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# Study of micronuclei frequency in human lymphocyte of local samples in Eshtar village and Al-Tameem region surrounding at Al-Tuwaitha site

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**Abstract**: The present study aimed to use the biological techniques in a genotoxicity assessment of DNA damage in peripheral lymphocytes due to ionizing radiation which include micronuclus assy in peripheral lymphocytes of local samples in Esther Village and Al-Tameem region surrounding at Al-Tuwaitha site. This technique of the most important cellular genetic indicators that depend on the International Atomic Energy Agency (IAEA) for radiation dose assessment. This number included thirty samples from Eshtar village, aged (18 - 55 years), and thirty samples from Al-Tameem region, aged (18 - 50 years), also the control group, which included twenty samples aged (16 - 58 years) from other regions of Baghdad was studied. The results of the average of micronuclei showed no significant differences ($p < 0.05$) in peripheral blood lymphocytes of local samples in Eshtar Village and Al-Tameem region surrounding at Al-Tuwaitha site as compared with the control group. In conclusion, the recorded results revealed no significant differences in the rate of MN for population living in the Eshtar village, Al-Tameem region compared with the control groups. Also, the level of micronuclei in binucleated lymphocyte cells of the samples within was normal value according the technical report series No.405, IAEA in 2001.

**Key words**: *Radiation , Biological dosimetry, , frequency of micronuclei.*

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## Introduction:

Ionizing radiation affects the blood and its components, such as red blood cells, leukocytes, and platelets, where erythrocytes and platelets are nuclei-free particles that are not directly affected by radiation (1). The decrease in their numbers is due to the destruction of their cellular content. The cells, especially the lymphocytes, are most susceptible to ionizing radiations (2). These cells are short-lived and often die when exposed to radiation doses (0.1-0.2) Gray followed by granular cells and monocytes (3,4). The IAEA confirm to use of cytogenetic analysis, which is one of the best modern standards used in biological dosimetry e and estimate radiation doses assessment, it is used to complement the routine physical measurements that used in the measurement of radiation doses (5.6) .Cellular genetic markers are used as indicators in the biological standardization of radiation workers such as chromosomal aberration, frequency of micronucleus assay in lymphocytes, and FISH. In addition using the gene expression to identification of possible candidate a biomarker for whole body radiation

exposure and contamination of radioactive materials (7).

Thecytokinesis-block micronucleus (CBMN) assay method is a complementary test of dicentric assay, which is a rapid, sensitive andquantitative biomarker of radiation damage in people exposed to radiation (8,9). Micronuclei (MN) are formed from lagging chromosomal fragments or whole chromosomes at anaphase which are not included in the nuclei of daughter cell. Micronuclei are formed when the cell is exposed to ionizing radiation induces the formation of acentric chromosome fragments and to a small extent malsegregation of whole chromosomes that are unable to interact with the spindle lag behind at anaphase (10, 11 , 12).The present study aims to use the biological techniques in a genotoxicity assessment of DNA damage in peripheral lymphocytes due to ionizing radiation which include micronuclus assy in peripheral lymphocytes of local samples in Esther Village and Al-Tameem region surrounding at Al-Tuwaitha site.

## Materials and methods:

This study was carried out on 60 apparently healthy individuals collected randomly from population living Esther Village and Al-Tameem region surrounding at Al-Tuwaitha site , including 30 individuals selected randomly from population living in the Eshtar village (aged 18-50 years) and 30 individuals selected randomly from population living in Al-Tameem region (aged 18-58 years), compared with a sample consisted of 20 healthy normal individuals collected randomly from population living in Baghdad a way from Al-Twaitha nuclear site (aged 16-68 years). They were asked to fill in an extensive questionnaire which included the following criteria (age, sex, smoking drink alcohol ,X-ray, abortion, time of living in these area, kind of their work).

## Blood Sampling:

Five ml of peripheral blood from all select subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

## Cytogenetic and lysis:

The MN was performed according to the description by Fenech, and Morley, 1985 (12). In short, whole blood cultures were prepared by adding 0.5 ml blood to 4.5 ml RPMI-1640 culture medium (sigma) supplemented with 20 % fetal bovine serum ( sigma ), 100 UI/ml penicillin ( Sigma-Aldrich) and 0.1 mg/ml streptomycin (Sigma-Aldrich). Phytohemagglutinin (PHA) at a concentration of 10 µl /ml was used to stimulate lymphocyte proliferation. Blood cultures were incubated at 37°C for 72 hours. Cytochalasin B (Sigma) was added after 44 h after PHA stimulation at a concentration of 4.5 µg/ml to block cells at cytokinesis.The cultures were harvested after incubation for 72 h.

## Treatment with hypotonic solution and fixation:

After a 72 h incubation period, cells were collected by centrifugation (1000 rpm, for 5 min), hypotonically treaded with cold 0.01M KCl for 3 min to lyse red blood cells. After removal of the supernatant, the pellet was fixed with a fixative solution containing

methanol:acetic acid (3:1). The cells were washed with four further exchanges of fixative solution, After fixation, the cells were gently resuspended, dropped onto wet clean glass slides and allowed to dry. Slides were stained in 4% Giemsa solution for 8 min after fixation, the cells were gently resuspended, dropped onto wet clean glass slides and allowed to dry. Slides were stained in 4% Giemsa solution for 20 min.

**Microscopic examination:**

Stained samples were evaluated using microscope; a total of 1000 binucleated cells was evaluated for the frequency of MN using 400 x magnification for surveying the slides while 1000 × magnification was used to confirm the presence or absence of MN in the cells (12).

**Data Analysis and Statistics:**

The data of this study were compiled into the computerized data file and frequency, distribution and statistical description (Mean, SE) were divided using SPSS statistical software. We used statistical analysis of variance (ANOVA) test and least significantly difference (LDS) test by probability of less than 0.05 ($p<0.05$) according to (13).

**Results and discussion:**

(Table 1) showed the rate of frequency of MN measured in the 1000 binucleated lymphocytes cell for a sample of the population residents living of Eshtar village , Al-Tameem region and control groups. There was no significant ($P< 0.05$) difference in MN frequency was observed in Eshtar village, Al-Tameem region as compared with the control. The cytokinesis-block micronucleus (CBMN) assay method is a complementary test of dicentric assay, which is a rapid, sensitive and uantitative biomarker of radiation damage in people exposed to radiation (8, 9).Micronuclei (MN) are formed from lagging chromoso-mal fragments or whole chromosomes at anaphase which are not included in the nuclei of daughter cells (Figure 1, 2 and 3). CBMN
assay is one of thesensitive methods in the evaluation of genetic and chromoso mal aberration. CB lymphocytes cells lymphocytes having one or more micronuclei are rendered evident to ALL risk according to the criteria reported by Fenech et al. (2007) (17).

They are therefore seen as distinctly separate small spherical objects that have the same morphology and staining properties of nuclei, within the cytoplasm of the daughter cells (14, 15,16 ).Micronuclei in mitotically active cells arise from structural chromosomal aberrations or disturbed function of mitotic spindle. That is why some authors consider that the follow up of MN frequencies in peripheral blood lymphocytes in the samples of human individuals could be a very effective test to estimate the effects of biological, physical and chemical agents (16) According to our results, the MN background level is 8- 35 which is in agreement with IAEA manual reporting the background MN values to range 2 to 40 per 1000 BN cells (17) and other studies ( 18, 19, 20).

**Table (1): Frequency of MN (Mean ± SE) in human peripheral lymphocyte resident living the Al-Twaitha region ( Eshtar Village , Al-Tameem) region and control group.**

| Study groups | No. of samples | Micronuclei frequency | | |
|---|---|---|---|---|
| | | No. of BN cells | Total of MN | MN / Cells (Mean ± SE) |
| Control (Baghdad) | 20 | 20000 | 184 | 0.0092 ± 0.00052 a |
| Eshtar Village | 30 | 30000 | 292 | 0.0097 ± 0.00068 a |
| Al-Tameem region | 30 | 30000 | 298 | 0.0099 ± 0.00046 a |

* Similar latter in a column (for comparison between regions) mean there is no significant difference ($p < 0.05$), according to Duncan test.

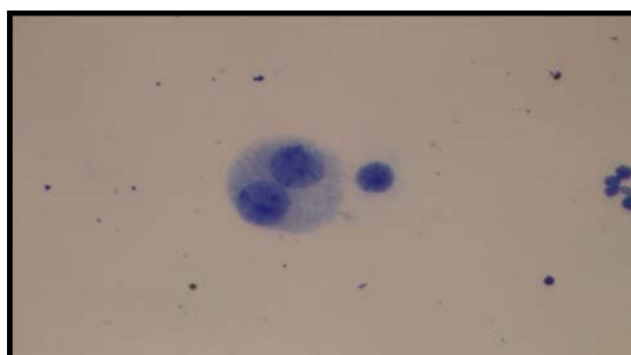

**Figure (1): Binucleated lymphocyte cell without MN (1000X).**

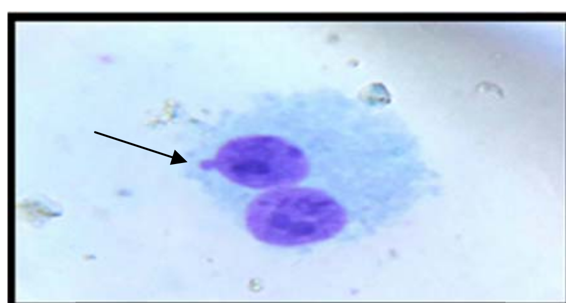

**Figure (2): Binucleated lymphocyte cell with 1 micronuclei (1000X).**

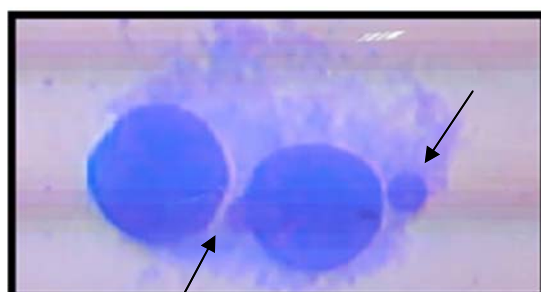

**Figure (3): Binucleated lymphocyte cell with 2 micronuclei (1000X).**

## Conclusions:

This results of MN frequencies within of normal values according to the technical report of International Atomic Energy Agency (IAEA) No. 405 , 2001.Also.the study showed presence of the no significant differences in the rate of MN for population living in the Eshtar village , Al-Tameem region compared control groups.

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