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RESEARCH

Effects of I-131 ablation/metastasis treatment on DNA damage parameters in patients with differentiated thyroid cancer: a pilot study

Diferansiye tiroid kanseri olan hastalarda I-131 ablasyonu/metastaz tedavisinin DNA hasarı parametreleri üzerindeki etkileri: pilot çalışma

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Abstract

Purpose: The purpose of this study was to ascertain how radioactive iodine therapy (RAIT). affected the DNA damage in the lymphocytes of differentiated thyroid cancer (DTC) patients.

Materials and Methods: Our study included 21 DTC patients in total, with a mean age of 45.09±10.02 years. Six (28.6%) of the 21 patients had a low dose of RAIT (≤30 mCi), eleven (52.4%) received a moderate dose of ablation (31 to 100 mCi), and four (%19) received a high dose (>100 mCi). Before, one week, and six months following treatment, venous blood samples were obtained from each patient. DNA damage was evaluated using the COMET assay. For a quantitative analysis of DNA damage, a number of characteristics were assessed, including head length, tail length, head intensity, tail intensity, and tail moment.

Results: Compared to before treatment (23.83±9.82), tail intensity levels were considerably lower after treatment (1st week and 6th months) (18.30±6.48 vs. 18.06±5.58). Following therapy (1st week and 6th months), the head intensity values were statistically substantially higher (81.69±6.48 vs. 81.93±5.58) than they were prior to treatment (76.16±9.82). Six months after therapy, the tail moment was lower than it was before treatment (5.24±2.51 vs. 8.04±3.86).

Conclusion: Treatment has a beneficial effect on peripheral blood cells, as seen by the increase in head intensity and decrease in tail intensity and moment after treatment. By triggering repair mechanisms, RAIT may have activated protective effects on the DNA of lymphocytes.

Keywords: Differentiated thyroid cancer, I-131, radioactive iodine theraphy, DNA damage, COMET assay

Öz

Amaç: Bu çalışmanın amacı, radyoaktif iyot tedavisinin (RAIT) farklılaşmış tiroid kanseri (DTC) hastalarının lenfositlerindeki DNA hasarını nasıl etkilediğini belirlemekti.

Gereç ve Yöntem: Çalışmamıza toplam 21 DTC hastası dahil edildi ve yaş ortalamaları 45.09±10.02 yıldı. 21 hastanın altısı (%28.6) düşük doz RAIT (≤30 mCi) aldı, on biri (%52.4) orta doz ablasyon (31 ila 100 mCi) aldı ve dördü (%19) yüksek doz (>100 mCi) aldı. Tedaviden önce, bir hafta ve altı ay sonra her hastadan venöz kan örnekleri alındı. DNA hasarı COMET testi kullanılarak değerlendirildi. DNA hasarının kantitatif analizi için baş uzunluğu, kuyruk uzunluğu, baş yoğunluğu, kuyruk yoğunluğu ve kuyruk momenti dahil olmak üzere çeşitli parametreler değerlendirildi.

Bulgular: Kuyruk yoğunluğu değerleri tedaviden sonra (1 hafta ve 6 ay) tedavi öncesine (23,83 ± 9,82) kıyasla önemli ölçüde daha düşüktü (18,30 ± 6,48'e karşı 18,06 ± 5,58). Baş yoğunluğu değerleri tedaviden sonra (1. hafta ve 6. ay) (81.69±6.48'e karşı 81.93±5.58) tedavi öncesine (76.16±9.82) göre istatistiksel olarak anlamlı derecede daha yüksekti. Kuyruk momenti tedaviden 6 ay sonra tedavi öncesine göre daha düşüktü (5.24±2.51'e karşı 8.04±3.86).

Sonuç: Tedaviden sonra baş yoğunluğunda artış ve kuyruk yoğunluğunda ve momentinde azalma, tedavinin periferik kan hücreleri üzerinde olumlu bir etkiye sahip olduğunu göstermiştir. RAIT'in onarım mekanizmalarını aktive ederek lenfosit DNA'sı üzerinde koruyucu etkileri aktive etmiş olabileceği düşünülebilir.

Anahtar kelimeler: DNA hasarı, radyoaktif iyot tedavisi, COMET analizi, I-131, Farklılaşmış tiroid kanseri.

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INTRODUCTION

The most common cancer of the endocrine system is thyroid carcinoma, which encompasses a spectrum of histologically distinct tumours. Among these, differentiated thyroid carcinoma (DTC) holds a prominent position[1]. With a steadily increasing global incidence, DTC primarily manifests as papillary, follicular and Hürthle cell thyroid carcinoma, representing approximately 90% of all thyroid malignancies[2,3]. While surgical intervention, often involving thyroidectomy, stands as a cornerstone in the management of DTC, adjunctive therapies play a pivotal role in reducing the risk of recurrence and improving long-term outcomes[4,5]. One such therapeutic modality that has revolutionized the approach to DTC is the use of Iodine-131 (I-131) treatment, commonly known as radioactive iodine therapy (RAIT)[6]. I-131, a radioisotope of iodine, exhibits unique properties owing to its ability to undergo beta decay, emitting therapeutic beta particles that selectively target thyroid tissues. This distinctive attribute makes I-131 an invaluable tool in the armamentarium against residual or metastatic thyroid cancer cells post-thyroidectomy [7,8].

The application of I-131 in the management of DTC is deeply rooted in the fundamental understanding of the unique capability of the thyroid tissue to actively accumulate and concentrate iodine[9]. The thyroid gland, being the exclusive organ in the human body with the ability to trap iodine, provides an inherent targeting mechanism for I-131, facilitating precise and targeted therapy[10] .

The primary goal of I-131 treatment in DTC is two-fold: to ablate any remaining normal thyroid tissue and, more critically, to eradicate microscopic residual disease, thereby reducing the risk of recurrence and improving the overall prognosis for patients[11]. The cytosolic effects of I-131 on thyroid cells, particularly through the formation of chain fractures in DNA, contribute to its therapeutic efficacy[12,13]. However, the landscape of I-131 treatment in DTC is not without its complexities and controversies[11,14]. Concerns linger regarding the potential genotoxic impact of RAIT on peripheral blood cells [15]. However, there are a few number of studies in the current literature on this regard[7,16-18].

The single cell gel electrophoresis assay, commonly known as the comet assay, is a straightforward and sensitive technique for identifying DNA damage at the level of a single eukaryotic cell[19,20]. This method has gained more attraction for genotoxicity testing, biomonitoring, and assessing DNA damage and repair[20].

The hypothesis of our study is that RAIT does not cause significant DNA damage in the peripheral lymphocytes of DTC patients and may instead activate cellular repair mechanisms which helps to preserve genomic integrity. The current study aimed to investigate the time dependent effects of I-131 treatment on systemic genotoxicity based on comet assay, which may potentially contribute valuable insights for refining and optimizing the current treatment modalities for DTC. In addition, this study also contributes to the literature regarding the genotoxic effects of RAIT on peripheral blood cells in DTC patients. Using the COMET assay, it was shown that RAIT does not cause permanent DNA damage in lymphocytes and may even promote DNA repair processes. These findings support the hematological safety of RAIT and provide new insights into its potential protective cellular effects.

MATERIALS AND METHODS

Sample

Based on power analysis using data from similar studies in the literature, it was calculated that a minimum of 20 participants would be required to achieve 95% power at a 95% confidence level. Our study comprised 21 individuals with a mean age of 45.09±10.02 years. The study included patients diagnosed with differentiated thyroid cancer (DTC) who had undergone total or near-total thyroidectomy and were scheduled to receive radioactive iodine (RAI) therapy; patients with liver and kidney failure, presence of chronic diseases (such as diabetes, hypertension, etc.), and/or a history of using medications containing salicylate derivatives were excluded. Although samples were initially collected from 27 patients at the start of the study, data from only 21 patients were available at the 6-month follow-up. An informed consent form was signed by each participant.

Treatment with radioactive iodine (RAIT)

An experienced nuclear medicine specialist analyzed twenty-one DTC patients. Thyroid stimulating hormone (TSH) blood level was evaluated prior to RAIT, and it was confirmed that all patients had

levels above 30 IU/ml. Following the amount of remaining tissue and the metastasis screening, the histological features were considered and the standard fixed dose calculation method was used to establish the RAIT dose for each patient. Based on the patient dosages, three groups were created. Six patients (28.6%) received low dosage (≤30 mCi) RAIT, while 11 patients (52.4%) received moderate dose (between 31 and 100 mCi), and 4 participants (19%) received high dose (>100 mCi) RAIT. In the Nuclear Medicine Department, I-131 oral capsules (Monrol Eczacibasi, Istanbul, Turkey) were administered for at least 4-6 weeks after thyroidectomy, before thyroid hormone replacement therapy started, or for 3-4 weeks after thyroid hormone preparations were discontinued if hormone replacement had already started. After the RAIT, each patient was given the recommended dosage of levothyroxine sodium, a thyroid hormone medicine.

Procedure

The study was conducted jointly by the Departments of Physiology and Nuclear Medicine at Pamukkale University Faculty of Medicine. RAIT procedures were performed by an experienced nuclear medicine specialist at the Nuclear Medicine Department of Pamukkale University Hospital. Blood collection, lymphocyte isolation, cell preservation, and COMET assay were carried out in the Physiology Department laboratory by trained researchers following standardized protocols. The hospital adheres to institutional quality control and data confidentiality standards. In compliance with the Helsinki Declaration, this single-center university-based clinical study was authorized by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (60116787-020/74536; 29.11.2016).

Each patient's venous blood samples were obtained at three different times: before treatment, seven days after treatment, and six months after treatment. Following the isolation of lymphocytes from venous whole blood, DNA damage was evaluated using the COMET assay. Currently, single and double strand DNA breaks are demonstrated using the COMET assay, a sensitive, quick, and simple gel electrophoresis technique. To assess DNA damage, 75 cells per slide/sample were scored. For a quantitative analysis of DNA damage, a number of characteristics were assessed, including head length, tail length, head intensity, tail intensity, and tail moment.

Steps of Comet assay

Whole blood collection and lymphocytes isolation

Peripheral venous blood was obtained from all subjects into a 10-mL vacutainer tube containing K3EDTA, and lymphocytes were separated using Histopaque-1077. Following the dilution of blood 1:1 with phosphate buffered saline (PBS) and transmission into leucosep tubes, the samples were centrifuged for 15 minutes at 800 g at room temperature. After extraction, the buffy coats were subjected to two PBS washes.

Cells cryopreservation prior to comet assay

As described by Visvardis et al. (21), the cell suspension was centrifuged at 200 g for five minutes. The cell pellet was then resuspended at a concentration of 3×10^5 cells/mL in a freezing medium consisting of 10% DMSO, 40% RPMI and 50% fetal calf serum. The cell suspension was placed in plastic freezing vials in $2x10^6$ cell aliquots. To obtain a chilling rate of -1°C/min, vials were first placed in a Cryo 1°C freezing container, then immediately moved into a -80°C freezer, and lastly stored at -80°C.

Comet assay

The procedure of Nandhakumar et al [19] was followed. To summarise, the vials were removed and placed in a water bath at 37°C until all the ice had melted. The thawed cells were promptly moved into conical centrifuge tubes filled with 15 mL of prechilled thawing media, which was made up of 10% dextrose, 40% RPMI, and 50% foetal calf serum. The cells were centrifuged at 200g for 10 minutes at 4°C in order to perform the comet assay. Next, ice-cold PBS pH 7.3 was used to resuspend the cell pellet.

Based on the modified Singh et al. method, the comet test was carried out in an alkaline environment [22]. A frosted glass microscope slide coated with 1% normal melting point agarose was pipetted with 100 µL of 1% low melting point agarose at 37°C and pH 7.4. In this way, the cells were suspended in the solution. In order to extract the cell proteins, the agarose was let to set on ice for ten minutes. After that, the slide was submerged in a lysis solution (containing NaOH to pH 10.0, 2.5 M NaCl, 10 mM Tris, 1% Triton X- 100 and 100 mM Na_2 EDTA) for one hour at 4°C.

In order to reveal the alkali labile sites (alkali unwinding) and unwind DNA strands, the slides were then placed in an electrophoresis tank and kept in the alkaline buffer (0.3 M NaOH and 1 mM Na EDTA) for 30 minutes. Later 30 minutes, 30 minutes of electrophoresis at 25 V and 300 mA at the same temperature was carried out. The slides were put on a staining tray after being carefully removed from the electrophoresis buffer. The slides were washed three times for five minutes each with the neutralization buffer (0.4 M Tris-HCl, pH 7.5). The fluorescent staining technique was then used to visualise the slides. To put it briefly, 50 µL of ethidium bromide stain was applied to each slide, and a new cover slip was then placed over it. Any excess stain was removed by blotting the back and edges of the slides before they were examined. Slides stained with ethidium bromide were viewed under a fluorescent microscope equipped with an 20X magnification, a barrier filter of 590 nm, and an excitation filter of 515 to 560 nm.

All processes, beginning with the isolation of lymphocytes, were conducted under yellow light to lower the possibility of destroying cellular DNA.Comet IV software for computer (Perceptive Instruments, UK) was used to analyse slides under a microscope.

Statistical analysis

SPSS 24.0 was used for statistical analysis. Continuous variables are presented as mean ± standard error (SE). When parametric assumptions were available, independent group differences were assessed using the Independent Samples t-test; if parametric assumptions were not available, the Mann–Whitney U test was applied for comparison between independent groups. The threshold for statistical significance was $p<0.05$.

RESULTS

This study included 21 patients diagnosed with DTC. When the entire cohort (n = 21) was evaluated, a significant reduction in DNA damage, as measured by tail intensity, was observed at both 1 week and 6 months post-RAIT. The tail intensity values decreased from 23.83±9.82 before RAIT to 18.30±6.48 at 1 week post-RAIT and 18.06±5.58 at 6 months post-RAIT (p=0.002). Similarly, head intensity values increased significantly from 76.16±9.82 before RAIT to 81.69±6.48 at 1 week and 81.93±5.58 at 6 months post-RAIT (p=0.002). Additionally, a significant reduction in tail moment was noted 6 months after RAIT, with values decreasing from 8.04±3.86 before treatment to 5.24±2.51 post-RAIT (p=0.005) (Table 1).

Table 1. DNA damage assay parameters of the patients before and after the radioactive iodine therapy (RAIT) (n=21)

COMET assay values	Before treatment (n=21) (mean±SE)	1 week after treatment (n=21) (mean±SE)	6 months after treatment (n=21) (mean±SE)
Tail Intensity (%)	23.83±2.14	**18.30±6.48***	**18.06±1.21***
Head Intensity (%)	76.16±2.14	**81.69±6.48***	**81.93±1.21***
Tail Moment (µm)	8.04±0.84	6.02±2.15	**5.24±0.54*,#**

Values are expressed as means± standard error (SE). *p<0.05: the difference from before treatment; #p<0.05: statistically significant from 1 week after treatment.

The DNA damage parameters were also analysed according to the RAIT dose groups, which were divided into low, moderate and high. In the low-dose group, tail intensity and tail moment values significantly decreased, while head intensity increased at 6 months post-RAIT compared to pre-treatment levels (p<0.05). However, in the moderate and high-dose groups, changes in DNA damage parameters between baseline and 6 months post-RAIT did not reach statistical significance (p>0.05) (Table 2).

Table 2. The changes in DNA damage assay (COMET) parameters in the low, moderate and high dose radioactive iodine therapy (RAIT) groups.

COMET assay values	Patients treated with a low (≤30 mCi) RAIT dose, n=6 (mean±SE)	Patients treated with a moderate (31-100 mCi) RAIT dose, n=11 (mean±SE)	Patients treated with a high (>100 mCi) RAIT dose, n=4 (mean±SE)
Tail Intensity (%) before treatment	29.55±4.69	20.63±2.71	24.06±3.53
Tail Intensity (%) 6 months post RAIT	**18.59±3.36***	18.36±1.21	16.47±3.02
Head Intensity (%) before treatment	70.46±4.69	79.30±2.71	75.93±3.53
Head Intensity (%) 6 months post RAIT	**81.40±3.36***	81.63±1.21	83.52±3.02
Tail Moment (μm) before treatment	9.91±1.88	7.33±1.13	7.21±1.28
Tail Moment (μm) 6 months post RAIT	**5.14±1.22***	5.63±0.73	4.34±1.22

Values are expressed as means± standard error (SE).; *p<0.05: difference from before treatment values of the concordant group. mCi: millicurie; RAIT: Radioactive Iodine Therapy

DISCUSSION

The use of RAIT in the treatment of DTC is highly effective for eradicating residual thyroid tissue and metastatic lesions after thyroidectomy. However, due to the systemic distribution of I-131, concerns are growing about its genotoxic effects, particularly the DNA damage it causes in non-thyroid tissues. This study aimed to evaluate the effects of I-131 therapy on DNA damage parameters-specifically, tail intensity, head intensity, and tail moment-in patients with DTC, and to explore whether the extent of DNA damage differ depending on the administered RAI dose.

The significant reduction in tail intensity observed in the overall patient group, as well as in the low-dose group, suggests that I-131 therapy induces initial DNA damage that is largely repaired over time. Tail intensity, which reflects the proportion of fragmented DNA in the comet assay, decreased significantly both at 1 week and 6 months after RAIT. This reduction suggests that DNA damage caused by I-131 is transient, with repair mechanisms effectively mitigating the damage. These findings are consistent with previous studies indicating that, while potentially harmful, radiation-induced DNA double-strand breaks (DSBs) are largely reparable through cellular DNA repair pathways[23,24]. The decrease in tail moment 6 months after treatment also supports the idea of effective DNA repair over time. Tail moment is a composite parameter that accounts for both the amount of DNA damage and the distribution of DNA fragments. The significant reduction in tail moment at 6 months in the entire group indicates that the DNA damage inflicted by I-131 in peripheral lymphocytes gradually resolves.

A short-term study by Unlu et al. demonstrated that DNA damage significantly increases one day after RAIT based on the tail moment measurements, which was improved at one week after RAIT in patients with papillary thyroid carcinoma[17]. Gutiérrez et al also evaluated the eventual DNA damage 1 week after RAIT based on COMET assay in a group of 28 patients with thyroid carcinoma [18]. They reported an insignificant increase in tail length one week after RAIT, and proposed that the absence of a statistically significant response one week after treatment was consistent with the repair kinetics found by Plappert et al., who showed that most of the DNA damage caused by RAI exposure was fully restored by one week[25]. Conversely, in the same study, Gutiérrez et al. discovered a noteworthy rise in the percentage of injured cells and the mean tail length one month following RAIT. They attributed this illness to the buildup of RAI in the thyroid gland, which served as an internal radiation source[18]. Signore et al. reported

that, in a group of 62 patients treated with 50 mCi RAIT for thyroid carcinoma, the COMET assay revealed a statistically significant worsening of DNA damage parameters at one week, followed by a significant reduction at three months, although these values were not yet back to baseline[7]. Using the Comet assay, Grzesiuk et al. assessed the impact of RAIT on DNA damage in hyperthyroid patients prior to, during, and 54 days following therapy. They found that the DNA damage was dramatically decreased 54 days following treatment[26]. Bitgen et al. examined the impact of RAIT on patients with papillary thyroid cancer prior to total thyroidectomy in 2024. They used the cytokinesis block micronucleus cytome (CBMN-Cyt) assay to analyse the effects of RAIT before treatment and at 1 week, 6 months and 1 year afterwards. The parameters used were micronuclei (MN) and nucleoplasmic bridges (NPB). They found that, MN levels in lymphocytes showed a significant increase 1 week after RAIT which gradually decreased at 6 months and 1 year, indicating ongoing DNA repair mechanisms [13]. In summary, the variability in DNA damage responses observed in high-dose RAIT studies can be attributed to differences in irradiation protocols, cell types, and methodological approaches.

Interestingly, our study showed that significant changes in DNA damage parameters were limited to the low-dose group, while the moderate- and high-dose groups did not experience statistically significant changes in tail intensity, head intensity, or tail moment after RAIT. The significant reduction in tail intensity and tail moment in the low-dose group suggests that lower RAI doses may result in manageable levels of DNA damage which can be effectively repaired by the DNA repair mechanisms. This aligns with findings from prior studies that suggest lower radiation doses induce less complex forms of DNA damage, which are more easily repaired, and may even stimulate adaptive repair responses. The significant increase in head intensity in this group may also further support the concept that non-damaged DNA remains intact and stable, highlighting the resilience of non-thyroid tissues when exposed to low-dose RAIT. On the other hand, the DNA damage induced by moderate and high doses of RAIT appears to be recovered by the DNA repair mechanisms, and the damage-repair balance remained consistent, preventing significant changes in the DNA damage markers. Conversely, if DNA damage had remained persistent or irreparable, one would expect increased levels of DNA damage parameters over time (higher tail intensity and tail moment). Studies have shown that high-dose-rate irradiation can lead to increased DNA damage, but the extent and reparability of this damage can vary depending on irradiation parameters and cell types[27].

While the data from the current study does not support the idea of increased long-term risks from high-dose RAIT based on DNA damage parameters, high-dose RAIT can still pose long-term safety concerns, such as an increased risk for secondary malignancies or other radiation-induced effects, as demonstrated in broader literature.

This study is subject to several limitations, including a modest sample size, which limits the generalizability of the findings. Additionally, the follow-up period of 6 months may not be sufficient to capture the full scope of long-term DNA damage or the risk of delayed genotoxic effects. Furthermore, although the current study used COMET assay to measure DNA damage, future research could benefit from incorporating additional markers of DNA damage and repair.

In conclusion, our study demonstrated that RAIT induces alterations in DNA in patients with DTC, with the evidence of repair and recovery over time, particularly in those receiving low doses of RAIT. Furthermore, the moderate- and high-dose RAIT seem not to lead detectable DNA damage in peripheral lymphocytes.

The relatively small sample size and the absence of control group can be considered as the limitations of the current study. Without a control group, it remains uncertain whether the alterations in DNA repair are exclusively caused by RAIT or influenced by other factors, including environmental exposures like UV radiation, ionizing radiation, chemicals, and endogenous reactive oxygen species. Additionally, the general health status of the patients, their symptoms, and other biological variables should have been considered, which also represents a limitation of our study. Further studies with larger patient populations and extended follow-up periods are required to assess potential secondary malignancies and other long-term effects of RAIT. Incorporating additional assays like the MN test in future studies could provide a more comprehensive evaluation of genotoxic effects.

In conclusion, this study demonstrates that radioactive iodine therapy induces transient DNA damage in patients with differentiated thyroid cancer,

with evidence of repair particularly at low doses over time. The absence of significant changes at moderate and high doses suggests a balance between DNA damage and repair mechanisms. Future studies with larger cohorts and extended follow-up periods are warranted to better understand the long-term effects of RAIT. Incorporating multiple DNA damage markers could further enhance the evaluation of treatment safety. These findings contribute valuable insights for optimizing therapeutic strategies.

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The first and second authors participated equally to this research.

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