Sultana et al., Afr J Tradit Complement Altern Med. (2016) 13(2):185-190
http://dx.doi.org/10.4314/ajtcam.v13i2.22

A CYTOGENIC MONITORING APPROACH OF HOSPITAL WORKERS OCCUPTIONALLY EXPOSED TO IONIZING RADIATIONS USING MICRONUCLEUS ASSAY

Salma Sultana[1], Sadia Tabassum[1], Tayyaba Sultana[1], K. A. Al-Ghanim[2], Komal Shah[3], Tehniat Shahid[4]and Shahid Mahboob[2,1]

[1]Department of Zoology, Government College, University, Faisalabad, Pakistan,
[2]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh, Saudi Arabia,
[3]Department of Pharmacy, Islamia University, Bahawalpur, Pakistan
[4]Department of Physical Therapy, University of Faisalabad, Pakistan
Corresponding author: E-mail: shahidmahboob60@hotmail.com

Abstract

Background: The objective of this study was to determine chromosomal damage in occupational workers of the radiation department from three different hospitals, Faisalabad, Pakistan exposed for a long term to ionizing radiations using micronucles (MN) assay. A comparison between exposed and non-exposed subjects (controlled) of same age exhibited a significant an increase in the number of micronuclei in occupational workers. MN frequency increases with an increase in age and duration of exposure in both sexes but higher in females.
Materials and Methods: The study was conducted at the District Head Quarter Hospital (DHQ), Punjab Institute of Nuclear Medicine (PINUM) and Allied Hospital, Faisalabad, Pakistan. The total 145 subjects were selected from these hospitals. The subjects were divided into two groups. The control group (N= 40) (20 males and 20 females) of healthy subjects (no exposure) and the second group of subjects (N=105) (68 males and 37 females) subjects of occupational workers who were indirectly exposed to radiation. Blood samples (2ml) were collected in sodium heparinised vaccutainer tubes through venipuncture from both the groups. Disposable syringes were used for this purpose. For the evaluation of MN yield, slides were prepared by following the method of Jorge et al. (2004).
Results: A significant difference in micro nuclear induction was observed between the occupational subjects and the control subjects and as well as in females and in males ($P < 0.01$). Females are more vulnerable to ionizing radiation than males. In females, MN yield was two times higher than males. MN frequency was increased with an increase in age and duration of exposure in both sexes, but higher in females and may be due to an increase in chromosomal loss in hospital workers. There is an individual response to the physical noxa, depending on sex, age and exposure. Smoking and drinking habits do not have a significant effect in increasing the number of MN in occupationally exposed workers.
Conclusion: It was concluded that females are more vulnerable to ionizing radiations than males. MN test can be used as a biomarker with a predictive value for the estimation in occupationally exposed subjects.

Key Words: Radiations; Hospital workers; Sex; Micronucleus assay; Chromosomal damage

Introduction

Over the years, X-ray radiation has been considered a basic and widely accepted diagnostic tool. Exposure to ionizing radiation (IR) shows a combination of various consequences, such as early physical symptoms at the prodromal stage of the acute radiation syndrome (nausea, vomiting, anorexia, reddening of skin, erythema, epiltion and central nervous system impairment, lymphocyte counts, metabolic and serum components, urinary components, somatic mutations and cytogenetic biomarkers (Ward 1997). The most harmful environmental substances are those that damage the genetic material. Genotoxicants have increased the frequency of spontaneous miscarriages and genetically induced birth defects (Dobias and Vit 1998). Ionizing radiation is one of the most harmful agents that induce a direct DNA breakage in a cell (Fenech 2000). DNA damage and abnormalities in human peripheral lymphocytes were assessed using the comet assay and a cytokinesis-blocked micronucleus test (Maluf et al. 2001; Bouraoui et al., 2014); a fourfold increase in the incidence of chromosomal aberration (CA) and micronuclei (MN) in the peripheral blood lymphocytes (PBL) in occupationally exposed subjects to X-rays and other nuclear medicine (Hagelstorm et al. 1995).

Millions of occupational workers in different occupational settings are exposed to radiation and other hazardous substances such as dust particles, fibers, and organic or inorganic chemicals, are raw materials, intermediates, by-products, or products in industrial processes. Most hospital workers have more chances of exposure to radiations and complex chemical mixtures, and illness in some of them was caused due to exposure to these substances (Keshava and Ong 1999). Cytogenetic damage has been studied among hospital workers exposed to IR and an increase in the frequency of micronuclei in peripheral blood lymphocytes (Bonassi et al., 2001; Popova et al., 2005). Micronuclei are small bodies in the cytoplasm resembling the nuclear material in morphology and staining pattern (Scmid 1975; Heddle 1973). Chromosomal breakage and dysfunction of the mitotic apparatus are the two basic phenomena for the formation of MN in mitotic cells. “MN is formed from acentric chromosome or chromatid fragments and whole chromosomes or chromatids that lag

http://dx.doi.org/10.4314/ajtcam.v13i2.22
behind in anaphase and are left outside the daughter nuclei in telophase. Some MN may have their origin in fragments derived from broken anaphase bridges formed due to chromosome rearrangements such as dicentric chromatids, intermingled ring chromosomes or union of sister chromatids" (Albertini et al., 2000; Bouraoui et al., 2014). The objective of this study was to assess the indirect effect of radiation in the hospital workers of radiotherapy from three different hospitals, Faisalabad, Pakistan through the micronucleus test (MNT).

Materials and Methods

Subjects

The study was conducted at the District Head Quarter Hospital (DHQ), Punjab Institute of Nuclear Medicine (PINUM) and Allied Hospital, Faisalabad, Pakistan. The total of 145 subjects was selected from these hospitals. The control subjects (N=40) were recruited from the administration department of a DHQ hospital (N=15), PINUM (N=10) and Allied hospital (N=15). The main emphasis was on exposure duration and subjects were divided into two groups. The first group of control consists of 40 healthy subjects without any exposure (20 males and 20 females) and 2nd group of 105 subjects which were indirectly exposed to radiation (68 males and 37 females) subjects of occupational workers who were indirectly exposed to radiation (Table 1). The experimental and control subjects were divided into 4 age groups (21-30, 31-40, 41-50 and 51-60 years) and their distribution is presented in Table 2. The subjects were recruited from the three ionizing radiation units of the District Head Quarter Hospital (DHQ), Punjab Institute of Nuclear Medicine (PINUM) and Allied Hospital, Faisalabad, Pakistan. Out of the selected subjects, 35 were from the DHQ hospital, 30 from the PINUM and 35 from Allied hospital. The workers were exposed to different kinds of radiation (X-ray, γ-ray) and many radioactive isotopes (125I, 131I, 57CO, etc.). A questionnaire on lifestyle, occupational history, smoking and drinking habits, health status, diet, etc., was distributed among the selected subjects prior to study among the subjects of both groups. The selection criteria for the subject was at least 6 years of indirect exposure (duration of service), maximum exposure of more than 30 years of 20-60 years. The occupational workers represent the group most consistently exposed to low doses of IR. All subjects were nonsmokers and were divided into four groups according to age, gender (two groups) and time exposure (five groups) (Table 1).

Table1: Distribution of male and female in experimental and control subjects

Sr. No	Experimental subjects	Control subjects	Total subjects
Males	68	20	88
Females	37	20	57
% of Males	64.76%	50%	60.68%
% of Females	35.24%	50%	39.32%

Experimental subjects =105 Control subjects=40

Table 2: Distribution of in experimental and control subjects according to age groups

Age range	Experimental subjects	Control subjects	Total subjects
21-30	15	10	25
31-40	35	10	45
41-50	30	15	45
51-60	25	5	30

Experimental subjects =105 Control subjects=40

Sample Collection and Micronucleus Test (MNT)

"Blood samples (2ml) were collected in sodium heparinised vaccutainer tubes through venipuncture from both the groups. Disposable syringes were used for this purpose. For the evaluation of MN yield, slides were prepared by following the method of Jorge et al. (2004). To determine the MN yield, nearly 1000 binucleated (BN) cells was scored for each experimental condition under magnifications of 400x and finally 1000x from 2 coded slides/culture. Identification of MN was, according to the criteria summarized by Jorge et al. (2004)". The statistical analysis was performed by using the two- tailed Student's "t"- test (Chaudary and Kamal 1996).

http://dx.doi.org/10.4314/ajtcam.v13i2.22

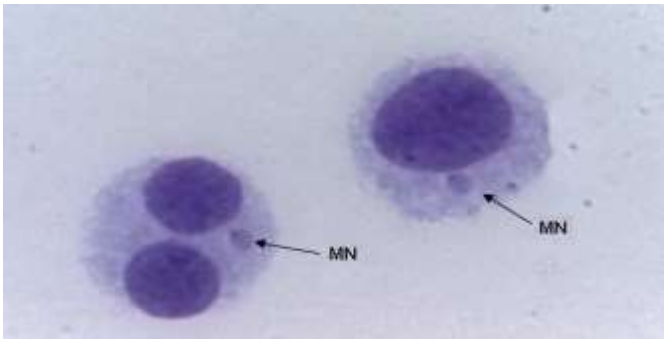

Figure 1: Showing mono-nucleated and binucleated lymphocytes with one micronuclei in their cytoplasm.

Results

In exposed subjects, a number of MN (±SE) in first age group (21-30) was 1.5±0.170 whereas in control group, it was 1.4±0.014. In the second age group (31-40), the number of MN (±SE) was 3.5±0.060. In control, number of MN was 1.8±0.026. In 3rd age group (41-50), the number of MN (±SE) was 3.6±0.074, whereas in the control, it was 3.4±0.037. In 4th age group (51-60), (Table 3), the number of MN (±SE) was 6.8±0.140 whereas in control was 6.2±0.098. Both the groups showed significantly higher difference ($p<0.001$) for MN induction. The data concerning the number of micronuclei analyzed in females (experimental and control subjects) according to different age groups. In exposed female group the number of MN (±SE) in the first age group (21-30) was 3±0.071 whereas in the control group was 1.6±0.04. In the second age group (31-40), the number of MN (±SE) was 9.3±0.271, whereas, in control was 4±0.067. In the third age group (41-50), the number of MN (±SE) was 13.5±0.346, whereas in control, it was 5.2±0.098. In 4th age group (51-60), the number of MN (±SE) was 14±0.180, whereas in control, it was 11.4±0.075 (Table 3). These groups showed significantly higher difference ($P<0.0001$) for MN induction. The number of MN increases with an increase in the exposure duration in both males and females (Table 3-4). As it were minimum 1.2 ±0. 026 and 2.8 ±0. 098 with exposure duration of 0-1 year in males and females, respectively. Number of MN was maximum 8.4±0.150 and 15.0±0.146 with the exposure duration of greater than 30 years. Table 5 Shows MN averages in both Experimental subjects (Male and Female) and in control subject (Male and Female). In experimental subjects, MN (±SE) were 6.97 ±0. 205 where 4.94 ±0. 125 in males and 10 ±0. 265 while in females, in controls the parameter was 4 ±0. 116 where 3.2 ±0. 081 in males and 5.5 ±0. 135 in females. A significant difference in micro nuclear induction was observed between the occupational subjects and the control subjects and as well as in females and in males ($P < 0.01$). Females are more vulnerable to ionizing radiation than males (Table 5). In females, MN yield was two times higher than males. The slides when observed showed induction of different number of micronuclei in mono and bi-nucleated lymphocytes. Figure 1 shows one micronuclei in both mono and bi-nucleated lymphocytes. Two micronuclei in a bi-nucleated lymphocyte have been observed (Figure 2), whereas a mono nucleated lymphocyte with three micronuclei (Figure 3).

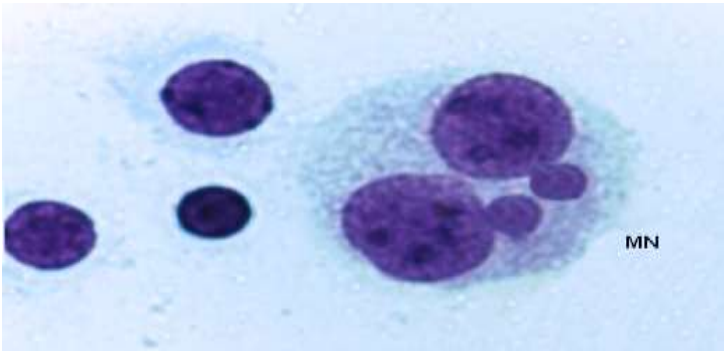

Figure 2: Showing a binucleated lymphocyte with two micronuclei near nuclear membrane.

http://dx.doi.org/10.4314/ajtcam.v13i2.22

Table 3: Distribution of mean MN in different age groups of male and female of experimental and control groups

S. No	Age range	Male Experimental group MN (±SE)	Male Control group MN (±SE)	Female Experimental group MN (±SE)	Female Control group MN (±SE)
1	21-30	1.5±0.170[b]	1.4±0.014[b]	3.1±0.07[a]	1.6±0.047[b]
2	31-40	3.5±0.060[c]	1.8±0.03[d]	9.3±0.27[a]	4.2±0.07[b]
3	41-50	3.6±0.07[c]	3.4±0.04[c]	13.5±0.35[a]	5.2±0.09[b]
4	51-60	6.8±0.14[c]	6.2±0.09[c]	14.3±0.180[a]	11.4±0.07[b]

± S.E= Standard Error; Means sharing similar letter in a row are statistically non-significant (P>0.05)

Table 4: MN yield (mean MN±SE) in males and females (occupational workers and Control) in response to the exposure duration

S. No	Exposure duration	Male Experimental MN (±SE)	Female Experimental MN (±SE)
1	0-1	1.2±0.03[b]	2.8±0.09[a]
2	1-10	3.3±0.06[b]	7.5±0.13[a]
3	11-20	4.6±0.13[b]	13.6±0.06[a]
4	21-30	6.4±0.01[b]	14.8±0.09[a]
5	>30	8.4±0.15[b]	15.2±0.15[a]

± S.E= Standard Error; Means sharing similar letter in a row are statistically non-significant (P>0.05)

Table 5: MN yield (±SE) in Occupational workers and control subjects from three hospitals

Gender	Experimental MN (±SE)	Control MN (±SE)
Male	4.96±0.12[a]	3.2±0.08[b]
Female	10.7±0.26[a]	5.55±0.13[b]
Male +Female	6.97±0.20[a]	4.37±0.12[b]

± S.E= Standard Error; Means sharing similar letter in a row are statistically non-significant (P>0.05)

http://dx.doi.org/10.4314/ajtcam.v13i2.22

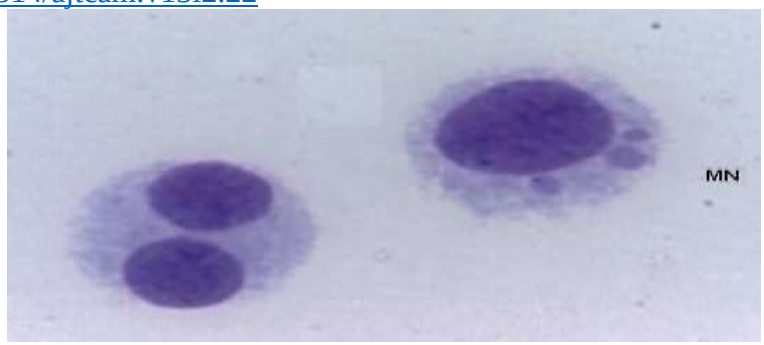

Figure 3: Showing a mono-nucleated lymphocyte having three micronuclei in the cytoplasm

Discussion

Ionizing radiation is widely known to cause mutations and cell transformations predominantly by causing single–stranded double-stranded DNA breakage, thereby leading to chromosomal instability and carcinogenesis (Preston, 2005; Bouraoui et al., 2014). In this study, no data were available on individual exact physical doses of radiation to these occupational workers due to no use of the dosimeter, for this reason, radiation exposure was considered as time/year a worker was working in the radiation department. The present study indicated that a long-term exposure of radiation causes damage in peripheral lymphocytes and induction of MN increased significantly, as compared with control subjects. These findings are in agreement with the findings of Cardoso et al. (2001) and Singh et al. (2005) who reported a significant increase in micronuclei count from pretreatment to successive weeks of external radiotherapy in cervical cancer patients with respect to local response. The frequency of MN was higher in nonsmoker – exposed workers are in line with the findings of Znoar et al. (2003). A micronucleus is formed when mutational agent has the ability to distort normal chromosomes (anugenic) or clatogenic agent with a potential to break the chromosome exposed to a cell under active cell division and decrease the movement of entire chromosomes or a part of it. Age has a significant effect on the production of MN in both sexes in this study (Maffei et al., 2002; Znaor et al.; 2003; Mihalache et al., 2007). We observed that females had a higher number of micronuclei than males (Mihalache et al., 2007), who also reported that in spite of the fact that both sexes showed an increase in production of MN but this effect is more pronounced in females. It has been observed that the control subject also showed an increase in no of MN with increasing age, but the frequency is much less when compared with exposed subjects (Maffei et al., 2002 because of apoptosis. The frequency of MN was increased with increase in duration of exposure (Mihalache et al., 2007; Gadhia et al. 2004). The earlier bio monitoring reports on hospital radiographers occupationally exposed to radiation exhibited mentioned an increase in MN frequencies (Angelini et al., 2005; Maluf et al., 2001). The DNA damage leading to the formation of micronuleated lymphocytes which were noticed among the occupational subjects may not be necessary because of the ionizing radiation. Bonassi et al. (1997; 2013) proposed that in addition to ionizing radiation, other chemical agents might also be involved in chromosomal aberration in hospital workers. “These chemical contaminants in hospital complex are present in addition to the ionizing radiation; several chemical contaminants may have genotoxic effects, and thus, there is possibility that the higher MN in hospital subjects may be the cumulative effect of IR and chemical pollutants. Ionizing radiation induces significant health effects such as cancer and genetic damage (Terzic et al., 2015). Ionizing radiation causes mutations and cell transformations, predominantly by causing single-strand and double-strand DNA breakage, leading to chromosome instability and carcinogenesis (Eken et al., 2010)”.

A number of aspects support the hypothesis of a direct correlation between MN frequency and cancer development (Fenech 2003). It is important to consider the micro nucleation test as a biomarker with a predictive value for the estimation to reduce the incidence in due course of time of cancer risk due to multiple reasons. The first one refers to the genetic material response being under the impact of the physical noxa in the occupational exposure, namely the nuclear structural changes (the micronuclei appearance, nucleoplasmatic bridges and “nuclear buds”). Micronuclei frequency increases naturally with an increase in age events. In addition, the ageing process is stimulated to become an indicator of noxiousness with a practical direct application in the case of the occupational exposures to chemical or physical noxae. A cumulative effect was observed in this study in the occupational subjects exposed to ionizing radiation in males. The intensity of the damage was more in females, where the MN frequency is even more intense. “The findings of this study confirm that the incidence of MN significantly increased for health workers in radiation departments occupationally exposed to ionizing radiation in comparison to control subjects. Our results are in line with what many others reported in literature (Sakly et al., 2012; Terzic et al., 2015)”.

Conclusions

We had concluded that occupation has a drastic effect along with age, gender and life style, which also play a crucial part in the status of human health. We are of the view that the duration of exposure of ionizing radiation causes induction of MN in peripheral blood lymphocytes (PBL). Smoking and drinking habits did not express any significant effect in term of increase in yield of MN in occupationally exposed workers.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

http://dx.doi.org/10.4314/ajtcam.v13i2.22

Compliance with Ethical Standards: To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, consent was obtained from each subject or a local human ethics committee of the Institute obtained subject's guardian after receiving approval of the experimental protocol. We also followed the principles of Helsinki Declaration.

Acknowledgement

The authors would like to express their sincere appreciation to the Deanship of Scientific at King Saud University for its funding of this research through the Research Group Project no. RGP-1435-012.

References

1. Alberitini, R. J. (2000).Biomarker responses in human populations; towards a worldwide map. Mutat. Res., 428: 217-226.
2. Angelini, S., Kumar, P., Carborne, F., Maffei, F., Forti, G. C. and Vio-lante, F. S. (2005). Micronuclei in humans induced by exposure to low level of ionizing radiation: influence of polymorphisms in DNA repair genes. Mutat. Res., 570: 105-17.
3. Bonassi, S., Forni, A., Bigatti, P., Canavarollo, N., Ferrani, M. D. and Lando, C. (1997). Chromosome aberrations in hospital workers: evidence from surveillance: Studies in Italy. Am J Ind. Med., 31:353–60.
4. Bonassi, S., Mougou, S., Drira, A., Tabka, F., Bouali, N., Mrizek, N., Elghezal, H. and Saad, A. (2013). A Cytogentic approach to the effects of low levels of ionizing radiation (IR) on the exposed Tunisian hospital workers. Int. J. Occu. Med. and Env. Heal. 26:144-154.
5. Chaudhry, S. M. and Kama,l S. (1996). Introduction to statistical theory. *Markaz kutub khana*, ed.6th
6. Cardoso, R. S., Hyodo, S.T, Neto, J. R. P. P. and Hojo, E. T. S. (2001).Evaluation of Chromosomal Aberrations, Micronuclei, and Sister Chromatid Exchanges in Hospital Workers Chronically Exposed to Ionizing Radiation Terato. Carcino. Mutag., 21:431–439.
7. Dobias, L., Vit, M., Malachova, K. and Harankova, J. (1998). Occupational exposure to heavy metals in industry. In Proceedings of the 2nd International Conference on Trace Elements Effects on Organisms and Environment, *Katowice, Poland*, pp. 5–10.
8. Dobrzynska, M. M. (2005). The effects of mice of combined treatments to X- rays and antineoplastic drugs in the comet assay. Toxicol., 207: 331-338.
9. Eken, A., Aydin, A., Erdem, O., Akay, C., Sana, H. T. and Soykut, B. (2010). Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation. Toxicol. Ind. Heal. 26: 273-280
10. Fenech, M. and Morley, A. (20000. Measurement of micronuclei in lymphocytes. Mutat. Res., 147: 29-36.
11. Fenech, M. (2003). Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes results of an international slide-scoring exercise by the Human project. Mutat. Res., 534: 45-64.
12. Gahia, P. K., Shah, N., Nahata, S., Patel, S., Patel, K., Pithawala, M. and Tamakuwala, D. (2004). Cytogenetic Analysis of Radiotherapeutic and Diagnostic Workers Occupationally Exposed to Radiations. Int. J. Hum. Genet., 4(1): 65-69.
13. International Atomic Energy Agency. (2001). Cytogenetic analysis for radiation dose assessment. A manual. Technical Report Series No. 405. Vienna (IAEA).
14. Heddle, J. A. (1999). A rapid in vivo test for chromosomal damage. Mutat Res 1973; 18: 187-190
15. Keshava, N. and Ong, T. M. (1999) Occupational exposure to genotoxic agents. Mutat. Res., 437: 175-194.
16. Maluf, S. W., Passor, D. F., Bacelor, A. and Speit, G. (2001). Assessment of DNA damage in lymphocytes of workers exposed to X-radiation. Environ. Mol. Mutagen., 38: 311-315.
17. Maffei, F., Angelini, S., Cantelli, G., Lodi, F. V. and Violante, F. S. (2002). Micronucleus frequencies in hospital workers occupationally exposed to low level of ionization radiation; influence of smoking status and other factors. Mutag., 7 : 404- 409.
18. Maluf, S. W., Passon, D. F., Bacelar, A., Speit, G. and Eordtmann, B. (2001). Assessment of DNA damage in lymphocytes of workers exposed to X-radiation using the micronucleus test and the comet assay. Env. Mol. Mutag. 38: 311-5.
19. Mihalache, D., Preoteasa, V. and Petrescu, A. (2007). Incidence of radiation-induced micronuclei in occupationally exposed subjects. Romanian J. Biophys., 17: 119–128.
20. Preston, R. J. (1975). Bystander effects: Genomic instability, adaptive response and cancer risk assessment for radiation and chemical exposures. Toxicol. Appl. Pharmacol., 207:550-6.
21. Sakly A, Ayed Y, Chaari N, Akrout M, Bacha H, Cheikh HB. (2012). Assessment of chromosomal aberrations and micronuclei in peripheral lymphocytes from Tunisian hospital workers exposed to ionizing radiation. Genet. Test Mol. Biomark., . doi:10.1089/gtmb.2012.0111.
22. Singh, S., Niloy, R., Datta, T., Krishnani, N., Lal, P. and Kumar, S. (2005). Radiation therapy induced micronuclei in cervical cancer—does it have a predictive value for local disease control? Gynecol. Oncol., 97: 764 – 771.
23. Terzic, S., Milovanovic, A., Dotlic, J., Rakic, B. and Terzic, M. (2015). New models for prediction of micronuclei formation in nuclear medicine department workers. J. Occup. Med. Toxicol., 10, 25
24. Ward, J. F. (1997). Radiation mutagenesis: The initial DNA lesions responsible. Radia. Res., 142: 362-8.
25. Znaor, A., Fuelae, A., Strand, M., Barkoviae, D., Skara, M. and Hozo, I. (2003). Micronuclei in Peripheral Blood Lymphocytes as a Possible Cancer Risk Biomarker a Cohort Study of Occupationally Exposed Workers in Croatia. Croat. Med. J., 44: 441 446.