Original Article

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Cytogenetic Studies of Chromosome Abnormalities in Thyroid Cancer Patients Before and After Iodine-131 Treatment

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ABSTRACT

Objective: Thyroid cancer is among the most common endocrine malignancies. Initial management consists surgery and ablation. I-131,which is used to damage surgically unremoved thyroid tissue or treat metastases, is a source for ionizing radiation (IR), also generates potential mutagenic and carcinogenic effects.The purpose of research is to investigate the damage on chromosomes at the I-131 treatment of thyroid cancer patients exposed to the same dose of genotoxic agent.

Methods: Peripheral blood of 20 patients and 20 healthy control individuals were used in study. Materials were collected from patients before the I-131 treatment and three times on the 15th day, the first and second month after the I-131 treatment. All chromosome abnormalities, spontaneous chromosomal breakages, and sister chromatid exchange (SCE) were analyzed by cytogenetic methods in the samples.

Results: The results obtained from peripheral blood of patiens who received ablation therapy revealed no significant increase for chromosome breakages, chromosome abnormalities, and SCE. Karyotyped metaphase spreads showed none of the structural and numerical chromosome abnormalities.The results of chromosome breakages and SCE have been statistically assessed by Aspin Welch test and did not differ from the control group.The t values of this variability were found to be -1.001,-1.654, respectively, and $p>0.05$.

Conclusion: Our results demonstrate that applied therapeutic clinical dose does not show cytogenetic abnormalities risks of the early period in patients. I-131 treatment is a good option for the treatment of thyroid cancer,but also we recommend that physicians should perform long term follow-up examinations for IR exposed patients and prevent possible complications.

Keywords: Thyroid neoplasms, cytogenetics, ionizing radiation, chromosome aberrations

Introduction

Thyroid cancer is the most common type of cancer among malignant tumors of the endocrine system and accounts for 1-2% of all cancers (1). Etiological factor could not identified in most of the patients, while radiation exposure to the neck region in childhood has been clearly shown as the only factor causing thyroid cancer. In the past the relationship between thyroid cancer and radiation was defined and in subsequent studies, it was demonstrated that radiation exposure to the neck region increases the risk of cancer (2-4).

In the treatment of the disease surgery (total or subtotal thyroidectomy) and in addition, in good differentiated thyroid cancers acoording to risk groups, iodine-131 (I-131) ablation/ treatment is applied. I-131 is used to destroy residue thyroid or tumor tissue or metastatic tumor tissue after surgery (5). I-131 is applied successfully in treatment due to its high affinity to the

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thyroid gland. I-131 is a radioisotope with 8-day half-life which emits beta particles and gamma rays and it is used in treatment of differentiated thyroid cancer as well as in thyroid tissue imaging and in treatment of hyperthyroidism. The basis of I-131 treatment is to establish a cytotoxic effect by ensuring I-131 involvement in the target tissue (6-8). I-131 leads to ionizing radiation (IR) and has the potential to produce mutagenic and carcinogenic effects in the genetic structure of cells (8). Ninety percent of the effect of radiation is considered to be due to beta particles (9). Investigation of the biological damage of I-131 exposure, used for therapeutic purposes in patients with thyroid cancer, still continues to be important in terms of clinical and therapeutic approaches.

Cytogenetic analysis of cell deoxyribonucleic acid (DNA) for detection of damage at chromosome level including sister chromatid exchange (SCE), chromosome abnormality and chromosome breakage analysis are considered as *in vitro* indicators of chromosome instability (10). Chromosome instability is the development of abnormalities in chromosomes due to errors in replication, recombination, DNA repair, chromosome separation or cell cycle checkpoints. Small errors are mostly repaired by DNA repair systems, while severe errors stimulate apoptosis, leading the cell to death. The accumulation of errors in the cell is the first step of carcinogenesis, causing mutations (11).

Because the effects of low levels of IR are difficult to be shown by epidemiological studies, it is preferred to be shown using cytogenetic studies (9). In our study, we aimed to perform cytogenetic analysis to determine the damage on chromosomes caused by the I-131 treatment in patients with thyroid cancer. Peripheral blood samples were taken from the patients with thyroid cancer admitting to the nuclear medicine department of our university hospital for ablation treatment, before the treatment and 15 days, 1 month and 2 months after the treatment of 100 milicurie (mCi) I-131. Blood culture was performed and SCE, chromosome abnormalities and chromose breakage were investigated by cytogenetic methods.

Methods

The peripheral blood samples of 20 patients who were diagnosed as having thyroid cancer, whose ages ranged between 18-72 years and who were decided to undergo I-131 ablation/treatment were used as study materials. None of the patients included in the study were exposed to IR. Blood samples were taken form the patients before the treatment and 15 days, 1 month and 2 months after the treatment. As a control group, peripheral blood of 20 healthy individuals who did not use cigarettes, alcohol and drugs, and who did not have viral disease recently were used. The study was approved by the Ethics Committee of Clinical Research of our university (no: 29619) and the study materials of the cases were obtained after the informed consent form was signed.

All chromosome abnormalities, spontaneous chromosome breakages and SCEs occurring in the samples were investigated with cytogenetic methods. Peripheral blood samples were taken into lithium heparinized tubes (BD Vacutainer® Tubes, BD diagnostics, NJ, USA) and were used for peripheral blood cultures which were administered for 72 hours. For blood culture; 20% fetal bovine serum (FBS), L-glutamine, penicillin, streptomycin and phytohemagglutinin containing RPMI 1640 (Biologic Industries, CT, USA) media was used. For the SCE method; during peripheral blood culture, 100 µL of solution of 5-Bromo-2'-deoxyuridine (5-BrdU) (Thermo Scientific, MA, USA), which is a thymine analogue, was added to the media. The tubes containing 5-BrdU were preserved in such a way that no light was emitted during and after the process. In accordance with the standard technique, spread slides were prepared from the cultured cells and were first left in the Hoechst solution and then shaken with McBouline solution and purified for SCE staining after the hypotonic shock and fixation phase. The slides were then placed in glass petri dishes and completely covered with McBouline solution and kept under UV lamb for 1 hour. The slides were then washed with buffer solutions and stained with 10% Giemsa stain (Figure 1) (12). For the analysis of chromosomal abnormalities; post-culture samples were spread to slides in accordance with conventional cytogenetic technique, aged at 90°C, and then stained (13). In the analysis, karyotyping was performed from at least 20 metaphase sites for

Figure 1. Microscopic imaging of sister chromatid exchange observed in metaphase plate of case 20 (SCE observed chromosomes are shown with a red arrow)

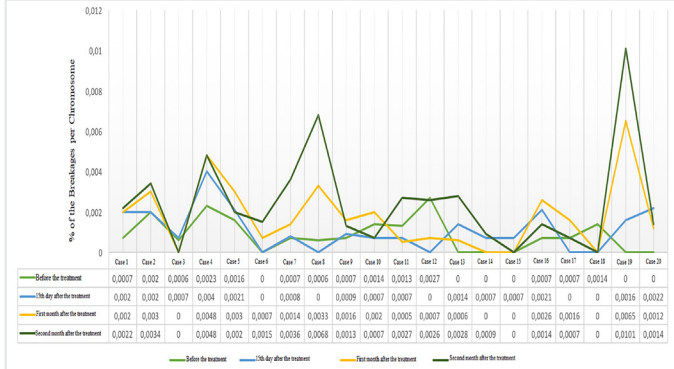

Figure 2. Change in chromosome breakage before and after treatment during follow-up in patients

each case with the investigated band level of at least 400 bands. The karyotyping was based on ISCN 2013 (An International System for Human Cytogenetic Nomenclature) (14). For the examination of chromosomal breakage, the preparations spread with solid staining method and dried were stained with 20% Giemsa (Merck, Darmstadt, Germany). All preparations that were ready for imaging after staining were examined with light microscopy (Nikon Eclipse E600, Nikon, Tokyo, Japan), and the fields in the preparations were scanned with the X10 objective lens and were analyzed with the X100 objective lens.

Statistical Analysis

Statistical analysis was done using the Statistical Package for the Social Sciences Statistics Versiyon 17.0 (SPSS Inc., IL, USA).

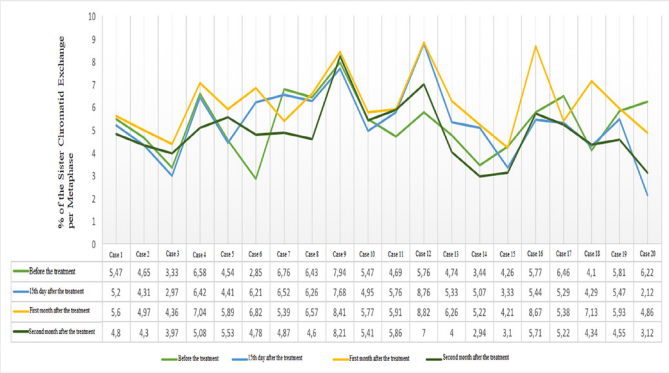

Figure 3. Change in sister chromatid exchange before and after treatment during follow-up in patients

Table 1. Demograpic data and karyotypes detected during follow-up of the patients

	Age/ Gender	Karyotype
Case 1	43, F	46, XX
Case 2	62, F	46, XX
Case 3	18, F	46, XX
Case 4	40, F	46, XX
Case 5	72, M	46, XY
Case 6	42, F	46, XX
Case 7	43, F	46, XX
Case 8	52, F	46, XX
Case 9	44, F	46, XX
Case 10	42, F	46, XX
Case 11	63, F	46, XX
Case 12	38, F	46, XX
Case 13	41, F	46, XX
Case 14	33, M	46, XY
Case 15	62, M	46, XY
Case 16	24, F	46, XX
Case 17	42, F	46, XX
Case 18	44, F	46, XX
Case 19	52, F	46, XX
Case 20	35, F	46, XX

The data analyzed were expressed as mean±standard error. At the end of the investigations, the chromosomal breakage rates and the results of the SCE were evaluated using the Aspin Welch test. The t values of variability of chromosome breakages and SCEs in the study group compared with the control group were -1.001 and -1.654, respectively. The p value was >0.05.

Results

Demographic data and the karyotypes of the patients were shown in Table 1. Chromosome breakage and SCE findings before I-131 treatment and 15 days, 1 month and 2 months after I-131 treatment and changes in them during process were shown in Figure 2 and 3. Of 20 patients included in the study, the mean age was 44.6 years and 85% of the them (n=17) was female and 15% (n=3) was male. Of 17 females, 35% reported smoking 3-4 cigarettes a day and 33% of 3 males smoked one packet a day. In this study, patients who received 10 mCi I-131 treatment were evaluated and no evidence of abnormality was observed in these patients in terms of cytogenetic analysis during the follow-up. The results of cytogenetic analyses and the clinical and demographic data of the patients were evaluated together. The mean chromosome breakage percentage was 0.0009 in the samples studied before the treatment, and the percentages were 0.0012, 0.0018 and 0.0024, respectively in the samples studies 15 days, 1 month and 2 months after the treatment. Although the percentage of chromosome breakage in patients showed an increase after treatment, it was not statistically significant when compared with control group. The mean percentage before the treatment was 5.26 in the samples studied, and the mean percentages of SCE in the samples 15 days, 1 month and 2 months after the treatment were 5.29, 6.16 and 4.87, respectively. There was no difference between SCE before and after the treatment in the patients. There was no significant difference between the patients treated with I-131 and the control group in terms of SCE frequency. During the follow-up period, no chromosomal abnormality was observed in karyotyped metaphase sites in terms of numeric and structure. There was no difference between the patient and control groups in terms of chromosome breakage and SCE.

Discussion

As a result of the gradual accumulation of many mutations in a normal cell, instability of the genome occurs. IR; by interacting with intracellular macromolecules and changing cellular metabolism; is a powerful stimulant of instability in cell DNA, indirectly causing oxidation of free radicals and directly causing damages such as base changes and DNA single/double chain breaks in cell (15,16). The examination of chromosomal breakages and SCE, which are indicators of chromosome instability, by various methods are the most effective methods used to determine the damage to cells (10). The loss of genomic stability resulting from gene rearrangement, leading to the formation of different types of cancer and at the same time, increasing the risk of secondary cancers seen after the treatment of cancer is one of the most important pathological stages (11).

I-131 has been used in medical field for diagnosis and treatment since 1942 (17). The benefit of I-131, especially for thyroid cancer patients, is undisputable. Also, it is easy and inexpensive to apply this method which makes it even more convenient. However, during I-131 degradation, radiated gamma rays and beta particles may be harmful to normal cells and tissues due to absorption.

In previous studies, blood samples of patients with thyroid cancer treated with I-131 were reported to show temporary leukopenia, anemia and thrombocytopenia, and persistent cytopenia was observed in patients exposed to high doses of I-131 (18-20). Also, a risk of cancer in patients who exposed to low dose IR for diagnosis and treatment was reported and development of acute and chronic myeloid leukemia was reported in patients who were exposed to IR for a long time. In patients receiving high doses (≥800 mCi) IR therapy, development of leukemia have been reported in various studies (21,22). The important chromosomal abnormalities in acute and chronic myeloid leukemia including t(8;21), t(15;17), inv(16), t(9;11), t(6;9) and inv(3) were not observed in this study.

In this study, we aimed to investigate the effect of 100 mCi I-131 treatment on the treatment of patients with thyroid cancer who were exposed to the same dose of genotoxic agents with cytogenetic tests. Since patients receiving treatment were exposed to harmful effects of gamma rays and beta rays directly affecting tissue, analyses were made to determine chromosome damage in peripheral blood before and after treatment. As in our study, the reasons for choosing peripheral blood as material to examine the effects of the IR are that lymphocytes are highly sensitive to the IR, circulate through the entire body and are numerous, it easy to obtain them and short-term culture techniques are easy to apply (9,11).

When the test results obtained from peripheral blood of patients receiving ablation treatment were evaluated, no significant increase was observed in chromosomal breakage, chromosomal abnormalities and SCE. Our findings suggest that this therapeutic dose did not involve the risk of causing cytogenetic abnormalities that might be observed in the early period. On the other hand, since our investigations were conducted in the metaphase phase of the second cell division in peripheral blood culture, it should be considered that IR-related chromosomal abnormalities in the first division might be overlooked. It should be taken into account that the cells that have been damaged after exposure to IR can not divide in cultures, the rate of division may slow down, or the damaged cells may be completely destroyed. All these may limit the actual damage rates of the analyzed cells. Parida et al. investigated chromosome damage in peripheral blood of 74 hyperthyroid patients before and 3 months after I-131 treatment with micronucleus method and did not find statistical significance similar to that of our study. They suggest that cytogenetic damage caused by low dose therapy may be temporary and reversible (23).

Smoking habits, age and gender factors were not found to affect spontaneous chromosomal damage in the cases we examined. Milosevic-Djordjevic et al. and Vrndic et al. compared the frequency of chromosomal instability between the groups in terms of age, sex, smoking habits and the type of cancer, and found no difference (24,25). When the results are evaluated together, it is thought that the chromosome damage in the treated patients with thyroid cancer is related to the I-131 accumulation and not related to the other factors examined.

Another issue to consider is that the sensitivity of the tissues to radiation varies. Several studies have reported that chromosome instability varies according to the amount and the repeat number of radioactive iodine taken. Also, it was shown in studies that genetic predisposition, occupational hazard, and life style of individuals exposed to IR can also affect the damage to the cell (26,27). Seo et al. (28) showed that incidence of leukemia increases with ≥100 mCi IR. Teng et al. suggested that ≥150 mCi IR increases the risk of secondary cancer development (28,29).

Although the modified effect of IR on chromosome and DNA structure is well known, the results are still in mystery. Various studies on atomic bomb victims have shown that increased chromosome abnormalities in affected individuals continue for at least twenty years (30-32). Although there are long-term studies on chromosomal abnormalities of individuals exposed to high doses of radiation, long-term studies on cytogenetic effects of low doses of IR exposure for diagnosis and treatment purposes are limited. Livingston et al. (11) followed up cytogenetic findings in a patients who underwent total thyroidectomy and ablation treatment, at certain intervals by taking peripheral blood for 20 years. The results showed that cytogenetic effects persisted after treatment due to I-131 exposure. The number of micronucleus was 10 times higher 5 years after treatment than the number of micronucleus before treatment (11). In their study published in 2017, they examined the same patient's peripheral blood sample by multicolor fluorescent in situ hybridization and found a higher proportion of reciprocal and non-reciprocal translocations compared with their previous study (33). These findings show the persistent effects of IR. The cause of the abnormalities in the case examined was explained by the long-lasting T-lymphocytes or hematopoietic stem cells being affected by the IR.

Study Limitations

Although our study supports the idea that the effects of IR exposure can be detected, it does not provide sufficient information on long-term results due to the evaluation of a limited number of cases and the short follow-up period.

Conclusion

In our study, DNA damage was analyzed at chromosome level, and there was no statistically significant cytogenetic finding in peripheral blood examinations in the short-term. We recommend that the use of I-131 is a good option in the treatment of thyroid cancer, but when patients are treated with IR, they should be monitored for a long time and the necessary controls should be performed by the physician to prevent possible complications. Cytogenetic methods and advanced analysis can be used to determine potential DNA damage. Based on the data reported

earlier in the literature, we believe that the effects of IR on the cell should be verified with long-term follow-up in larger number of case-control groups.

Ethics

Ethics Committee Approval: İstanbul University Cerrahpaşa Faculty of Medicine, Ethics Committee of Clinical Research/29619.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer Review: Externally peer-reviewed.

Authorship Contributions

Concept: S.Ö., S.A., M.D., A.D., Design: S.Ö., S.A., M.D., A.D., Data Collection or Processing: S.Ö., S.A., Analysis or Interpretation: S.Ö., S.A., M.D., A.D., Literature Search: S.Ö., S.A., Writing: S.Ö., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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