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Research Articles

Evaluation the Synergistic Effect of High Dose Radiation of Radioiodine on the Immune System Suppressed By Cyclosporine

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Abstract: Cyclosporine is an immunosuppressant drug and iodine-131 is the diagnostic and therapeutic radioisotope in nuclear medicine. In this study, we aimed to evaluate the synergistic effect of high-dose radiation of radioiodine on the suppression of the immune system of BALB/cmice after receiving cyclosporine. A total of 50 mice BALB/c were randomly divided into two groups (control and test). Then, 50 mg kg^{-1} body weight cyclosporine was given to both groups. After 24 h, 0.5 mL iodine-131 was given to the test group and in the control group the same volume of normal saline was given. Data analysis was done using SPSS V.20 and p value <0.05 was considered significant. After 30 days of monitoring, only 2 deaths (8%) occurred in the control group, however 16 deaths (64%) were observed in the test group. Statistical analysis of the data indicated that there is a significant relationship (p value <0.0005) between injecting radioiodine and the mortality rate in mice receiving cyclosporine and proves the synergistic effect of these two factors. Based on the results, iodine-131 can be introduced as a stimulating and synergistic factor on the suppression of the immune system of BALB/cmice and subsequent death.

Keywords: Cyclosporine, Radioiodine, Synergistic Effect, High-Dose Radiation

Introduction

It's about a century that human knows ionizing radiation is harmful for biological tissues and in adequate doses can lead to irreversible insult, cancer and even death. Nowadays, with the advancement of the science, the use of radioactive materials has increased dramatically in various fields including industry, agriculture, medicine and research (Mortazavi *et al*., 1999).

High levels of ionizing radiations from radioactive substances are dangerous for living organisms, including human kind. The first people who worked with X-rays and radioactive substances clearly observed that these substance scan cause burns or scarring and there is a possibility of chromosomal mutation and subsequent cancer even at low levels (NRPB, 1999).

In addition to the benefits of early detection and treatment of diseases, the risk of exposure must also be considered in medical applications of ionizing radiation such as radiology, nuclear medicine and CT scan. Iodine-131 or radioiodine is a radioactive substance that is widely used in nuclear medicine for both diagnosis and treatment. The dose of iodine-131 used for diagnosis is about 5 mCi, however, the dose of medical treatment is higher (approximately 20 to 250 mCi and even more in some cases) (Li *et al*., 2001).

One of the major differences of iodine-131 with other radiopharmaceuticals used in nuclear medicine is its Beta

(β) radiation that has more applied biological effect compared to Gama (γ) radiation (Li *et al*., 2001).

So far, there have been many studies on the biological damages after radioiodine treatment, however, there are very few reports regarding the effects of radiation emitted by radioactive iodine on the immune system (Baugnet-Mahieu *et al*., 1994).

Effects of ionizing radiation and radioactive substance son the immune system was studied first by a committee in 1972. The UNSCEAR committee concluded that the levels of radiation can stimulate or suppress the immune system, with high doses of radiation having immunosuppressive effects (UNSCEAR, 1972).

The biological effect of ionizing radiation on human body depends on the total dose of radiation received and dose rate of exposure. High-dose ionizing radiation, provided extremely at high-dose rate, is commonly considered to be harmful, resulting in apoptosis, DNA damage and formation of tumor cells. Radiation triggers DNA repair pathways and cell cycle checkpoints in normal cells and results in recovery or cell death (Park *et al*., 2014).

Apoptosis is the process of Programmed Cell Death (PCD) that may occur in multi cellular organisms in response to threatening symptoms and poisoning including radiation. This phenomenon can be observed in the cells especially bone marrow stem cells and Peripheral Blood Mononuclear Cells (PBMCs) after exposure to the radiation (Bordon *et al*., 2010; Schnarr *et al*., 2009; Zhang *et al*., 2012).

Cyclosporine is an immunosuppressant drug widely used in organ transplantation in combination with corticosteroids to prevent rejection. The drug is also used in a cute rheumatoid arthritis, atopic dermatitis, alopecia and psoriasis (Brown *et al*., 1988).

Synergistic immunosuppressive effects of high dose radiation and cyclosporine can lead to irreparable damage and even death in patients receiving these. This study aimed to investigate the synergistic immunosuppressive effect of high-dose radiation of radioiodine on the immune system suppressed by cyclosporine.

Methodology

The Study Population

In this experimental study, carried out at the Center of Experimental Animals of the Shiraz University of Medical Sciences, we used 50 consanguine, male, BALB/c mice in weight range of 20-25 gram. Mice were purchased from the Stem Cell and Transgenic Technology Research Center of the Shiraz University of Medical Sciences. They were kept in a room with a temperature of 22-24°C and constant humidity, in a cycle of 12 h light and 12 h dark. Adequate water and food was provided. The room was disinfected before the study began by Sterl-STAT. To create further adaptations of animals to a new environment, all of the tests were carried out 7 days after the new cage. The ethics committee of SUMS approved the study.

Experimental Stage

Mice were randomly divided in 2 equal groups (Control and Test) and were coded. Decoding was done at the end of the study for bias prevention. All the mice were maintained under the conditions described before. According to SUMS ethical codes regarding the care and use of laboratory animals, all possible measures were taken into account to avoid animals' suffering at each stage of the experiment.

Initially, 50 mg kg^{-1} body weight cyclosporine was administered orally to both groups (Oral solution: 100 mg mL^{-1}). The dose of cyclosporine was calculated according to weight and with the viewpoint of an expert pharmacologist in order to the severe weakening of the immune system.

After 24 h, 0.5 mL radioactiveiodine-131 (radioiodine) was given to the test group and in the control group the same volume of normal saline was given. All mice were monitored immediately after receiving iodine and the results were recorded each day. Every 6 h the number of dead and alive mice was counted for 30consecutive days in both groups.

Statistical Analysis

The gathered data were analyzed by SPSS V.20 software; Chi-Square nonparametric test was used for comparison of the groups and Kaplan-Meier test was done in order to analyze the survival rate. In all cases, p value <0.05 was considered statistically significant.

Results

According to the data, as can be seen in Fig. 1, among 25 mice of the test group which received radioiodine after treating with high dose of cyclosporine, 16 (64%) mice died and 9 (36%) mice survived after passing 30 days. In contrast, in the control group, only 2 (8%) mice died and 23 (92%) mice survived.

Statistical analysis of the data by the Chi-Square test indicated a significant relationship (p value <0.0005) between receiving radioiodine and mortality among mice that received the high dose of the immunosuppressant drug (cyclosporine) and showed the synergistic effect of these two factors.

The results of analyzing Phi and Cramer's V test that measures the power of the relationship between the variables, representative a robust correlation (p value <0.0005) between the variables of receiving radioiodine

and mortality of mice after giving high dose of cyclosporine and confirmed the synergistic immunosuppressive effects of cyclosporine and radioiodine (Table 1).

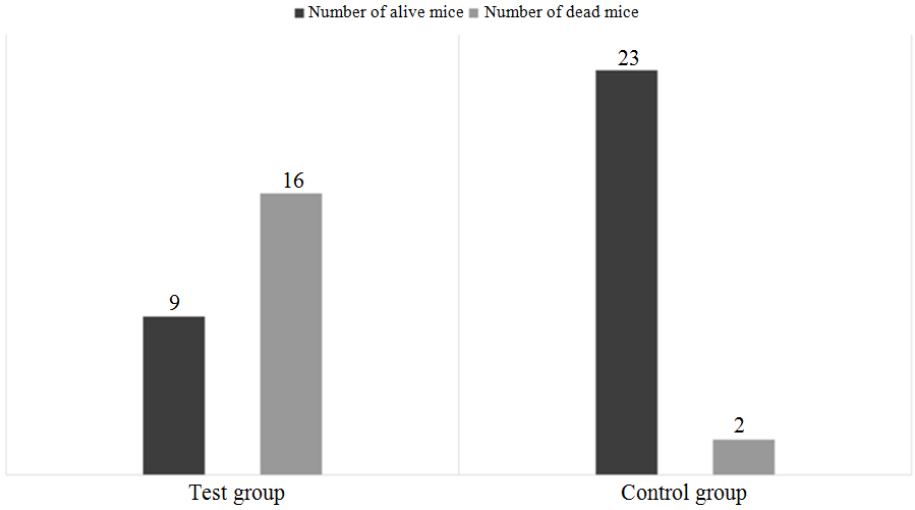

Fig. 1. Comparison of the number of alive mice in both test (radioiodine injected) and control groups after receiving a high dose of cyclosporine after passing 30days

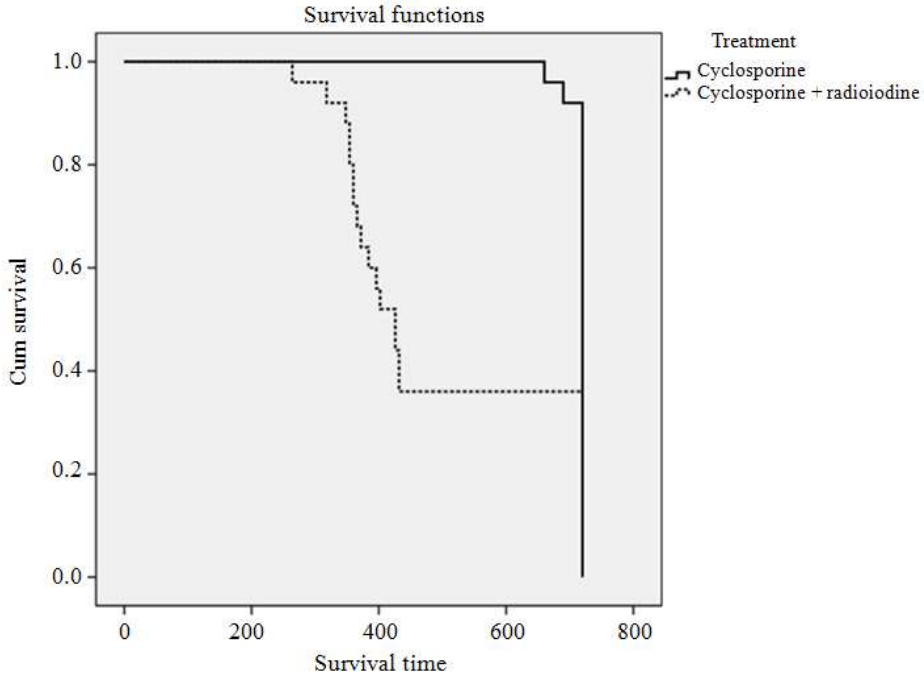

Fig. 2. Plot of the cumulative survival proportion against time for control and radioiodine injected group

Table 1. Phiand cramer's vtest

Symmetric measures			
		Value	Approx. Sig.
Nominal by nominal	Phi	-0.583	0.000
	Cramer's V	0.583	0.000
N of valid cases	50		

Table 2. Estimated means and medians for survival time

	Mean				Median			
			95% confidence interval				95% Confidence interval	
Treatment	Estimate	Std. Error	Lower bound	Upper bound	Estimate	Std. Error	Lower bound	Upper bound
Cyclosporine	716.400	2.638	711.229	721.571	720.000	0.000	.	.
Cyclosporine + Radioiodine	498.960	34.587	431.170	566.750	426.000	24.819	377.354	474.646

A log rank test was run to determine if there were differences in the survival distribution for the different types of intervention: Cyclosporine administered group (Control) and the group that cyclosporine and radioiodine were given (Test). The survival distributions for the two treatments were statistically significantly different, $X^2(1) = 19.201$, $p<0.0005$. The estimated survival time were 716.4±2.638 and 498.96±34.587 h in control and test group respectively (Table 2). Regarding the survival curve, it can be seen that radioiodine increase the molarity rate and reduce the survival time considerably (p value <0.0005) (Fig. 2).

Discussion

Our findings showed that radioiodine can increase the rate of mortality among mice that received the high dose of the immunosuppressant drug (cyclosporine). Regarding Kaplan-Meier analysis, the estimated survival time in cyclosporine administered group (716.4±2.638 h) was significantly more compared to the group that received cyclosporine plus radioiodine (498.96±34.587) and this figure confirmed the synergistic immunosuppressive effects of cyclosporine and radioiodine.

Cyclosporine injection is given together with a steroid medicine to prevent the body from rejecting a transplanted organ (e.g., kidney, liver, or heart). It belongs to a group of medicines known as immunosuppressive agents (Van Buren, 1986).

Cyclosporine is a strong immunosuppressive drug. Early biological studies revealed that Cyclosporine inhibits T cell activation by blocking the transcription of cytokine genes, including those of IL-2 and IL-4. Moreover, it is proven that Cyclosporine prevents the phosphatase activity of calcineurin and subsequently blocks the activation of NFAT transcription factors. Additionally, recent studies demonstrate that Cyclosporine also inhibits the activation of JNK and p38 signaling pathways (Matsuda and Koyasu, 2000).

Among NFAT family members, NFAT1, NFAT2 and NFAT4 are involved in the transcriptional activation of genes encoding cytokines including IL-2 and IL-4 and CD40L. By preventing phosphatase activity of calcineurin, Cyclosporine inhibits the nuclear translocation of NFAT family members and consequent gene expression in activated T cells (Matsuda and Koyasu, 2000; Graham, 1994).

Cyclosporine mainly prevents the activation cascade essential for particular immune functions by blocking T cell biosynthesis of cytokines, especially IL-2. Moreover, Cyclosporine terminates intrathymic T-cell development and inhibits some B-cell responses (Van Buren, 1986; Graham, 1994).

Radioiodine (I-131), an isotope of iodine that emits radiation, is used for medical purposes. High levels of radiation of iodine-131 isotope to the thyroid is almost always associated with hypothyroidism and thyroid cells destruction. Hence, after exposure to high doses ofiodine-131, very small population of thyroid cells persists and the risk of thyroid cancer becomes extremely low. When a small amount of radioiodine is swallowed, it is absorbed into the blood circulation in the GI tract and concentrated from the blood by the thyroid gland, where it initiates destroying the gland's cells by the high levels of radiation. Consequently, very small population of thyroid cells persists after exposure to high dose radiation related to radioiodine and the risk of thyroid cancer become extremely low (Alimanovic-Alagic *et al*., 2009).

In contrast, a large number of children exposed to a lower dose of iodine-131 and other short-lived isotopes of iodine related to the Chernobyl incident in 1986 and they were suffering from thyroid cancer after only a few years. There are several possible reasons for the differences between the medical use of radioactive iodine and exposure to radioactive iodine during a nuclear event and thyroid cancer development (NIH, 1999).

As noted above, high levels of iodine-131 can cause thyroid cell death. In contrast, exposure to low doses caused injury and damages to thyroid cells, but do not destroy them, hence results in nuclear damages and cause thyroid cancer by inducing mutations. Radioactive iodine that released into the atmosphere, possibly involving short-lived isotopes of iodine (in addition to iodine-131) and these isotopes can be carcinogenic. Since the thyroid cells of children are more in cell division and proliferation than adults, there is a higher chance of mutations and thyroid cancer when expose to lower content of radioactive iodine (NIH, 1999).

In a study that was conducted by single-cell gel electrophoresis in order to evaluate DNA damage in circulating lymphocytes of papillary thyroid cancer

patients who received I-131 by oral administration, high levels of DNA damage observed (Unlu *et al*., 2013).

In another study investigating the damages caused by ionizing radiation on the structure of hemoglobin, high levels of radioiodine was shown to induce severe structural damages (de Oliveira *et al*., 2013).

Many studies have been performed about the damages caused by high levels of radiation on the immune system, specifically white blood cells. That the results can help people exposed to these radiations, such astronauts exposed to cosmic radiation and Gamma (γ) radiation, which can cause a significant decrease in white blood cells and immunosuppression (Maks *et al*., 2011).

Ionizing radiation exposure leads to induction of apoptosis in T and B lymphocytes and mature NK cells. It also cause lethal damage in bone marrow stem cell precursors of monocytes and granulocytes (Park *et al*., 2014). In individuals receiving high doses of radiation, both mature lymphocytes and bone marrow stem cells were severely damaged, causing significant reduction of granulocytes and NK cells, which together defend against microbial invasion. Accordingly, many people died from active infections (UNSCEAR, 2006).

Apoptosis plays a crucial role within the immune system, in particular in negative regulation and is the key mechanism in the response to ionizing radiation. Lymphocytes die by apoptosis immediately after exposure (interphase death) or by reproductive cell death (Jonathan *et al*., 1999; Meijer *et al*., 1999). There are at least two pathways for radiation-induced apoptosis: One is mediated by mitochondrial factors and is p53-dependent and the other is mediated by cell surface receptors (Fas/CD95) (Shankar and Sainis, 2005). Several studies have considered B-cells to be more radiosensitive than T-cells (Prosser, 1976; Rivett and Hearn, 2004; Schmitz *et al*., 2003; Wuttke *et al*., 1993). However, progenitor/steam cells and NK cells seem to be more radio resistance compared to T- and B-cells (Meijer *et al*., 1999).

When cells are exposed to high levels of radiation, macrophage system quickly activate (Lorimore *et al*., 2001). Derived NO from activated macrophage plays a major role in inducing apoptosis and suppressing mitosis in cells around (Brown and Savill, 1999; Duffield *et al*., 2000) and this increases the genetic mutation, DNA base changes and detachment of the two strands of DNA in the cells and all of the secellular factors cause the immune system becomes weakened (Dizdaroglu *et al*., 1993).

To conclude, the results of this study indicate that high morbidity and mortality is associated with the combined use of radioiodine and cyclosporine. Therefore, it is imperative that the risks and benefits are taken into consideration in patients requiring both agents clinically and they should be presented with alternatives. The biological and possible immunosuppression side effects of high-dose radiation related to therapeutic radioisotopes must be seriously studied, especially in radiotherapy by radiopharmaceuticals in order to avoid irreparable damage and even death.

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Author's Contributions

Mehrosadat Alavi: Coordinate and supervisor.

Mohammadali Okhovat: Executive Member (feeding drug and …).

Kourosh Bamdad: Data-analysis.

Motahareh Motazedian: Scientific supervisor and technical help.

Fatemeh Ebadi: Coordinated the mouse work.

Ahmad Movahedpour: Coordinated the mouse work.

Faezeh Hekmatara: Coordinated the mouse work.

Mohammad Atefi: Corresponding Author designed the research plan and organized the study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

Conflict of Interest

The authors report no declarations of interest.

References

Alimanovic-Alagic, R., E. Kucukalic-Selimovic and M. Mekic, 2009. Efficiency and safety of radioactive iodine I-131 in treatment of thyroid disease. Medicinskiarhiv, 63: 295-6. PMID: 20380134

Baugnet-Mahieu, L., M. Lemaire, E.D. Leonard, A. Leonard and G.B. Gerber, 1994. Chromosome aberrations after treatment with radioactive iodine for thyroid cancer. Radiat. Res., 140: 429-31. DOI: 10.2307/3579122

Bordon, E., L.A. Henriquez-Hernandez, P.C. Lara, A. Ruiz and B. Pinar *et al*., 2010. Prediction of clinical toxicity in locally advanced head and neck cancer patients by radio-induced apoptosis in Peripheral Blood Lymphocytes (PBLs). Radiat. Oncol., 5: 4-4. DOI: 10.1186/1748-717X-5-4

Brown, M.W., E.M. Bolton, I.A. More and J.A. Bradley, 1988. Immunohistological observations of rat fetal pancreas allografts transplanted into unmodified and cyclosporine-treated recipients. Transplantation, 46: 800-6. DOI: 10.1097/00007890-198812000-00003

Brown, S.B. and J. Savill, 1999. Phagocytosis triggers macrophage release of Fas ligand and induces apoptosis of bystander leukocytes. J. Immunol., 162: 480-5. PMID: 9886423

de Oliveira, G.C., G.A. Maia, V.F. Cortes, H.D.L. Santos and L.M. Moreira *et al*., 2013. The effect of γ-radiation on the hemoglobin of stored red blood cells: The involvement of oxidative stress in hemoglobin conformation. Annals Hematol., 92: 899-906. DOI: 10.1007/s00277-013-1719-z

Dizdaroglu, M., R. Olinski, J.H. Doroshow and S.A. Akman, 1993. Modification of DNA bases in chromatin of intact target human cells by activated human polymorphonuclear leukocytes. Cancer Res., 53: 1269-72. PMID: 8383005

Duffield, J.S., L.P. Erwig, X. Wei, F.Y. Liew and A.J. Rees *et al*., 2000. Activated macrophages direct apoptosis and suppress mitosis of mesangial cells. J. Immunol., 164: 2110-9. DOI: 10.4049/jimmunol.164.4.2110

Graham, R.M., 1994. Cyclosporine: Mechanisms of action and toxicity. Cleveland Clinic J. Med., 61: 308-13. DOI: 10.3949/ccjm.61.4.308

Jonathan, E.C., E.J. Bernhard and W.G. McKenna, 1999. How does radiation kill cells? Curr. Opin. Chem. Biol., 3: 77-83. DOI: 10.1016/S1367-5931(99)80014-3

Li, L.B., J.P. Wang, X.R. Yu, S.S. He and F.H. Yu *et al*., 2001. Medical radiation usage and exposures from medical X ray diagnosis in Shandong province of China. Radiat. Protect. Dosimetry, 93: 261-6. DOI: 10.1093/oxfordjournals.rpd.a006437

Lorimore, S.A., P.J. Coates, G.E. Scobie, G. Milne and E.G. Wright, 2001 Inflammatory-type responses after exposure to ionizing radiation *in vivo*: A mechanism for radiation-induced bystander effects? Oncogene, 20: 7085-95. DOI: 10.1038/sj.onc.1204903

Maks, C.J., X.S. Wan, J.H. Ware, A.L. Romero-Weaver and J.K. Sanzari *et al*., 2011. Analysis of white blood cell counts in mice after gamma- or proton-radiation exposure. Radiat. Res., 176: 170-6. DOI: 10.1667/RR2413.1

Matsuda, S. and S. Koyasu, 2000. Mechanisms of action of cyclosporine. Immunopharmacology, 47: 119-25. DOI: 10.1016/S0162-3109(00)00192-2

Meijer, A.E., A.B. Saeidi, A. Zelenskaya, S. Czene and F. Granath *et al*., 1999. Influence of dose-rate, post-irradiation incubation time and growth factors on interphase cell death by apoptosis and clonogenic survival of human peripheral lymphocytes. Int. J. Radiat. Biol., 75: 1265-73. DOI: 10.1080/095530099139421

Mortazavi, S.M.J., T. Ikushima and A.A. Sharafi, 1999. Radiation hormesis and adaptive responses induced by low doses of ionizing radiation. J. Kerman Univ. Med. Sci., 6: 50-60.

NIH, 1999. Exposure of the American people to iodine-131 from Nevada nuclear-bomb tests: Review of the National Cancer Institute Report and Public Health Implications. The National Academies Collection: Reports funded by National Institutes of Health. Washington.

NRPB, 1999. Guidelines on patient dose to promote the optimisation of protection for diagnostic medical exposures: Report of an advisory group on ionising radiation. National Radiological Protection Board, Great Britain, DidcotEngland.

Park, B., C. Yee and K.M. Lee, 2014. The effect of radiation on the immune response to cancers. Int. J. Molecular Sci., 15: 927-43. DOI: 10.3390/ijms15010927

Prosser, J.S., 1976. Survival of human T and B lymphocytes after X-irradiation. Int. J. Radiat. Biol. Related Stud. Phys. Chem. Med., 30: 459-65. DOI: 10.1080/09553007614551271

Rivett, A.J. and A.R. Hearn, 2004. Proteasome function in antigen presentation: Immunoprotea some complexes, Peptide production and interactions with viral proteins. Curr. Protein Peptide Sci., 5: 153-61. DOI: 10.2174/1389203043379774

Schmitz, A., J. Bayer, N. Dechamps and G. Thomas, 2003. Intrinsic susceptibility to radiation-induced apoptosis of human lymphocyte subpopulations. Int. J. Radiat. Oncol. Biol. Phys., 57: 769-78. DOI: 10.1016/S0360-3016(03)00637-0

Schnarr, K., D. Boreham, J. Sathya, J. Julian and I.S. Dayes, 2009. Radiation-induced lymphocyte apoptosis to predict radiation therapy late toxicity in prostate cancer patients. Int. J. Radiat. Oncol. Biol. Phys., 74: 1424-30. DOI: 10.1016/j.ijrobp.2008.10.039

Shankar, B. and K.B. Sainis, 2005. Cell cycle regulators modulating con a mitogenesis and apoptosis in low-dose radiation-exposed mice. J. Environ. Pathol. Toxicol. Oncol., 24: 33-43. DOI: 10.1615/JEnvPathToxOncol.v24.i1.40

Unlu, S., S. Ozdemir, S. Sumer, E. Saglar and S. Tastan *et al*., 2013. Investigation of DNA damage by the alkaline comet assay in 131I-treated thyroid cancer patients. Analytical Quantitative Cytopathol. Histopathol., 35: 36-40. PMID: 23469622

UNSCEAR, 1972. A report of the United Nations Scientific Committee on the effects of atomic radiation to the general assembly, with annexes. Manhattan, New York City, United Nations.

UNSCEAR, 2006. Effects of ionizing radiation on the immune system. Effects of Ionizing Radiation (United Nations Scientific Committee on the Effects of Atomic Radiation UNSCEAR 2006 Report to the General Assembly, with scientific annexes) 2. Manhattan, New York City: United Nations.

Van Buren, C.T., 1986. Cyclosporine: Progress, problems and perspectives. Surgical Clin. North Am., 66: 435-49. PMID: 3520887

Wuttke, K., C. Streffer and W.U. Muller, 1993. Radiation induced micronuclei in subpopulations of human lymphocytes. Mutat. Res., 286: 181-8. DOI: 10.1016/0027-5107(93)90182-F

Zhang, H.M., J.S. Nie, X. Li and Q. Niu, 2012. Characteristic analysis of peripheral blood mononuclear cell apoptosis in coke oven workers. J. Occupat. Health, 54: 44-50. DOI: 10.1539/joh.11-0155-OA