Original Article

Micronucleus frequencies in groups receiving external or internal radiation

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ABSTRACT

Objective: In the current study, we aimed to explore whether there is alteration between pre- and post-treatment micronucleus (MN) frequencies induced by internal and external ionizing radiation. **Materials and Methods:** The study enrolled a total of 67 patients including patients admitted to our hospital for treatment of hyperthyroidism (n = 17), scanning with low-dose I-131 (n = 15), and ablative therapy with high-dose I-131 (n = 15) at Department of Nuclear Medicine as well as patients with different diagnoses receiving external radiotherapy with various doses and durations at Department of Radiation Oncology (n = 20). Thirty-two patients who received radioactive iodine and returned for a follow-up visit at 1 month. **Results:** Considering both pre- and post-treatment MN frequencies of each group, lowest MN frequencies were detected for patients undergoing screening with low-dose I-131, and highest MN frequencies were found in radiotherapy patients. Comparison of pre- and post-treatment MN frequencies among hyperthyroidism, when pre- and post-treatment MN frequencies compared among hyperthyroidism, I-131 whole body scanning, ablation, and radiotherapy patient groups differences between MN frequencies were significant for each group ($P < 0.05$). **Conclusion:** Our study showed that MN analysis might be of value in determining chromosome damage that could potentially occur in patients exposed to internal and external radiation.

Keywords: Ionizing radiation, micronucleus, radioiodine

INTRODUCTION

Protecting the health of current and future generations is of paramount importance when using the radiation for the benefit of humanity. Thus, it is essential to have knowledge about the level of radiation that individuals are exposed to.

Dose-related chromosome damage can be demonstrated by micronucleus (MN) analysis, one of the biological dosimetry methods. Micronuclei form during mitotic division of the cell and contain whole chromosomal or acentric chromosomal fragments which are not incorporated into the cell nucleus. An increased number of micronuclei is considered an indirect indicator of numerical and structural chromosomal aberrations in the cells induced by several agents and MN analysis has become a widely used technique for this purpose.[1,2]

In the current study, we aimed to explore whether there is alteration between pre- and post-treatment MN frequencies induced by internal and external ionizing radiation.

Examples of similar studies published in the literature including Gutiérrez *et al.*[3] In this study, they used MN method to examine the cytogenetic damage following I-131 treatment in patients with hyperthyroidism and thyroid cancer and showed a dose-proportional significant posttreatment increase in the number of micronuclei.

In a separate study, Erselcan *et al.* evaluated chromosome damage using "sister chromatid exchange" method at baseline and in acute and late phases among 15 patients receiving various doses of

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I-131 for thyrotoxicosis or ablative therapy. They found a marked increase in the chromosome damage in the short-term and partial improvement achieved within 6 months following the treatment.[4]

Also, Jagetia *et al.* performed an MN analysis before fractionated treatment, in the middle and at the end of the treatment in fifty patients undergoing radiotherapy. They found a more than 2-fold increase in the MN frequency among cancer patients in comparison to the untreated healthy volunteers.[5]

In this study, we aimed to determine pre- and post-treatment MN frequencies in a group of patients who received radiotherapy for different diagnoses.

In contrast to above-mentioned studies, the current study is original in terms of cytogenetic effects of internal and external ionizing radiation were evaluated together.

MATERIALS AND METHODS

The study enrolled a total of 67 patients including patients admitted to our hospital for treatment of hyperthyroidism, scanning with low-dose I-131, and ablative therapy with high-dose I-131 at Department of Nuclear Medicine as well as patients with different diagnoses receiving external radiotherapy with various doses and durations at Department of Radiation Oncology.

Female patients with possibility of pregnancy who were previously exposed to radiotherapy and those receiving concomitant chemotherapy in addition to radiotherapy were excluded from the study.

The mean age of enrolled patients ($n = 67$) was 50.8 ± 15.6 years (minimum: 23, maximum: 83); 49 (73.1%) were female and 18 (26.9%) were male.

Peripheral venous blood samples were drawn into blood collection tubes containing 0.1–0.2 ml heparin using 5 ml sterile injectors before and after treatment for all 67 patients and for 32 patients who received radioactive iodine and returned for a follow-up visit at 1 month. Blood specimens immediately inoculated into previously prepared culture media to obtain lymphocyte cell culture. Preparations were performed according to the procedures as described by Fenech and Morley;[6] for each culture, the number of micronuclei counted per 1000 binucleated lymphocytes was recorded as per Fenech's MN identification criteria.[7]

Statistical method

Study data were analyzed using Statistical Packages for the Social Sciences (version 14.0) software package (IBM, NY, USA). Wilcoxon test was used to compare pre- and post-treatment MN values for all groups and the differences between pretreatment, posttreatment, and at 1 month MN frequencies were investigated using Friedman test for all groups.

RESULTS

Of the study patients, 17 had hyperthyroidism (25.4%), 15 underwent I-131 whole body scanning (22.4%), 15 received ablative therapy (22.4%), and 20 were exposed to radiotherapy (29.9%).

I-131 was orally administered at a dose of 11.7 ± 3 mCi (431.5 ± 112.3 MBq) to patients with hyperthyroidism, at a dose of 5.0 ± 0.9 mCi (185.2 ± 22.1 MBq) to patients undergoing screening with low-dose I-131, and 117.5 ± 18.8 mCi (4347.2 ± 693.8 MBq) to patients receiving ablative therapy with high-dose I-131.

Considering both pre- and post-treatment MN frequencies of each group, lowest MN frequencies were detected for patients undergoing screening with low-dose I-131, and highest MN frequencies were found in radiotherapy patients.

Mean pre- and post-treatment MN frequencies are summarized in Table 1 for each group.

Comparison of pretreatment versus posttreatment MN frequencies among hyperthyroidism, when pre- and post-treatment MN frequencies compared among hyperthyroidism, I-131 whole body scanning, ablation, and radiotherapy patient groups differences between MN frequencies were significant for each group ($P < 0.05$) [Figures 1 and 2a].

Although a significant difference was found between pre- and post-treatment MN levels in the subgroup of 32 patients from hyperthyroidism, screening, and ablation groups with follow-up blood sample available at 1 month ($P < 0.05$), there was no significant difference between hyperthyroidism and screening groups with respect to changes in posttreatment MN frequencies compared to MN frequencies obtained at 1-month follow-up visit ($P > 0.05$). For ablative therapy patients, both pretreatment to posttreatment difference and difference between posttreatment and 1-month follow-up MN frequencies were statistically significant ($P < 0.001$) [Figure 2b].

Table 2 summarizes mean pretreatment, posttreatment, and 1-month MN frequencies for 32 patients whose blood samples could be collected at 1-month follow-up visit.

Table 1: Comparison of pretreatment micronucleus and posttreatment micronucleus frequencies in patient groups

Groups	Mean±SD	
	Pretreatment MN	Posttreatment MN
Hyperthyroid	2.5±5.5	10±12.6*
Scan	0.6±2.3	5.8±8.3*
Ablation	1.9±5.1	28.5±17*
Radiotherapy	4.7±7.1	33.1±10.8*

*P<0.05. MN: Micronucleus, SD: Standard deviation

DISCUSSION

In recent years, much attention has been focused on the effects of ionizing radiation on chromosomes resulting from increased use of various types of therapeutic radiopharmaceuticals and stochastic effects of radiation are discussed.

Table 2: Comparison of pretreatment, posttreatment, and 1st month control micronucleus frequencies in each patient groups

Groups	Mean±SD		
	Pretreatment MN	Posttreatment MN	1 month MN
Hyperthyroid (*n*=10)	2.6±5.5	12.5±11.5*	9.4±9.3
Sca (*n*=12)	0.8±2.6	6.4±8.9*	2.8±5.2
Ablation (*n*=10)	1.3±4.1	24.6±17*	12.5±10.6*

*P<0.05. MN: Micronucleus, SD: Standard deviation

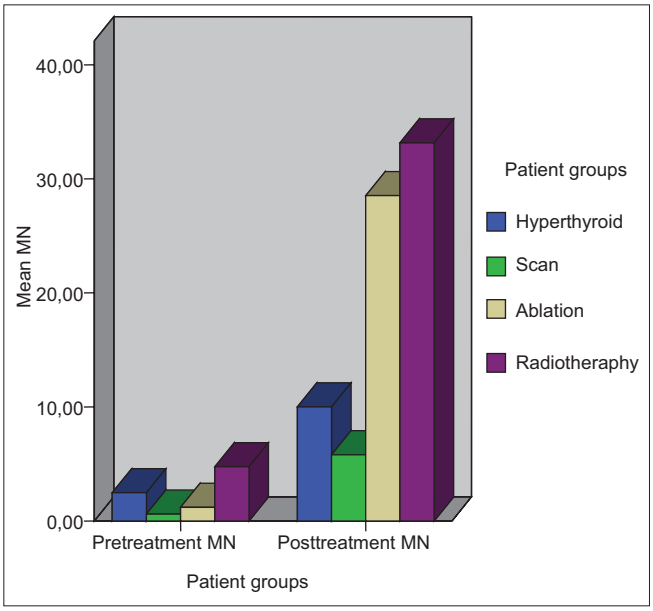

Figure 1: The bar chart showing the micronucleus frequencies in pretreatment and posttreatment groups

Although there is no clear epidemiological evidence indicating an increase in the incidence of late effects of radiopharmaceuticals used in nuclear medicine, there is a general consensus that radiation at lower doses may cause genetic effects in living organisms that may even occur many years after the exposure. The effects of radiation, particularly at lower doses are being investigated by examining the chromosomes which encode all the genetic material of the living organisms.[8]

The use of MN analysis for determination of biological damages has gained a wide acceptance in demonstrating total biological effect, in other words in detecting cytogenetic damage in case of exposure to chemical and physical mutagens because of several advantages including its ease of use compared to chromosome analysis, allowing MN scoring in a larger number of cells, and achieving more statistically significant results.[9-11]

In a study involving workers at Çekmece Nuclear Research and Training Center, an increase in MN frequency was determined which was dependent on the dose of radiation. Relevant *in vitro* studies have found a direct relation between the number of micronuclei formed in lymphocytes that were exposed to radiation and radiation dose and showed that a quantitative relationship existed between the dose of ionizing radiation and MN frequency which can be used for purposes of biological dosimetry.[12,13]

In the current study, a difference was observed between groups with regard to pretreatment MN frequency. MN frequency was high among radiotherapy patients. High-MN frequency was an expected finding because some of the patients with cancer have been previously exposed to chemotherapy. The mean age of hyperthyroid patients was greater compared to all other groups, and this may explain the highest baseline MN frequency in the group receiving radioiodine.

In our study, we first determined the MN frequency of our patients before treatment to optimize individual variations while evaluating posttreatment changes in MN frequencies and to look

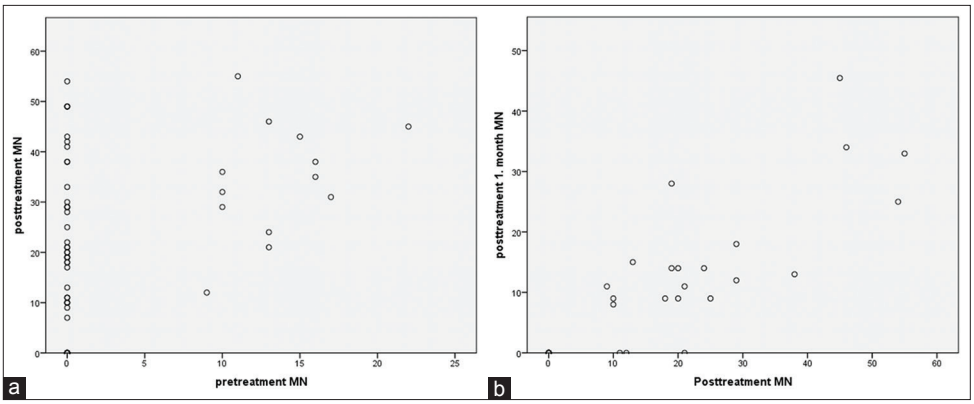

Figure 2: (a) Scatter diagram of pretreatment micronucleus and posttreatment micronucleus frequencies. (b) Scatter diagram of posttreatment micronucleus and posttreatment 1. Month micronucleus frequencies

at the effects of ionizing radiation only by making pretreatment–posttreatment comparisons.

In a study by Peace and Succop reported that changes in MN frequency showed a wide variation in a normal population consisting of different groups.[13]

Huber *et al.* examined the response of unstimulated peripheral lymphocytes to a single dose of 3 Gy of ^{137}Cs gamma rays in blood samples from thirty donors by a conventional MN analysis and from 14 donors by the cytokinesis-block method. Significant interindividual variations could be detected for the baseline frequency and for induced frequency of micronuclei. They also investigated the relation between age and MN frequency and found a 3.4% ± 1.3% increase in the MN number per year. They concluded that if cell proliferation kinetics is reliably taken into account, the MN assay could be helpful for diagnosing potential radiosensitive individuals.[14]

In a study in elderly people, MN frequency was shown to increase with advancing age among females and the identification of chromosomes that forming micronuclei was determined using a fluorescence *in situ* hybridization technique. The authors demonstrated that X chromosome was involved in MN formation more extensively compared to autosomal chromosomes. Loss of X chromosome was also confirmed by karyotype analysis of monosomic cells, and a direct relation was found between MN formation and chromosomal aberrations detected by karyotype analyses.[15]

Fenech *et al.* have studied MN assay for long years and to examine the effects of different laboratory protocols, scoring criteria, and lifestyle on baseline MN frequency to guide optimization of the procedure. In this study, criteria described by Fenech were adopted for scoring of micronuclei.[16-18]

In our study, we determined a statistically significant increase in posttreatment MN frequency in each patient group. This finding is consistent with many studies in the literature which reported increased MN frequencies following treatment with radioactive iodine.[3,9,19] Considering with other relevant reports, our findings support the result that MN assay is a sensitive method for demonstrating chromosome damage induced by internal and low-linear energy transfer (low-LET) ionizing radiation.

Popova *et al.* showed an increase in MN frequency over a period of 1 month in patients undergoing radioiodine therapy for differentiated thyroid carcinoma and suggested that it could be used to demonstrate genotoxic activity.[19]

Federico *et al.* conducted an MN analysis in 11 adolescent patients with differentiated thyroid carcinoma aged 14.8 ± 3.1 years who received ablative therapy with I-131 (1.11–4.44 GBq) and evaluated expression of some genes involved in DNA repair or the apoptosis pathways.[20] For the analyses, blood samples were obtained from each patient before and 24 h and 48 h after treatment. They emphasized that administration of I-131 may induce early genome damage indicated by the presence of micronuclei in stimulated T lymphocytes and/or activation of some genes involved in DNA repair or in the apoptosis pathways. They explained that since in patients with total thyroidectomy, the maximum serum level of I-131 was reached within 2.5 h from the end of the therapy and its half-life in blood varied from 12 to 48 h, so they performed a gene expression analysis at the same sampling times at which the potential genotoxic effects were investigated in cultured lymphocytes. No considerable change was found in MN frequency at 24 h and 48 h compared to baseline. Nine out of 11 patients showed a marked increase in the expression of genes involved in DNA repair and apoptosis pathways at 24 h after radioiodine therapy and a reduction in the expression of the previously altered genes at 48 h compared to 24 h. They did not show chromosome damage within 48 h after the treatment and suggested that this may be due to activation of the cell machinery that maintains the integrity of the genome to prevent harmful double-strand breaks from progressing to chromosome mutations, either by repairing the lesions or by apoptosis.

Taking into account previous studies published in the literature, we performed posttreatment blood sampling about 1 week after administration of radioactive iodine therapy. Federico *et al.* suggested that the negative results, they obtained in their study were associated with early blood sampling.[20] They reported that I-131-induced damage generally occurred 1 week after the treatment and was maintained over a long period of time and suggested that damage mechanism could occur due to the presence of circulating lipid peroxidation products and cytokines such as tumor necrosis factor-α, and an increased release into the plasma of superoxide radicals.

In our study, an additional MN analysis was performed 1 month after treatment to monitor short-term changes in the MN frequency in a subset of patients (n = 32 of 47 patients) who received I-131. There was not a marked change in the MN frequency at 1 month versus posttreatment MN frequency in patients with hyperthyroidism and screening patients. However, although a statistically significant decrease was observed in the MN frequency among ablative therapy patients at 1 month, it was still higher compared to baseline.

There are studies in the literature that monitored MN frequency over long periods of time. Gutiérrez *et al.* monitored MN frequency over a period of 1 year following completion of therapy in hyperthyroid patients and patients with thyroid cancer.[3] In the study group comprising 47 hyperthyroid and 39 thyroid cancer patients, the micronuclei frequency was determined before I-131 therapy and 1 week, 1 month, and 3 months after therapy. Furthermore, MN analysis was repeated 6 months after treatment for 17 hyperthyroid patients and at 6 months and 1 year for four patients with thyroid cancer. In the hyperthyroidism group, a significant increase in the MN frequency was found posttreatment with a gradual increase for

3 months followed by persistently high values through 6. Month in the thyroid cancer group, a 2-fold increase in the MN frequency was seen 1 week after therapy and although this value decreased over time, the MN frequency obtained 1 year after I-131 therapy remained higher than the previous values.

M'Kacher *et al.* followed 50 thyroid cancer patients over a period of 2 years and found a decrease in the frequency of chromosomal aberrations at 3 months versus posttreatment 4th day.[21] They also observed that the number of chromosomal aberrations detected at 2 years posttreatment was still greater compared to baseline. They suggested that the lower frequency of chromosomal aberrations at 3 months might be attributed to the decrease in lymphocyte counts after I-131 administration and to make up for the decrease in lymphocyte counts, either the stem cells might accelerate their mitotic activity or lymphocytes from other organs such as the spleen, which are less exposed to irradiation may enter circulation.

In their respective studies, Gutiérrez *et al.* and M'Kacher *et al.* underscored that mean half-life of the lymphocytes in the peripheral circulation was 3 years and that chromosome damage could be detected within this time period.[3,21] This supports the feasibility of using MN analysis at a late period after radiation accidents. On the other hand, Watanabe *et al.* reported that the level of MN frequency returned to baseline 1 year after treatment in a study involving 25 thyroid cancer patients.[22]

Although the follow-up period of 1 month employed in our study was relatively short, the reduced MN frequency observed at 1 month after administration of high-dose I-131 suggests that "the DNA repair process begins faster after usage of high doses compared to lower doses." We believe that further studies with a larger patient group series and longer follow-up are needed to corroborate this finding.

In a study in 22 thyroid cancer patients treated with 3.7 GBq I-131, Watanabe *et al.* reported that compared with the MN analysis of all lymphocytes, an MN analysis performed in B lymphocytes may more sensitively detect cytogenetic damage.[23] Several *in vitro* studies have supported their findings. Wuttke *et al.*[24] showed that B-lymphocytes were more radiosensitive compared to T-lymphocytes and Vral *et al.*[25] reported that B-lymphocytes exhibited a highly radiosensitive behavior in MN formation *in vitro*. They also showed that B-lymphocytes were more radiosensitive than other lymphocyte subpopulations with regard to immune function and apoptosis. However, Streffer *et al.* suggested that MN analysis performed in B-lymphocytes was less sensitive than conventional procedures in a dose range of 0.3–5 Gy.[26] Taken together, it is obvious that these studies have reported contradictory findings. We were not able to perform an MN analysis in isolated B-lymphocytes due to lack of equipment.

In a study in cancer patients, measurements during curative radiotherapy have shown a dose-related increase in MN frequency among all patients.[27]

Jagetia *et al.* performed an MN analysis in blood samples obtained from 27 patients with various types of cancer before administration of scheduled radiotherapy treatment with 36–66 Gy ^{60}Co for 5–6 weeks.[5] In these patients, they found that pretreatment MN frequency was significantly higher compared to the untreated healthy volunteers. During the middle of the radiotherapy, a 2 or >2-fold increase was observed in the MN frequency in most of the patients when compared with the concurrent pretreatment samples. Immediately after the completion of treatment, the MN frequency further increased, and this increase was significantly higher than that of pre- and mid-treatment samples. The authors suggested that increased MN frequency might be related to altered immune status or impaired DNA repair process.

Catena *et al.* investigated the relationship between lymphocyte decrease and cytogenetic response in individual patients undergoing radiotherapy.[28] Initially, they identified that decrease in lymphocytes varied individually. They found that the relationship between the lymphocyte decrease ratio and whole-body dose is associated with the radiosensitivity of individual lymphocyte pool. At equivalent doses, MN values obtained by *in vivo* irradiation were lower than the *in vitro* values. The authors suggested that this might be related to cytogenetic recovery factor.

CONCLUSION

Our study showed that MN analysis might be of value in determining chromosome damage that could potentially occur in patients exposed to internal and external radiation. We believe that further studies in larger patient series are needed to establish the long-term effects of irradiation.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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