The cytokinesis-blocked micronucleus assay: Good choice for detection and evaluation of genotoxicity in human cells

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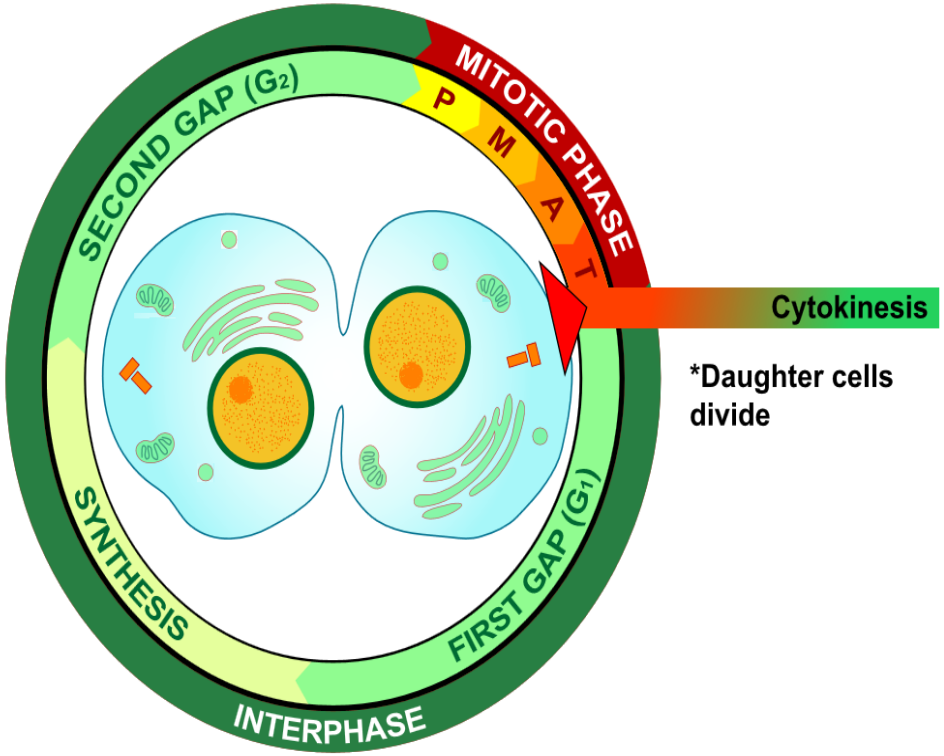
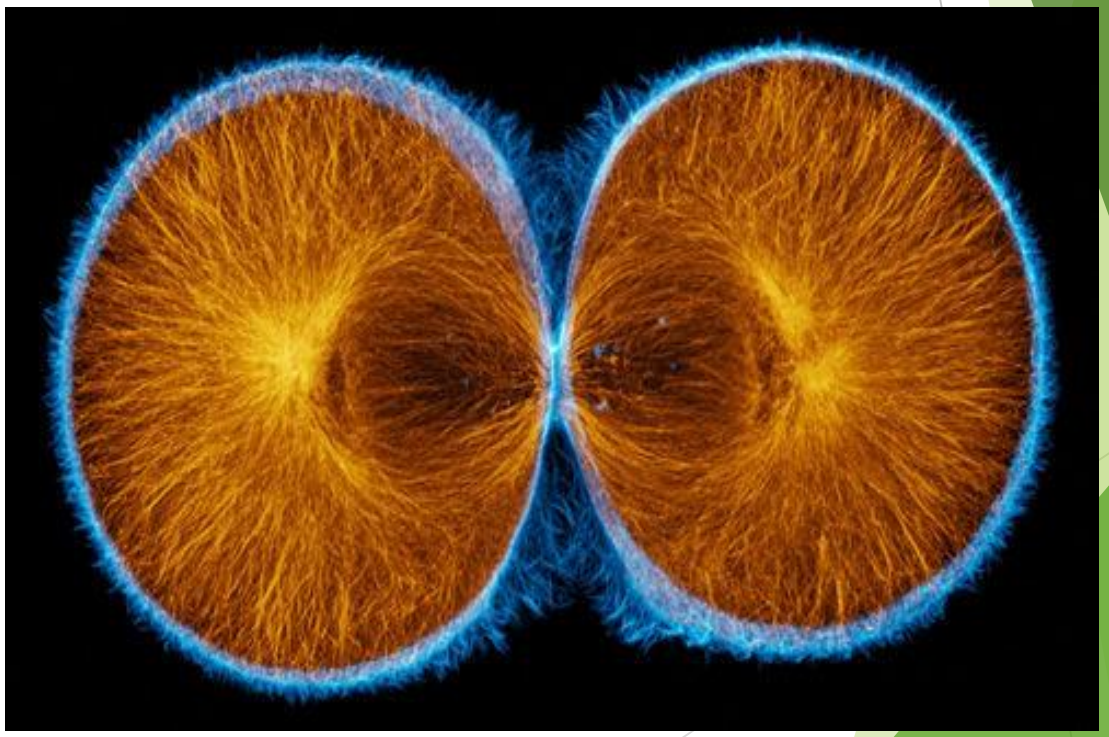
INTRODUCTION

- CYTOKINESIS-blocked micronucleus assay





INTRODUCTION

- Cytokinesis-BLOCKED micronucleus assay

- **Cytochalasin B** is a cell-permeable mycotoxin which strongly inhibits network formation by actin filaments. It inhibits cytoplasmic division by blocking the formation of contractile microfilaments

INTRODUCTION

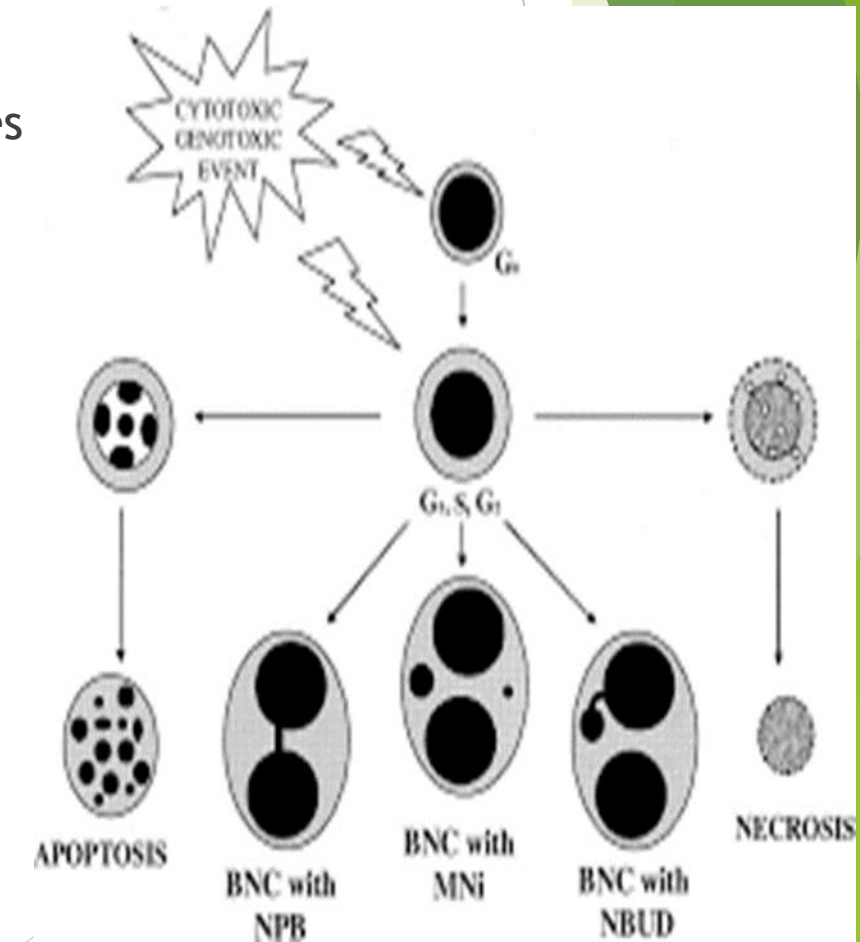
- Cytokinesis-blocked MICRONUCLEUS assay

- **Micronucleus (MN)** is the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division.

INTRODUCTION

- The cytokinesis-block Micronucleus (CBMN) assay is the preferred method for measuring MNi in cultured cells.

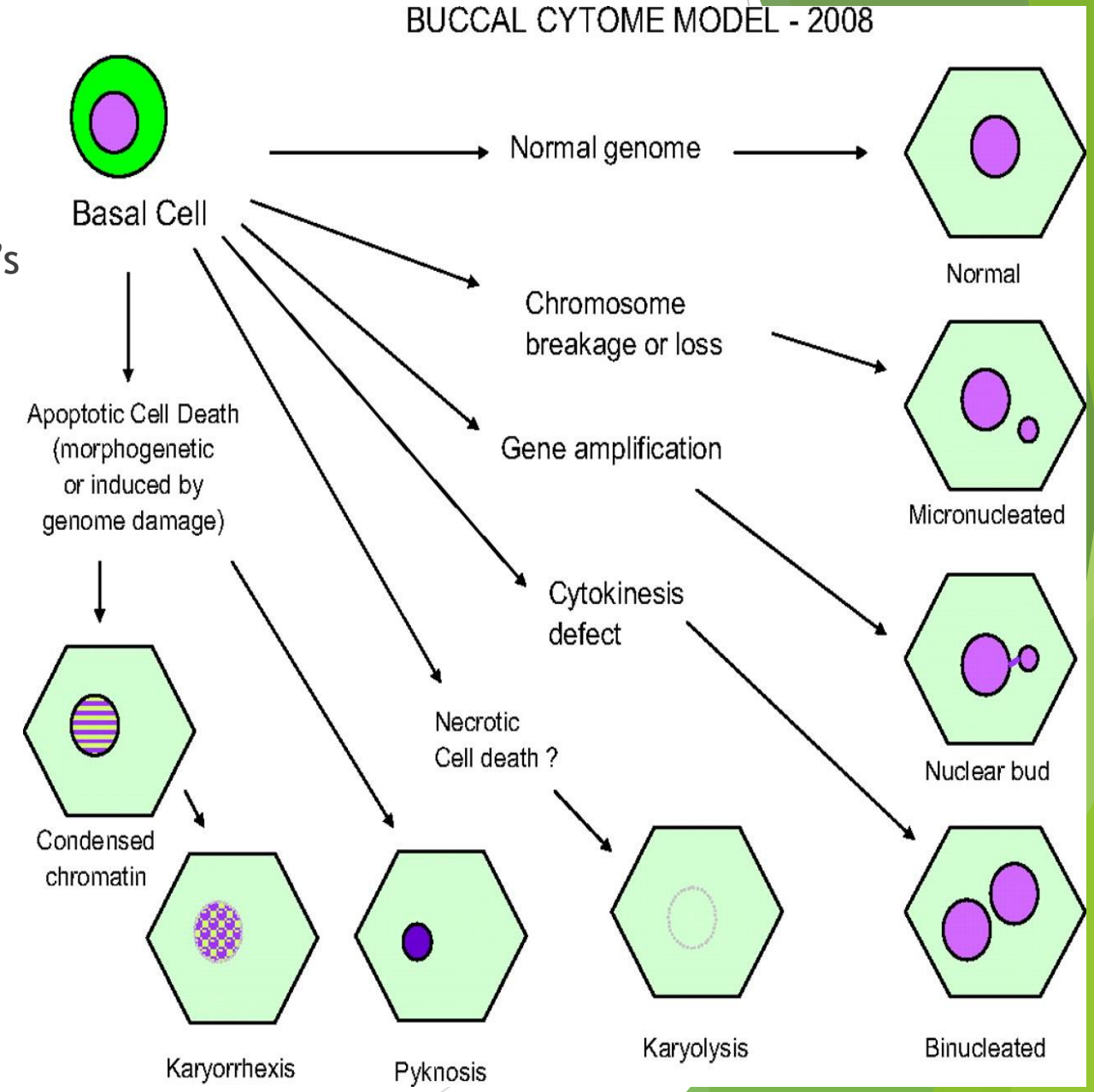
- In recent years it is used to measure nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs)



INTRODUCTION

Cytome concept: every cell is scored on it's

- Viability status
- Mitotic status
- Chromosomal damage status



INTRODUCTION

Reaserch project:

Examination of the genotoxicologycal effect of ionizing radiation using cytogenetic methods (micronucleus test) in occupationally exposed healthcare workers

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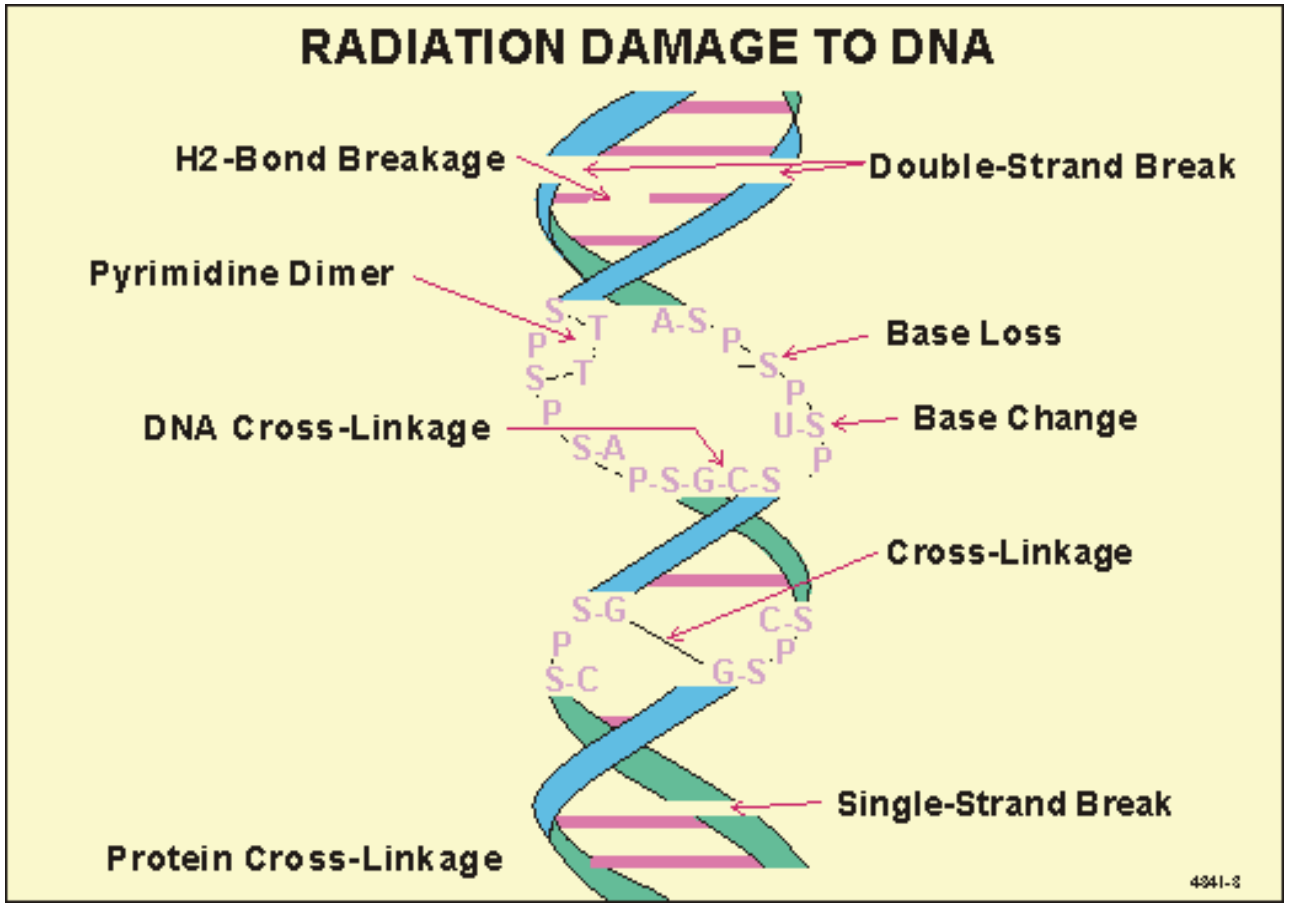
Genotoxicity – the toxic effect of any physical agents (radiation), chemical agents (hydrocarbon, benzene) and biological agents (retrovirus) on the genetic material

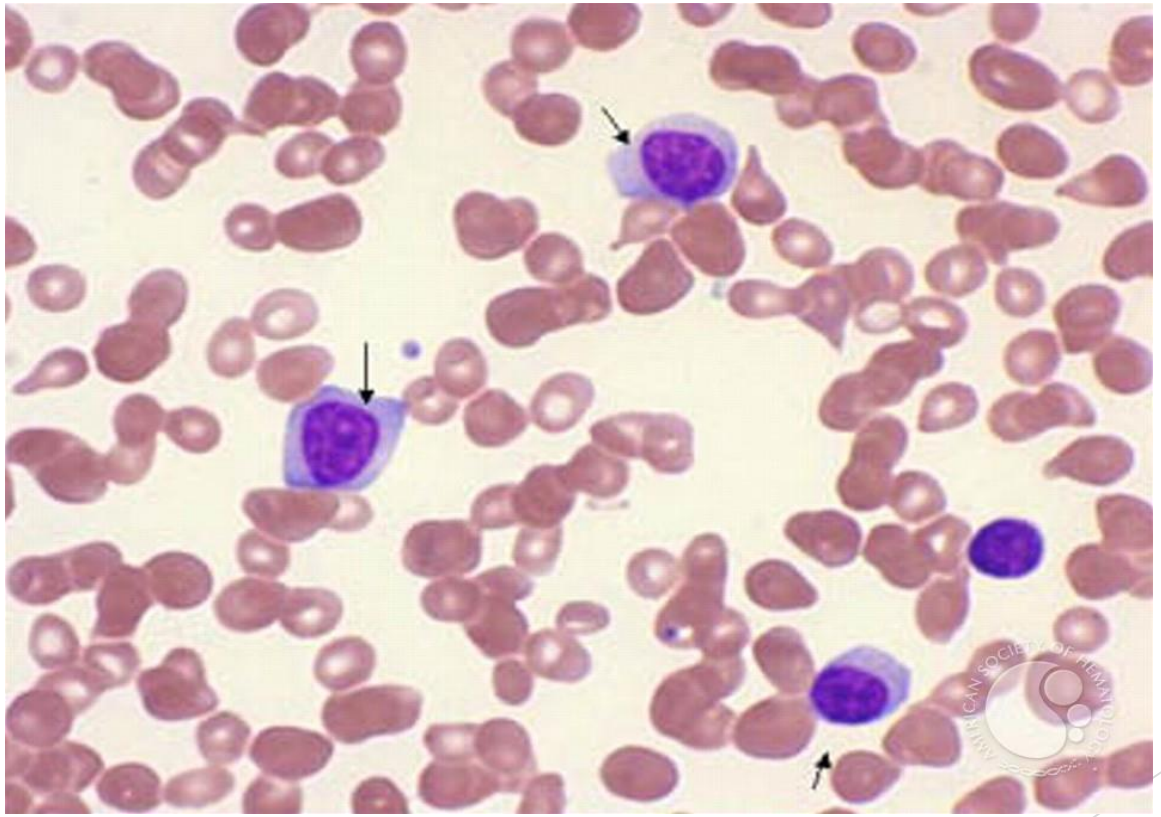
INTRODUCTION

Many radiation sources, including 60 Co represent significant hazards to the cells genetic material



INTRODUCTION

- Hematological tissues, particularly lymphocytes, are the most sensitive tissues to ionizing radiation



AIM

- In this study we wanted to evaluate the genotoxicity of ionizing radiation using the CBMN assay and determine it’s health risk.

- We analyzed the results of lymphocyte MNi for chromosomal damage, NPBs for DNA miss repair and telomere end-fusions and NBUDs for amplified DNA

STUDY GROUPS

- The study population included 20 individuals in the exposed group

- medical personnel exposed to ionizing radiation on daily bases (radiologists, technicians and nurses in radiology department)

- and 20 individuals in the control group

- healthy people (who have never been exposed to ionizing rays or othe physical or chemical toxic agents)

METHODS

- O,5 ml of blood sample was added to culture tubes containing 4.5 ml of RPMI 1640 media enriched with 20% fetal bovine serum, L-glutamine and 0.2 **phytohemaglutinin** 1% PHA. Each tube was supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin.

- The tubes were incubated for 44 hours at 37 °C in a slant position.

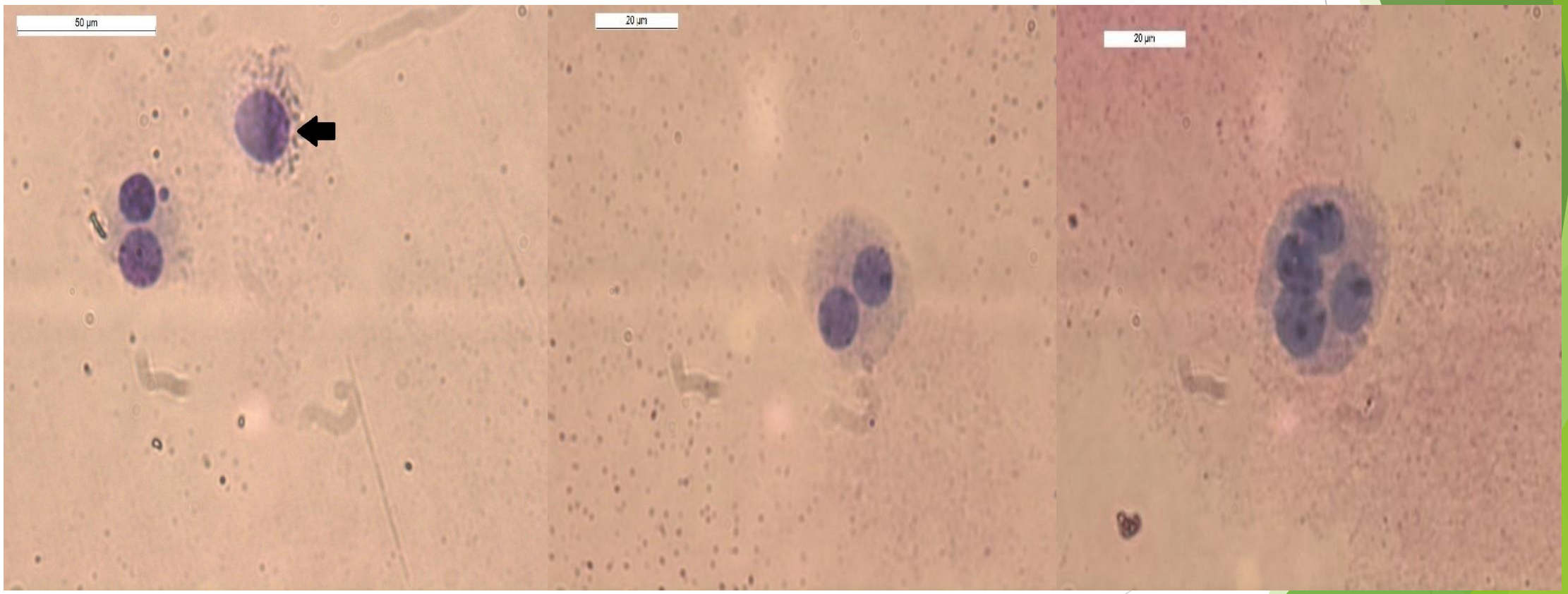
3 µg/ml of **Cytochalasin B** was then added to each culture to block cell cytokinesis and then the tubes were reincubated at 37 °C for another 28 hours.

METHODS

- Harvesting of cells by centrifugation at 1000 rpm for 10 min. Then Warm KCl hypotonic solution was added to each tube.
- Fixation with series of centrifugation with glacial acetic acid and methanol mix fixative
- Slide preparations by cell dispersion on microscope slides and staining them with 2% alkaline Giemsa
- Counting BN cells under microscope.

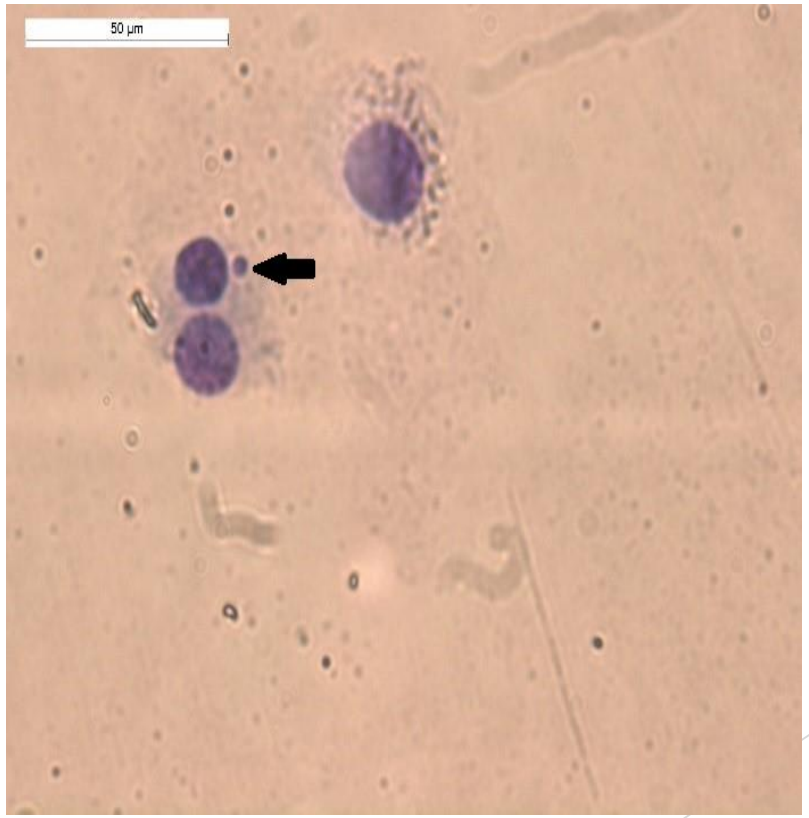
RESULTS

- The cells, according to their mitotic status, were classified as mononuclear, binuclear or multinuclear cells.



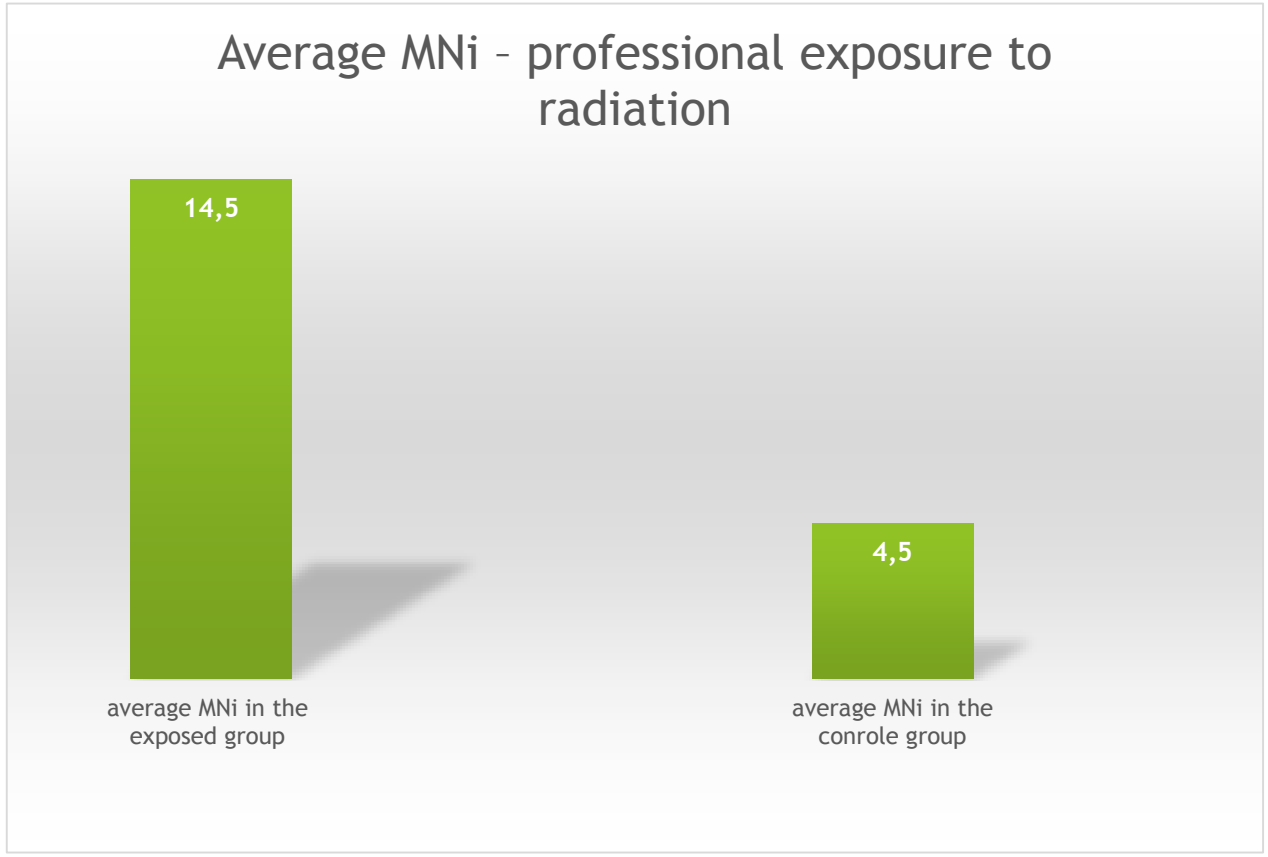
RESULTS

- The analyses of MN were carried out on 1000 BN lymphocytes per sample. MNi are defined as small, round nuclei clearly separated from the main cell nucleus



RESULTS

- Paired Student's t-test showed significant statistical differences between the total number of BN cells with MNi within the two groups (the exposed and the control) ($t=6,812$; $p<0,05$)



RESULTS

- The observed MN frequencies were compared with the criteria for spontaneous MNi (4.4±2.6 per 500 BN cells) given by Fenech and Morley

- In the exposed group, 12 individual samples (60 %) showed an increase in the MN frequency while in the control group, the increased frequency of MNi was found in 1 of the blood samples (5%)

- The samples with long time of exposure on ionizing radiation have much more MNi than the other samples. In both analyzed samples of women in the exposed and the control group, age represents serious factor for the MNi values

RESULTS

- 10 subjects with increased MN frequency in the exposed group and 1 in the control group smoked tobacco. In other words, this study revealed that the increased number of MNi in the exposed group was more common in smokers.

- We have also found an enormously high frequency of MN in control women in comparison with men in the same group

CONCLUSION

- Our results show that mean MN frequencies in the exposed group increased in comparison with mean MN frequencies in the control group
- The formation of small and large MNi, NPBs, NBUDs etc. indicates that medical personnel are exposed on clastogenic and aneugenic agents like ionizing radiation and have chromosomal instability and risk of cancer.
- Other variables that should be taken in consideration are age, gender, smoking, life stile etc.

IN FUTURE

- Other types of studies that this method can be applied

for example

Patients on cytostatic, dialysis patients, other professions exposed to genotoxic agents etc.

THANK YOU
FOR YOUR ATTENTION

ANY QUESTIONS?