Atom Indonesia

Journal homepage: https://atomindonesia.brin.go.id

Preliminary Study of Micronucleus Frequencies and Responses in Thyroid Cancer Patients After Treatment with ^{131}I Therapy

I. K. H. Basri[1*], Y. Lusiyanti[2], D. Ramadhani[1], D. Tetriana[2], A. R. Dewi[3], S. Purnami[2], V. A. Suvivan[2], M. R. A. Gani[3], T. Kisnanto[1]

[1]*Research Center for Radioisotope Technology, Radiopharmaceuticals and Biodosimetry, National Research and Innovation Agency (BRIN), Tangerang Selatan 15314, Indonesia*
[2]*Research Center for Safety, Metrology dan Nuclear Quality, National Research and Innovation Agency (BRIN), Tangerang Selatan 15314, Indonesia*
[3]*Dharmais National Cancer Center Hospital, Jl. Letjen. S. Parman No.84-86, Jakarta 11420, Indonesia*

ARTICLE INFO

Article history:
Received 27 December 2023
Received in revised form 21 March 2024
Accepted 21 March 2024

Keywords:
Micronuclei
Activity
Dose rate
Thyroid cancer
Follow up
Response

ABSTRACT

Radioiodine has become the most widely used to treat an overactive thyroid (hyperthyroidism) and thyroid cancer worldwide. The present research aimed to study the association between micronuclei (MN) frequencies, and follow-up responses after treating thyroid cancer patients with iodium-131(^{131}I). The detection of the MNs assay was carried out by Giemsa staining from lymphocytes obtained from twenty-four thyroid cancer patients one week after receiving ^{131}I treatment at Dharmais Cancer Center Hospital, Jakarta, Indonesia. Follow-up for clinical and laboratory responses grouped into good (stable) and bad (progressive, refractory, and dropout patients) responses, was observed one and six months after treatment. All patients received radioiodine with an activity dose of 30 - 200 μCi. The mean MN frequency in the good response group was 14.22, and that of bad response patients was 17.22. There was no statistically significant difference in MN frequency ($p>0.05$) between the two groups of patients after six months of treatment.

INTRODUCTION

Thyroid cancer accounts for 2.1 % of all cancer diagnoses worldwide and is the most common endocrine cancer. This cancer is one of the most common diseases in teenagers and young adults (586.202 new cases in 2020), with a median age at diagnosis that is lower than that of other cancer types [1-3]. Its incidence is roughly two to four times higher in women than in men. For the past three decades, its incidence has been steadily rising [1].

Thyroid cancer is primarily treated with surgical resection (total or near-total thyroidectomy), post-thyroidectomy radioiodine (RAI) therapy, and suppression of thyroid stimulating hormone [3,4]. The majority of cases of differentiated thyroid cancer (DTC), one of the subgroups of cancer tissue/cell differentiation, are indolent in nature, iodine-avid, and respond favorably to standard therapy. The prognosis for almost all cases is generally favorable, resulting in high long-term survival and low death rates from the disease [4,5].

The ability of ^{131}I to be preferentially absorbed and concentrated in normal or neoplastic thyroid follicular cells, taking advantage of these cells' specialized iodide uptake and mechanism of accumulation, is one reason that RAI therapy is widely used in the DTC treatment management [3,6,7]. The ^{131}I that has accumulated in thyrocytes decays into [β and γ], releasing high-energy electrons that cause severe local DNA damage. Radiation cytotoxicity causes cancer thyroid cells to die, which makes it possible to remove any remaining tumor cells and ablate any remaining

*Corresponding author.
E-mail address: iink001@brin.go.id
DOI: https://doi.org/10.55981/aij.2024.1409

normal cancer tissue [3,6]. Unfortunately, other normal tissues may subsequently fixate ^{131}I, which is known to have DNA-damaging effects. This increases the risk of RAI-closed secondary tumors, such as leukemia, salivary tumors, colorectal cancer, and soft tissue tumors, in addition to the thyroid gland [3,7]. There is concern that overdiagnosis of DTC could put patients at risk of overtreatment, as the rising incidence of TC is typically caused by an increase in the detection of stationary subclinical lesions [2].

Given the slow-growing nature of thyroid cancer, its high rate of long-term survival, and the average age at diagnosis, such therapy-related morbidity may not be warranted, since most patients will have many years to experience the side effects of the treatment [2]. This concern is reflected for the first time in the updated American Thyroid Association (ATA) clinical practice guidelines for the management of DTC [8], which advise a more cautious diagnosis and treatment strategy to minimize RAI use and radiation exposure, particularly in adolescent patients. The use of lower RAI doses (30-50 mCi.) in patients with low-risk DTC is crucial. Other examples of this include stricter criteria for diagnosis upon nodule detection, molecular-based risk grading for better treatment decisions, personalized disease management, and long-term surveillance strategies [2,8,9].

From the standpoint of assessing the possible risk associated with internal radiation exposure, the standard cytokinesis-block micronucleus assay is useful for analyzing DNA damage related to ^{131}I therapy. The cytokinesis-block micronuclei assay is a commonly employed method that is characterized by its simplicity, speed, dependability, and affordability [9-12]. Contradictory results from limited studies on cytogenetic damage in thyroid cancer patients following ^{131}I therapy have been reported [13-19]. Ionizing radiation exposure is known to increase the amount of clastogenic factors in the bloodstream as a result of oxidative stress, which may worsen DNA damage in vivo [20]. According to certain reports, treatment with 70 mCi ^{131}I in patients with DTC is consistently associated with higher levels of DNA damage in peripheral lymphocytes [21,22].

The purpose of this current study is to confirm the association between the cytokinesis-blocked micronucleus (CBMN) formation, as a biomarker of genome damage in peripheral lymphocytes of thyroid cancer patients, after one week of treatment with ^{131}I radionuclide, and the response of the patient after six months of therapy.

MATERIALS AND METHODS

Subjects

The present study was conducted at the Nuclear Medicine Department of Dharmais National Cancer Center Hospital and Cytogenetic and Radiobiology Laboratory, Research Center for Radiation Safety and Metrology, National Nuclear Energy Agency, before reorganization became the National Research and Innovation Agency in Jakarta, Indonesia, between March 2019 and September 2019.

Before the study started, the research protocol was approved by the Ethics Committee of the National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia no. LB.02.01/2/KE.071/2019, and all the subjects provided written informed consent.

Blood isolation

The majority of the external dose exposure measurements from the patients and blood sample collection were conducted within a week or during this period (the patients were admitted to our ward and kept in the hospital for a minimum of three days, or until the external dose rate fell below 3.5 mSv/h at a distance of 2.5 m while they were there). A total of 24 subjects met the main inclusive criteria, all of whom had never received any chemotherapy or radiotherapy before taking part in this study and had undergone radioiodine therapy for differentiated thyroid cancer. Blood samples were collected intravenously on a heparin tube (BD Vacutainer, USA).

Micronucleus assay

The micronucleus assay was performed according to the method explained by Fenech [23]. In brief, 500 µL of blood sample was added to 4 mL of Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with-Glutamine with 25 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 100 µL of PenStrep (Penicillin-streptomycin), 500 µL of Fetal Bovine Serum (FBS), and the addition of phytohemagglutinin (PHA; 100 µL/500 µL) (Gibco, Thermofisher, Waltham, Massachusetts, USA) to the culture medium and incubated it at 37°C to stimulate the cell division. After culture for 44 hours, 100 µL of cytochalasin B (Sigma-Aldrich, Sint Louis, Missouri USA) 6 µg/ml) was added to block the cell mitosis at the end of the metaphase of the cell cycle.

Following 72 hours, the cells were harvested, resuspended in 6 mL of cold KCl (0.075 M), and fixed with a 6:1 methanol/acetic acid fixative solution. The fixed cells were placed onto cold glass slides, allowed to air dry, and then stained for fifteen minutes using a 10 % Giemsa/PBS solution. The number of binucleated cells with micronucleus (MN) existence was calculated per 1000 binucleated cells and counted by expert researchers (YL, SP, and VAS).

Response one and six months after treatment

The analysis for response to radioiodine therapy was conducted one and six months afterward, with the criteria stated in the 2015 American Thyroid Association Management Guideline for Adults with Thyroid Nodules and Differentiated Thyroid Cancer [8,9]. In this study, the response to therapy was classified as follows: the good response shows non-evidence of disease (NED), the bad response, and the dropout group. The good response group consisted of stable patients without overt tumor symptoms, no RAI imaging evidence (no uptake outside the thyroid bed on the first post-treatment whole body scan (WBS), if performed, or if uptake outside the thyroid bed had been present), no imaging evidence of tumor on a recent diagnostic or post-therapy, neck the US, and/or low serum Tg levels during thyroid-stimulating hormone (TSH) suppression (Thyroglobulin <0.2 ng/mL) or after stimulation (Thyroglobulin <1 ng/mL) in the absence of interfering antibodies. While those with progressive disease and/or iodine refractory during follow-up were classified as the bad response group. Toxicity was evaluated according to World Health Organization criteria [8,9,24]. This clinical response evaluation was carried out by one of the authors (ARD).

Dose rate

Every patient had their dose rates measured at intervals of one meter from the anterior mid trunk during admission, prior to being discharged from the hospital, and for a maximum of eleven days following discharge. In certain patients, measurements were also taken at 0.1 and 0.5 meters, by using Surveymeter RedEye-Gamma Scout, (RadonTec GmbH Hauptstraße 589426 Wittislingen, Germany) [25]. This measurement and calculation were done by one of the co-authors (MAG).

Statistical analysis

All analyses were done with Excell Microsoft Office 15 for Windows 10, And MedCalc Version 22.005-64-Bit. Categorical variables, presented as frequencies and percentages, were compared between group responses (good and bad) and dropout subjects. The Shapiro-Wilk was utilized to assess the normality of micronucleus frequency, while the Levene tests were used to evaluate the homogenity of variances. The mean ± standard deviation of dose rates was calculated. For longitudinal comparisons, the paired sample t-test or the Wilcoxon signed-rank test was used depending on the distribution data. The parametric Student t-test was used for normal distributions or the t-test for independent samples was used for comparisons between genders. Additionally, the Correlation Test was used to analyze the relationship between age and MN frequency.

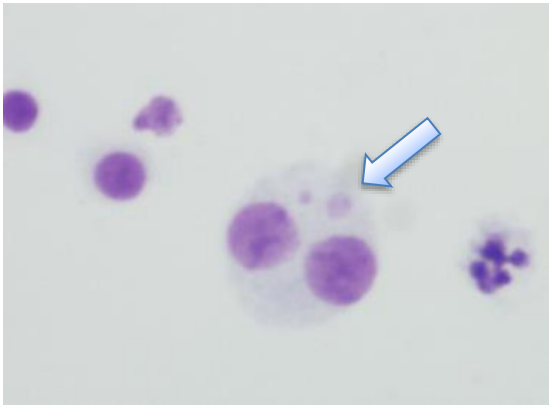
RESULTS AND DISCUSSION

Twenty-four subjects (7 men and 17 women) with a mean age of 46.50 ± 13.64 years were recruited and included in this study. Most of the subjects (n = 23) presented with papillary thyroid cancer and 1 subject had mixed cell and hyperplasia thyroid. One subject received 7.40×10^9 Bq, 8 subjects received 5.55×10^9 Bq, 14 subjects received 3.70×10^9 Bq, and 1 subject received 111×10^7 Bq. Radiation exposure rate (dose rate), sexes, ages, histology, activity of ^{131}I, and, micronuclei frequencies (after one week of treatment) and responses observed after one and six months are shown in Table 1.

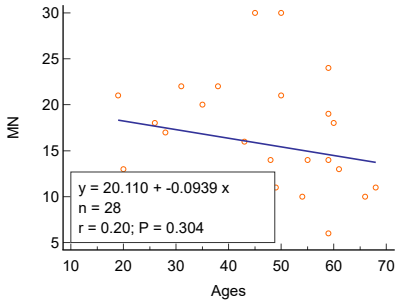
After one month of treatment, 15 (62.5 %) subjects showed a stable or good response, 3 subjects were progressive, 1 subject was refractory or unresponsive, and 5 subjects were dropped out. In this study, refractory or progressive responses were classified as bad responses and modified responses for dropout subjects were also classified as bad responses.

The MN frequency in the subject after one week of radioiodine treatment was 6 - 30 per lymphocyte cell in Fig. 1a. No correlation was found between MN and the ages of the subjects ($p > 0.05$) as shown in Fig. 1b. The mean MN index in women was 17.32 ± 6.27 and in men, 12.56 ± 5.50 ($p = 0.06$), indicating that the level of MN frequency in women almost reached a statistically higher level than MN in men (Fig. 1c). No statistical difference in MN of subjects after receiving treatments 1.11×10^9, 3.7×10^9, 5.5×10^9 and 7.4×10^9 Bq, but a tendency

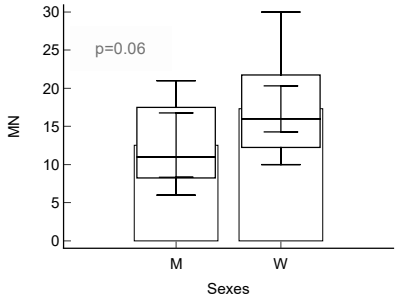
toward higher MN was found in subjects treated with 3.7×10^9, 5.5×10^9 and 7.4×10^9 Bq, compared to only receiving 1.11×10^9 Bq. If grouped by the response after one month of therapy (good response compared to bad response and dropout subjects), the MN in the good response subjects was 14.22 ± 6.14 and those with bad response were 18.60 ± 6.00 p = 0.08 (Fig. 3a). It seems consistent that MN in the good response group was lower than in the bad response subjects (Fig. 3b).



(a)



(b)



(c)

Fig. 1. (a) Micronucleus (arrow) on lymphocytes of patients in giemsa staining assay (magnification of 400 times); (b) Correlation between ages and MN frequency of the subjects; (c) MN frequency in men (M) and women (W) subjects.

Table 1. Characteristic the subjects, MN index, and response six months after treatment.

No	ID Subjects	Sexes	Ages	Histology	I-131 Activity (Becquerel)	Dose Rate After One Week (mSv/hour)	No. Micronuclei	Response
1	A	W	38	Ca Thy Papillary	555.10^7	3.17	22	Progressive
2	B	W	50	Ca Thy Papillary	370.10^7	7.2	30	DO *)
3	C	W	31	Ca Thy Papillary	555.10^7	1.7	22	DO
4	D	W	50	Ca Thy Papillary	370.10^7	10.68	21	DO
5	E	W	20	Ca Thy Papillary	555.10^7	17.3	13	Stable
6	F	W	55	Hyperplasia	555.10^7	14.29	14	Stable
7	G	W	26	Ca Thy Papillary	370.10^7	15.47	18	Stable
8	H	W	60	Ca Thy Papillary	370.10^7	14.89	18	DO
9	I	W	59	Ca Thy Papillary	740.10^7	2.8	14	Stable
10	J	M	28	Ca Thy Papillary	370.10^7	24.8	17	Stable
11	K	W	59	Ca Thy Papillary	370.10^7	27.2	24	Stable
12	L	M	19	Ca Thy Papillary	370.10^7	10.85	21	Stable
13	M	W	45	Ca Thy Papillary	370.10^7	16.11	30	Stable
14	N	W	35	Ca Thy Papillary	370.10^7	4.25	20	DO
15	O	M	47	Ca Thy Papillary	111.10^7	8.5	9	Stable
16	P	M	48	Ca Thy Papillary	370.10^7	6.5	14	Stable
17	Q	W	49	Ca Thy Papiller	370.10^7	11.2	11	Refractory
18	R	M	68	Ca. Mixed	370.10^7	10.5	11	Stable
19	S	W	45	Ca Thy Papillary	370.10^7	10.2	11	Stable
20	T	W	61	Ca Thy Papillary	555.10^7	8.8	13	Progressive
21	U	W	66	Ca Thy Papillary	555.10^7	3.7	10	Stable
22	V	M	54	Ca Thy Papillary	555.10^7	4.7	10	Progressive
23	W	W	59	Ca Thy Papillary	370.10^7	22.1	6	Stable
24	X	M	44	Ca Thy Papillary	555.10^7	7.12	10	Stable

*) DO: drop out

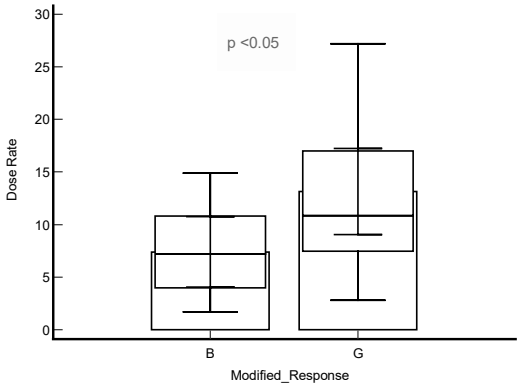
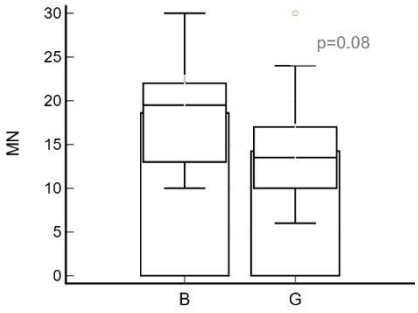
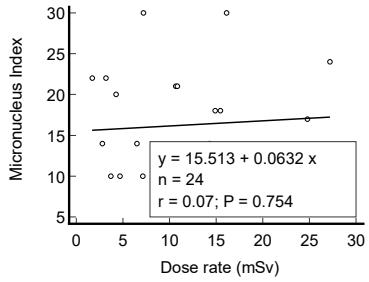


Fig. 2. Subject dose rate exposure on bad (B) and good response (G) after six months of treatment.



(a)



(b)

Fig. 3. (a) The MN frequency in six months response (Good (B) and Bad (B)); (b) Correlation between MN Frequency and dose rate exposure after one-week radionuclide treatment.

Related to the limitation of this current study, there was no micronuclei (MN) frequency data for the subjects before treatment with ^{131}I. Omrani et al. [26], found that before treatment, the MN frequency Fig 1. within 1000 lymphocyte cells in a thyroid cancer subject was 11.35 ± 1.88 and increased almost three times after radionuclide treatment. Similar to this current study, there was no significant association observed between the MN frequency applied radiation dose 30 - 200 mCi. and age, gender [27]. Watanabe et al. [22], showed that the MN before treatment was 5.4 ± 1.4 and increased to 15.7 ± 2.7 after one week of treatment (in 1000 lymphocyte cells).

There was a statistical difference in dose rate exposure between subjects who showed a good response and those who showed a bad response after six months of therapy as shown in Fig. 2. Following a week of treatment, the routine procedure involves measuring the subjects' dose exposure. The estimation of the patient's whole-body radioactive clearance is based on the measurement of the external dose rate, which is indirectly correlated with the patient's radioactive clearance [27]. High dose rate exposure from the subjects may also be related to the high dose of radionuclide exposure in the patients that are irradiated to the cancer tissue, which can kill the cancer cells and prevent metastasis [28]. Cancer cells' DNA is harmed either directly or indirectly by beta radiation released from ^{131}I decay. When ^{131}I causes DNA damage to cancer cells, these cells may not be able to continue dividing and may instead undergo apoptosis [29]. Although it was not statistically significant, the consistency data on the higher MN frequency tends to give a bad response or dropout compared to the subjects with the lower MN frequency. In our opinion, higher MN frequency is related to failed DNA damage repair and aggressivity in the invasion of metastasis in cancer cells. An indicator of chromosome damage, such as breakage and loss following exposure to ionizing radiation, is the MN induction assay [30]. It may be related to unrepaired or improperly repaired DNA damage since it shows the failure of appropriate chromosome segregation into daughter cell nuclei. According to reports, a significant portion of the baseline and induced MN frequencies are determined by genetic factors [26]. This assay is regarded as a biomarker of individual radiosensitivity because the radiation-induced MN varies significantly between individuals [31,32].

Subjects with lymph node, pulmonary, and skeletal metastases had a significantly higher frequency of micronuclei in their reports compared to those without metastases. The response to treatment was further stratified based on the extent of disease progression and the presence of metastases in different organs. This finding demonstrates how the disease's spread has led to an increase in DNA damage in peripheral blood lymphocytes [33]. Leal-Garza et al. [33] also reported similar results, with subjects exhibiting a progressive increase in MN frequency with cervical cancer. The increased MN frequency in these subjects could be attributed to either a clastogenic product released by the tumor cells or metabolic stress caused by the growth of the tumor. In advanced-stage anaplastic large-cell lymphoma, Lones et al. [34] also found complex additional chromosome abnormalities that impact the regulation of other oncogenes or tumor suppressor genes. The alterations mentioned above might be linked to a bad prognosis or the disease's

advancement. A similar situation might arise in the current study group's metastasis subjects, as they have been found to have higher levels of DNA damage [35].

High numbers of micronuclei are found in many cancers, which are also related to the failure of apoptosis. The higher MN frequency in this current study may also be caused by unsuccessful apoptosis formation through inhibition of caspase DNA-ase and blocking mitochondrial apoptosis [36]. Micronuclei are also important bottlenecks during tumor evolution [37,38].

The mean dose exposure rate of the subject after one week of treatment was recorded at 11.00 ± 6.92 mSv/h. There were statistically different dose exposure rates for subjects between the good (G) and bad responses after six months of therapy, as shown in Fig. 2. High dose rate exposure from the patient after one week of treatment may be related to DNA damage that induced the death of cancer cells and prevented metastasis after six months of treatment.

CONCLUSION

There was no statistically significant difference in micronuclei frequency between good response and bad response patients after six months of treatment. The existence of micronuclei after one week after ^{131}I treatment may indicate a higher incidence of progressive or thyroid cancer cells.

ACKNOWLEDGMENTS

This paper had been presented at Conrad 2023 Munich, Germany, with PAPER ID 83119 and granted by the IAEA CRP Medbiodose 35010 FY 2022. We thank Dr. Uta Eberlein (Department of Nuclear Medicine, University Hospital Würzburg, Germany) for any suggestions she has given to this manuscript. This study is supported by the Research and Development Grant of the Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency, Indonesia. Fiscal Year 2019 (before reorganization to the National Research and Innovation Agency in 2021) and IAEA CRP 35010 FY 2019.

AUTHOR CONTRIBUTION

I. K. H. Basri, A. R. Dewi, and Y. Lusiyanti have major contribution on experiment design, conducted the subject selection, micronucleus staining and data analysis and write the manuscript. D. Ramadhani, D. Tetriana, S. Purnami, V. A. Suvivan, M. R. A. Gani, and T. Kisnanto, supported on patient selection, blood analysis and supported the staining in laboratory as minor contribution.

REFERENCES

1. IARC, Cancer Today - International Agency for Research on Cancer. https://gco.iarc.fr/today/en. Retrieved in June (2023).
2. C. M. Kitahara, J. A. Sosa, Nat. Rev. Endocrinol. **12 (**2016) 646.
3. A. H. Lebastchi and G. G. Callender, Curr. Probl. Cancer **38** (2014) 48.
4. M. H. Khosravi, A. Kouhi, M. Saeedi *et al*., *Thyroid Cancers: Considerations, Classifications, and Managements*. In: Diagnosis and Management of Head and Neck Cancer, Akarslan, Z. (Ed.), Intech, UK (2017) 57.
5. C. Wild, E. Weiderpass and B. W. Stewart (Eds.), World Cancer Report: Cancer Research for Cancer Prevention, International Agency for Research on Cancer, Lyon (2020) 1.
6. S. E. Mayson, D. C. Yoo and G. Gopalakrishnan, Oncol. **88** (2015) 247.
7. L. S. Santos, O. M. Gil, S. N. Silva *et al*., Genes **11** (2020) 1083.
8. B. R. Haugen, E. K. Alexander, K. C. Bible *et al*., Thyroid **26** (2016) 1.
9. B. R. Haugen, Cancer **123** (2017)372.
10. N. Chatterjee and G. C. Walker, Environ. Mol. Mutagen. **58** (2017) 235.
11. S. P. Collins and A. Dritschilo, Cancer. Biol. Ther. **8** (2009) 1164.
12. U. Eberlein, M. Peper, M. Fernández *et al*., PLoS One **10** (2015) 1.
13. U. Eberlein, H. Scherthan, C. Bluemel *et al*., J. Nucl. Med. **57** (2016) 173.
14. V. Vinnikov and O, Belyakov, Semin. Nucl. Med. **52** (2022) 114.
15. S. Monzen, Y. Mariya, A. Wojcik *et al*., Mol. Clin. Oncol. **3** (2015) 692.
16. I. K. Khvostunov, E. Nasonova, V. Krylov *et al*., Int. J. Mol. Sci, **24** (2023) 5128.
17. G. K. Livingston, I. K. Khvostunov, E. Gregoire *et al*., Radiat. Environ. Biophys. **55** (2016) 203.
18. G. K. Parida, C. Bal, R. Dada *et al*., Nucl. Med. Commun. **37** (2016) 800.
19. C. C. Montero, G. L. C. Leandro, M. A. M. Martinez *et al*., Clin. Transl. Med. **12** (2022) e1001.

20. M. D. Jelic, A. D. Mandic, S. M. Maricic *et al*., J. Can. Res. Ther. **17 (**2021) 22.

21. R. Sabharwal, P. Verma, M. A. Syed *et al*., Indian J. Med. Paediatr. Oncol. **36** (2015) 212.

22. N. A. Selcuk, T. Toklu, S. Beykan *et al*., J. Appl. Clin. Med. Phys. **19** (2018) 134.

23. M. Fenech, Genes **11** (2020) 1203.

24. P. K. Julka, D. C. Doval, S. Gupta *et al*., Br. J. Radiol. **81** (2008) 444.

25. S. F. Barrington, A. G. Kettle, M. J. O'Doherty *et al*., Eur. J. Nucl. Med. **23** (1996) 123.

26. V. Omrani, R. Fardid, M. Alavi *et al*., J. Cancer. Res. Ther. **20** (2024) 304.

27. K. D. Gray, S. Bannani, C. Caillard *et al*., Surg. **165** (2019) 37.

28. S. R. Cherry, J. A. Sorenson and M. E. Phelps, Physics in Nuclear Medicine, 4th ed., Saunders Philadelphia (2012) 1.

29. S. M. Jhiang, P. Cheng, F. A. Nabhan *et al*., Fac. Opin. **10** (2021) 1.

30. S. Sommer, I. Buraczewska, M. Kruszewski, Int. J. Mol. Sci. **21** (2020) 1534.

31. B. Pardini, C. Viberti, A. Naccarati *et al*., Br. J. Cancer. **116** (2017) 202.

32. R. A. El-Zein, S. A. Rahman, K. J. Santee *et al*., Cytogenet. Genome Res. **152** (2017) 122.

33. S. K. Reddy, S. K. Verma, S. E. Jacob *et al*., Ann. Pathol. Lab. Med. **4** (2017) A615.

34. M. A. Lones, N. A. Heerema, M. M. L. Beau *et al*., Cancer Genet. Cytogenet. **171** (2006) 89.

35. A. Haimovici, C. Höfer, M. T. Badr *et al*., Cell Death Dis. **13** (2022) 315.

36. J. M. Sheltzer, J. H. Ko, J. M. Replogle *et al*., Cancer Cell **31** (2017) 240.

37. B. A. Weaver, A. D. Silk, C. Montagna *et al*., Cancer Cell **11** (2007) 25.

38. K. Rowald, M. Mantovan, J. Passos *et al*., Cell Rep. **15** (2016) 2679.