THE IMPORTANCE OF NUCLEAR DIVISION INDEX IN BIOMONITORING HUMAN STUDIES USING THE MICRONUCLEUS ASSAY

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ABSTRACT

Radiation as a physical agent can cause major alteration to the genetic material, chromosomal instability and cell damage.

In this study the micronucleus assay (MN) is being applied for biomonitoring of the hospital workers exposure to ionizing radiation or *in vitro* genotoxicity testing of radiation as a predictor of cancer risk, because chromosomal mutation and cell alteration is the main reason for carcinogenesis.

The frequency of micronuclei (MNi) and nuclear division index (NDI) are very important factors and usefull indicators for genotoxicity, mitogenic response and proliferative status of the lymphocytes.

It was a prospective observational study when MN assay was being applied on control and exposed group (hospital workers with different time of exposure and work activity).

Micronucleus in binuclear lymphocites

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INTRODUCTION

Micronucleus assay (MN) is suitable and standardized test in genotoxicology used for assessment of genotoxicological effects of different chemical or physical agents and it is the most commonly used method for measuring DNA damage in human lymphocytes.

The NDI (nuclear division index) provides a measure of the proliferative status of the viable cell fraction. It is therefore an indicator of cytostatic effects and in the case of lymphocytes, it is also a measure of mitogenic response, which is useful as a biomarker of immune function.

The NDI and the proportion of binucleated cells are useful parameters for comparing the mitogenic response of lymphocytes and cytostatic effects of agents examined in this assay.

Nuclear division index (NDI)

NDI= (M1 + 2M2 + 3M3 + 4M4)/N,

M1–M4 -represent the number of cells with 1–4 nuclei and N is the total number of viable cells scored (excluding necrotic and apoptotic cells).

The aim of this research was to evaluate and confirm the genotoxicity of ionizing radiation using the MN assay and to determine the human health risk.

METHODS AND MATERIALS

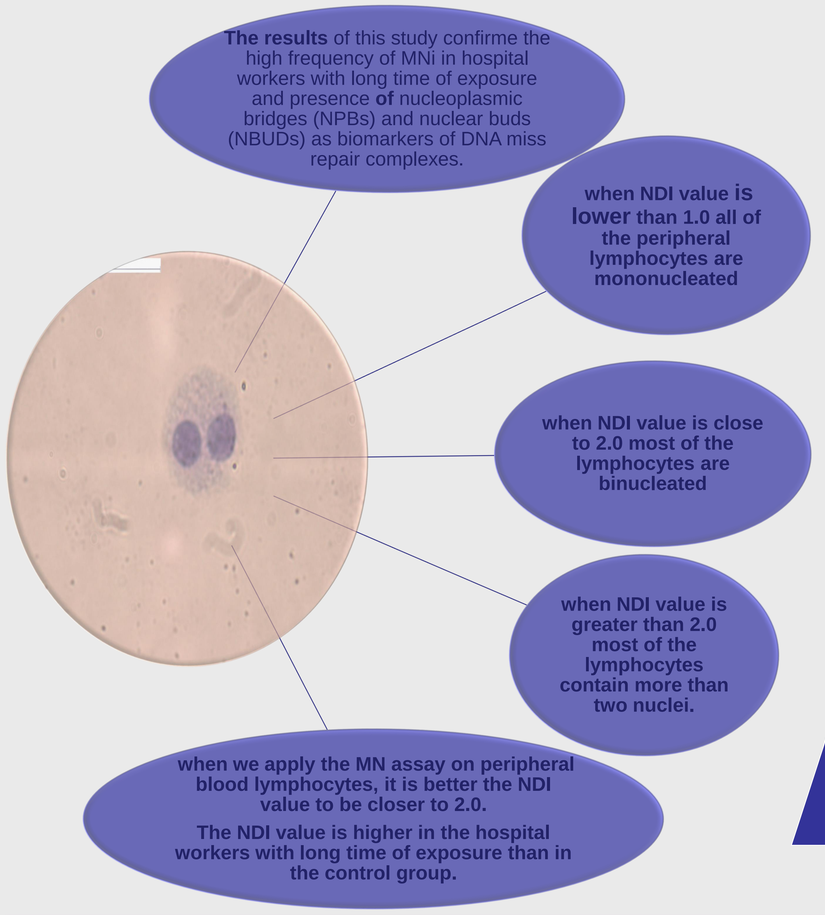
Blood culture protocol was done according to Fenech (5).

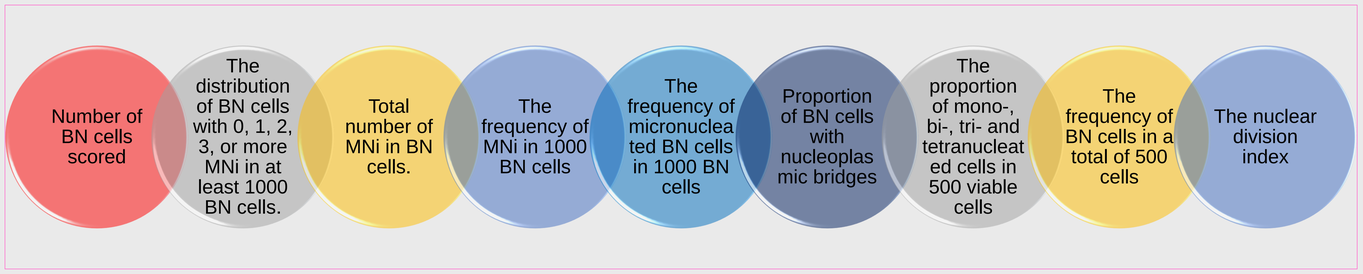
0.5-ml of blood sample was added to the culture tubes containing 4.5 ml of RPMI 1640 media enriched with 20% fetal bovine serum, L-glutamine and 0.2 ml of phytohaemagglutinin 1 % and each supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin.

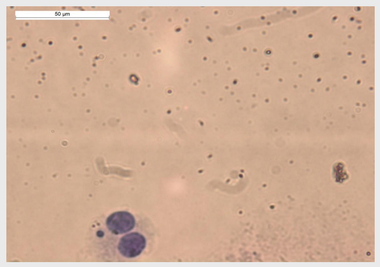
The lymphocytes were cultured and the tubes were mixed gently by inverting for a few minutes and incubated for 44h at 37 °C in a slant position.

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.
The 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. The slides were stained by 2% alkaline Giemsa for 8 minutes and examined by light microscope **Leica DM4500 P (×40 and×100).**

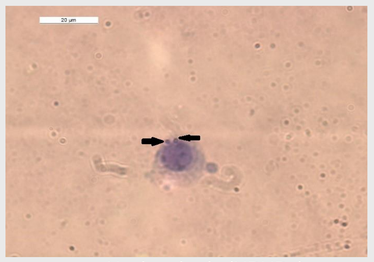
RESULTS



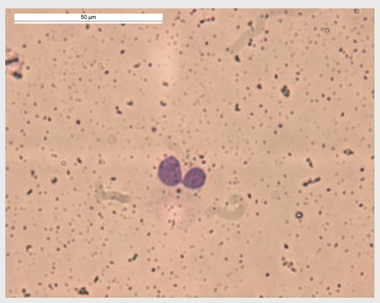




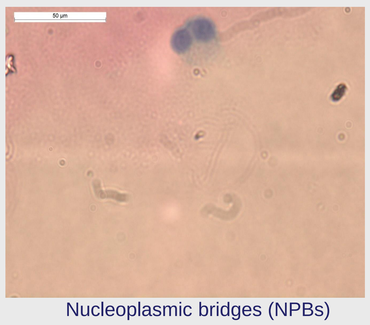
Micronucleus in binuclear lymphocites



Nuclear buds (NBUDs)



Nucleoplasmic bridges (NPBs)



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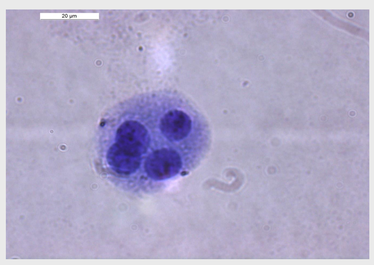
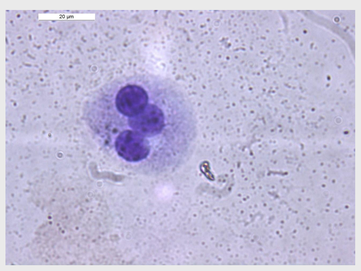
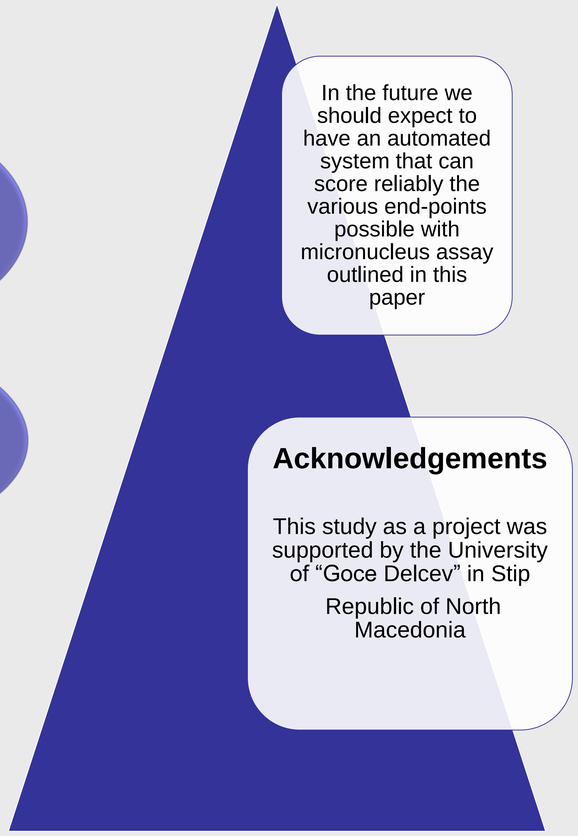


Multinucleated lymphocites



Multinucleated lymphocites

FUTURE PERSPECTIVE



Acknowledgements

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CONCLUSION

The frequency of MNi is higher in peripheral lymphocytes in exposed hospital workers than in control group. NDI is useful parameter and could provide better observation of genotoxicity from radiation or other agents in similar studies which use biomonitoring of the human cells using MN assay.

REFERENCES

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

BONASSI, S., NERI, M., LANDO, C., CEPPI, M. et al. HUMN COLLABORATIVE GROUP (2003): Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human Micro Nucleus project. Mutat Res;543:155-66.

BONASSI, D., EL-ZEIN, R., BOLOGNESI, C., FENECH, M. (2011): Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis, 26(1):93–100.

GARAJ-VRHOVAC, V., DURINEC, M., KOPJAR, N., ORESČANIN, V. (2008): A survey on the cytogenetic status of the Croatian general population by use of the cytokinesis-block micronucleus assay. Mutat Res;649:91-100.