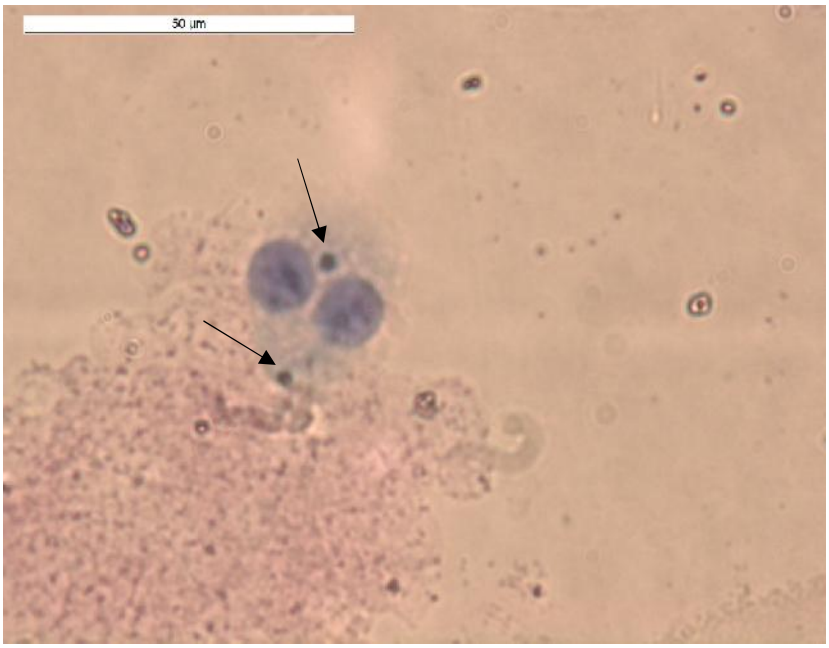

Fig. 2 (binuclear lymphocyte with two micronucleus)

Results and Discussion

The first (group) of 5 subjects were radiotechnicans, 50-55 years old men with 20-24 years of work experience on the same work place. One of them, was receiving a therapy with euthyrox because he had hypothyroidism. The observed MN frequencies were compared with the criteria for spontaneous MNi (4.4±2.6 per 500 BN cells) (Fenech and Morley, 1985). Results showed that the average number of MNi in PBL was **16,4** per 1000 binuclear cells. In according to this we confirmed the increased MN frequency in PBL. The secund group (5 subjects) were radiotechnicans, 40-46 years old men with 10-16 years work experience or exposore on IR. The average number of MNi in PBL was **14,5** per 1000 binuclear cells. Beside males as subjects in the study we observe 60 years woman as radiologist, with 30 years of occupational exposure on IR. The results confirme **19** MNi per 1000 binuclear cells. The time of exposure is a significant variable that influences the rate of MNi in lymphocytes. MN frequency in the BN cells is compatible (or well-matched) biomarker on mutagen or cytostatic effects of different agents on human cells (Bonassi et al., 2011; El-zein et al., 2011; Kocaman et al., 2008). Also, this frequency can represent an indicator for chromosome damage, instability and can assess the risk of cancer, acquired mutations and genetic susceptibility (Fenech, 2000; Joseph et al., 2009). In according to evaluation of increased number of MNi in one male (with hypothyroidism) other study (Al faisal et al., 2012) also evaluated the evidence of increased MN frequencies in patients with thyroid disorder, compared to healthy population. So many factors like age, gender, smoking etc. can cause MN frequency variation, but gender is one of the main factors estimated in many studies, which confirmed that MNi frequencies are higher in women than in men (Al faisal et al., 2012; Fenech et al., 1999) generally by 1.2 to 1.6 times (Fenech et al., 1999). This was explained by over-prevalence of X chromosome in the female organism (Bolognesi et al., 2002; catalan et al., 1998). The other possible factor which increases the number of MN in PBL is smoking habit. All subjects in the study are long time smokers (more than 15 years). Some studies (Hando et al.,1997) confirmed a significant effect of smoking on MN frequency but other studies (Bonassi et al., 2003; Hessel et al., 2001; Costa et al., 2006) observed no relation between smoking habits and MN frequency. We concluded that all subjects in the study who had long time of exposure on IR have much more MNi. These results suggest that chromosomal instability is in correlation with MNi frequencies. This study has a practical importance because it indicates the necesety of introducing a permanent genotoxicological and other type of monitoring of medical personnel exposed to IR and suggests the need of application of different cytogenetic methods and genotoxicological test (like MN assay) that can confirm the chromosome rearrangements and risk of cancer.

Ethical considerations:

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgments

The authors declare that there is no conflict of interest.

This study like project was supported by the University "Goce Delcev" in Stip, R. of Macedonia.

References

Al faisal, AHM., Al-ramahi, IJK., Abdul-hassan, IAR: Micronucleus frequency among Iraqi thyroid disorder patients, Comp. Clin. Pathol. DOI 2012;10.1007/s00580-012-1671-7 (2012)

Bolognesi, C, Perrone, E., Landini, E.: Micronucleus monitoring of a floriculturist population from western Liguria, Italia. Mutagenesis;**17**, 391-7 (2002).

Bonassi, S., Neri, M., Lando, C., Ceppi, M., Lin, YP., Chang, WP., Holland, N., Kirsch-volders, M, Zeiger, E., Fenech, M. Humn collaborative group: Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human Micro Nucleus project. Mutat Res; **543** 155-66 (2003).

Bonassi, D., El-zein, R., Bolognesi, C., Fenech, M.: Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis, **26(1)** 93–100 (2011).

Catalan, J., Autio, K., Kuosma, E., Norppa, H.: Age-dependent inclusion of sex chromosomes in lymphocyte micronuclei of man. Am J Hum Genet; **63** 1464-72 (1998).

Costa, C., Teixeira, JP., Silva, S., Roma-torres, J., Coelho, P., Gaspar, J., Alves, M., Laffon, B., Rueff, J., Mayan, O.: Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticides. Mutagenesis;**21**, 343-50 (2006)

El-zein, RA., Vral, A., Etzel, CJ.:Cytokinesis-blocked micronucleus assay and cancer risk assessment. Mutagenesis;**26(1),** 101–106 (2011)

Fenech, M., & Morley, A.A.: Measurement of micronuclei in human lymphocytes. Mutat. Res:**147,** 29–36 (1985)

Fenech, M., Crott, J, Turner, J., Brown, S.: Necrosis, apoptosis, cytostatis and DNA damage in human lymphocytes measured simultaneously within the cytokinesis-block micronucleus assay: description of the method and results for hydrogen peroxide. Mutagenesis;**14(6**), 605-12 (1999).

Fenech, M., Holland, N., Chang, WP., Zeiger, E., Bonassi, S.: The Human Micronucleus project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res;**428,** 271–283 (1999)

Fenech, M.: The in vitro micronucleus technique, Mutation Research;**455,** 81–95 (2000).

Fenech, M.: In vitro micronucleus technique to predict chemo sensitivity. Methods Mol. Med.;**111**: 3-32 (2005).

Fenech, M.: Cytokinesis-block micronucleus cytome assay, Nature protocols,**Vol.2, No.5** 1084-1104 (2007)

Hando, JC., Tucker, JD., Davenport, M., Tepperberg, J., Nath, J.: X chromosome inactivation and micronuclei in normal and Turner individuals. Hum Genet **100,** 624-8 (1997)

Hessel, H., Radon, K., Pethran, A., Maisch, B., Grobmair, S., Sautter, I., Fruhmann, G.: The genotoxic risk of hospital, pharmacy and medical personnel occupationally exposed to cytostatic drugs- evaluation by the micronucleus assay. Mutat Res;**497** 101-9 (2001).

International Atomic Energy Agency Vienna. Technical Reports series nr. 405. Cytogenetic Analysis for Radiation Dose Assessment - A Manual; (2001).

Joseph, LJ., Bhartiya, U.S., Raut, Y.S., Kand, P., Hawaldar, R.W., Nair, N.: Micronuclei frequency in peripheral blood lymphocytes of thyroid cancer patients after radioiodine therapy and its relationship with metastasis. Mutat Res;**675(1– 2),** 35–40, 30 (2009)

Kirsch-volders, M., Elhajouji, A., Cundari, E, Van hummelen, P.: The in vitro micronucleus test: a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. Mutat Res; **392,** 19-30 (1997)

Kirsh-volders, M., Fenech, M.: Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. Mutagenesis;**16,** 51–8 (2001)

Kirsch-volders, M., Sofuni, T., Aardema, M.: Report from the in vitro micronucleus assay working group. Mutat. Res.,**540**, 153–163 (2003).

Kocaman, A.Y., Rencuzogullari, E., Basriila, H., Topaktas, M.: The genotoxic effect of potassium metabisulfite using chromosome aberration, sister chromatid exchange, micronucleus tests in human lymphocytes and chromosome aberration test in bone marrow cells of rats. Environ Mol Mutagen;**49 (4)**, 276–282 (2008).

Sahin, A., Tatar, A., Oztas, S., Seven, B., Varoglu, E., Yesilyurt, A., Ayan, A.K.:
Evaluation of the genotoxic effects of chronic low-dose ionizing radiation exposure on nuclear medicine workers, , Nuclear Medicine and Biology, Vol. 36, Issue 5, 575-578 (2009).

Sakly, A., Gaspar, J.F., Kerkeni, E., Silva, S., Teixeira, J.P., Chaari, N., Ben, Cheikh, H.: Genotoxic damage in hospital workers exposed to ionizing radiation and metabolic gene polymorphisms, Journal Toxicol Environmental health 75(13-15):934-46. doi: 10.1080/15287394.2012.690710. (2012)

Access this Article in Online	
	Website: www.ijcrcps.com
Quick Response Code	Subject: Medical Sciences
DOI: 10.22192/ijcrcps.2017.04.05.005	

How to cite this article:
Velickova, N., Milev, M.(2017). Micronucleus Assay as Genotoxicity Method to Determine the Human Health Risk. Int. J. Curr. Res. Chem. Pharm. Sci. 4(5): 31-35.
DOI: http://dx.doi.org/10.22192/ijcrcps.2017.04.05.005