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ORIGINAL ARTICLE

Radioiodine Therapy Induced Cytotoxicity in Patients with Differentiated Thyroid Carcinoma

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Cytological radiation damage to lymphocytes can result in augmentation of cells with micronuclei. In this study we investigated cytological radiation damage to peripheral blood lymphocytes using the the micronuclei assay (MNA) method, considering the value of ^{131}I in diagnostic and therapeutic nuclear medicine and high absorbed dose of ^{131}I radioiodine in comparison with gamma emitters and the effect of type of radiation, dose and species on radiosensitivity of patient. At present no similar investigation from Iran has been reported evaluating the cytological radiotoxicity of therapeutic radiotracers such as ^{131}I.

Materials and Methods: We studied 22 patients with differential thyroid carcinoma referred for treatment with 100 or 150 mci ^{131}I. Peripheral lymphocytes were harvested and isolated by a cytological method and assayed for frequency of micronuclei as a marker of cytological radiotoxicity before and one week after treatment.

Results: The means for micronuclei per one hundred binuclear lymphocytes were 6. 3±2.2 before treatment and 9. 6±3.1 after treatment. These differences in the number of micronuclei was statistically significant (p<0. 05).

Conclusions: High doses of radioiodine therapy used after surgery in differentiated thyroid carcinoma can increase micronuclei among peripheral lymphocytes as an indirect marker of chromosomal aberrations and cytotoxic radiation damage.

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Introduction

In medical applications of ionizing radiation including radiology, nuclear medicine and CT scan, both the advantages such as identification and cure of patients as well as the dangers of radiation must be considered. Radioactive ^{131}I is one of the radioactive materials applied in nuclear medicine such as identification and cure of patients; The dose of applied ^{131}I for identification is about 5 miliCuri, but that used for therapy is approx 20 to 250 milicuri or higher[1]; this is used for treatment of thyroid cancers at doses of over than 100 milicuri and for treating thyroid hyperactivity at lower doses. One of main differences between radioactive ^{131}I and other radiotracers applied in nuclear medicine is the type of radiation; it is a Beta emitter which has more biological effects than Gamma emitters[1], one of the effects of Beta radiation being the rate of chromosomal aberrations and disorders evaluated by different methods.[1] Given the high number of patients who use the radioactive iodine method annually and the fact that there have been no

studies conducted in Iran on the biological effects of radioactive materials, makes this study so important. One of the harmful environmental effects of medical applications of ionizing radiations is the resulting numeral and structural chromosomal disorders in patients; the rate of structural disorder is evaluated by different methods such as micronuclei and FISH[1]; evaluation of the structural disorder rate has been used as a standard method to determine radioactive sensitivity.[1]

There have been many dosimetry studies to evaluate the rate of radiation in bone and bone marrow after radioiodine therapy[2], but there have been less reports on its relationship with chromosomal damage caused by radioiodine.[3,4]

In one study, cytological damages caused by ^{131}I were evaluated by the cytological method micronuclei assay (MNA).[5] Micronucleis are round intra cytoplasmic bodies which are composed of part of or all the chromosome. These are a result of a chromosomal break or the separating of a chromosome from spindle strings.[5] Counting the number of lymphocyte cells including the micronucleus showed that there is different radaiosensitivity in human groups; no comprehensive studies are documented, evaluating the effect of high doses of ^{131}I. Weral et al, reported that evaluation of peripheral blood lymphocytes by MNA may be a useful method for evaluating the cytotoxical radiation damages;[6] this evaluation is utilized to study different reactions to radiation. The biologic effect of radiation is begun at the time of injection but the Watanoba et al study reported one week after administration of ^{131}I as being the appropriate time for evaluating radiological cellular damages.[5]

Given that peripheral blood lymphocytes are more sensitive to radiation and since chromosomal damages of lymphocytes due to radiation can increase the number of cells including micronuclei,[7,8] in this study, we applied the MNA method to evaluate cytological damage of peripheral lymphocyte cells after ^{131}I therapy.

Materials and Methods

Between June and October 2006, 22 patients with thyroid cancer who were referred to the nuclear medicine department of Taleghani hospital were investigated. In all patients, thyroid cancer had improved pathologically and all of them were operated before radioiodine administration and on the other hand, TSH of all patients was higher than 30 UI/mL; 8 patients, received 100 Mci ^{131}I and 14 patients, 150 Mci respectively. Blood samples were taken before and one week after radioiodone administration, and were examined promptly as follows:

1**) Separating peripheral blood mononucleus cells**: This was done by a concentration gradient which is the same as the Faikol Hepak. First the blood was diluted and transferred by pastor pipettes. Pipes were centrifuged with round of 400 g for 20 minutes.

2) **Lymphoblast transformation test**: After centrifuging, the region of mononucleus cell collection between the Faikol layer and sample plasma was suctioned with a sterilized pastor pipette; this was gathered and transferred to the washing pipe, for cells to be prepared for culture and then be resuspended in 1 milliliter of complete culture media. Cells are counted by Neobar lam, and the 20 landa of PHA solution is applied for every 1 million lymphocyte cells and added to cells as well as cytoclasin B (6 ugr/ml) and the suspension is transferred to a 5% Co_2 incubator to be incubated for 48 hours.

3) **Investigating the condition of cultured cells for MN and other cellular indices**: At the end of incubation, cell culture pipes were removed and centrifuged for 2 minutes, when the cell condition is considered in viability and duplication. A semithick part of cells is provided which will be painted by the Gimsa method. Lams are put in special box after drying to perform cellular studies.

4) **Microscopic evaluation of samples**: Lams were examined using a light microscope and immersion method and the percentage of binuclear cells and numbers of binuc-

lear lymphocytes were determined. The procedure is repeated for apoptotic cells, differentiated according to the 4 following signs: Crumpling of the cell in plasma and membrane volume; pressing and separating of cell nuclei; changes of plasma membrane and its components into different vesicles; non-exclusion of materials inside the cell to out. Data were analyzed using SPSS software and P value less than 0.05 was considered statistically significant.

Results

Figure 1 shows micronucleus radiation after in lymphocytes. Average number of micronuclei in every 100 binuclear lymphocytes was 6.3±2.2 and 9.6±3.1, before and after treatment with radioiodine respectively, $p<0.05$. In 8 patients, who received 100 mci of radioiodine, these values were 6.5±2.5 and 9.8±3.4 before and after therapy and in 14, these were 6.0±2.7 and 6.0±1.5 respectively, before and after treatment. These differences in both groups were significant, $p<0.05$.

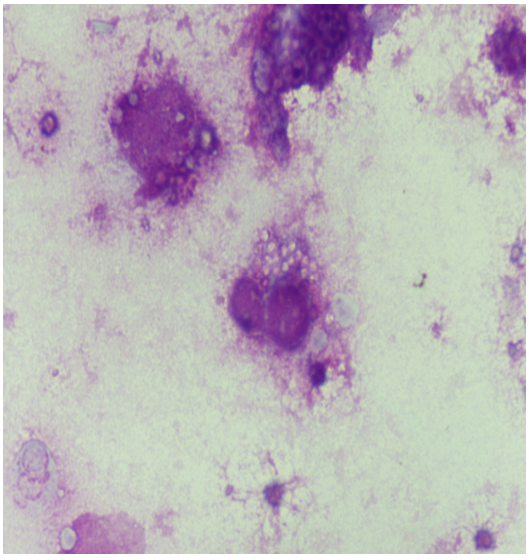

Fig. 1. Binuclear lymphocytes containing micronucleous bodies, secondary to radiation

Average percentages of increase for the 100 and 150 milicouri administered groups were 50.7 and 53.3%, showing no meaningful difference between amount of peripheral blood lymphocytes and apoptotic cells.

Discussion

There have been few studies documented about relationship between applied radiotracers and their potential biological effects. According to studies of thyroid cancer patients, the chromosomal disorders of these individuals and external radiation were compared and findings showed that chromosome disorders in such patients were more than in those treated without radioiodine.[12] In another study, the relationship between chromosome disorders and Alfa radiations was evaluated, and a direct relationship between beam amounts and translocated chromosomes was reported. The Reiners and Sonnenschein study, investigating the rate of chromosome disorders of blood lymphocytes found a linear relationship between beam dose and rate of chromosomal disorders, which was less than the amount related to fission Neutrons.[14] One study using the FISH method, examined chromosome disorders caused by Gamma radiations. [15]

Ionized radiation can damage DNA directly and indirectly and causes structural disorders of chromosomes; to do so, there should be 1 or more bi-chain breakage in DNA[16]. Of the disorders mentioned, all are unstable chromosome disorders, except for translocation because the cell including this type of chromosome goes toward apoptosis because of P53 activation. [17]

In our study, the MNA method has been applied for evaluating cytotoxic damages of radiation to environmental blood lymphocyte cells. Previous studies shows that the frequency rate of micronucleus in lymphocytes was higher compared with the control group prior to treatment.[18] Wuttke and his colleagues found that peripheral blood lymphocytes, compared to other blood cells, have more sensitivity to ionized or radioactive radiations and they also showed that high sensi-

tivity of peripheral blood lymphocytes to radioactive beams may be evaluated.[19] According to study reports on safety cells in apoptosis, peripheral blood lymphocytes cells have more sensitivity to radiation compared with the other blood subdivisions. This rate of sensitivity to lymphocyte radiation has been examined by radioactive materials.[20,21] In radioiodine therapy for thyroid cancer patients, the rate of radiation dose in different people varies being 0.32 Gy, on an averge.[22]

Watanoba examined 3 groups of lymphocytes B, T, NK, using PHA and found that this has most effect in lymphocyte B cells mentioned.[22]

The above findings show that evaluation of lymphocyte cells has high sensitivity to evaluate radiation damage and that amount of damage in lymphocyte cells is more than in other cells.[22] making the examination of cellular damage in lymphocyte cells by the MN method more valuable than in other blood cells.

In our study, there was a meaningful increase of binuclear cells including micronuclei and this could cause chromosomeal disorders because of internal radiation.

In a study, done by Gutiérrez et al, the number of lymphocyte cells including micronuclei was evaluated one week after therapy. Findings show coupling of cells, including micronuclei, to be as much as 2.4%.[23]

In a similar done study by Watanoba et al, on 25 patients treated by radioiodine,[24] the average increase of number of lymphocytes including micronuclei before and after therapy was 3.6 times that of our study and this shows that other factors such as race influence sensitivity to radiation; average percentage of increase in amount of micronuclei after and before therapy was 50.7 and 53.3% respectively, indicating no significant linear relation between increased number of micronuclei and radioactive iodine dose. It should be noted that most cells in which binuclear cells include micronuclei will face cellular death or apoptosis. These findings show possibility of chromosome disorders.

Although these results show radiotoxicity caused by radioiodine therapy, more studies on thyroid cancer patient are needed. The studies of chromosomal translocation rate using accurate genetic methods such as FISH are complementary for comprehensive informatin of micronucleous method as a marker of cytological radiation damage.

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