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COMPARISON OF THE CYTOGENETIC EFFECTS OF ^{131}I IN PATIENTS WITH DIFFERENTIATED THYROID CANCER WITH AND WITHOUT PRIOR TREATMENT WITH RHTSH

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ABSTRACT

The effects of internal irradiation caused by radiopharmaceuticals being utilized in nuclear medicine are very little studied at the cellular level. In this study, cytogenetic effects of ^{131}I thyroid remnant ablation were analyzed in lymphocytes of thyroidectomized differentiated thyroid cancer patients (DTC) through the chromosome aberration technique. The chromosome damages radioinduced in euthyroid patients submitted to rhTSH (Thyrogen) therapy (Group A) were compared to those induced in patients maintained in hypothyroid condition through levothyroxine withdrawal (L-T_4) (group B), before, 24h, one week and one month after radioiodine administration (4995 – 7030 MBq). In parallel, *in vitro* assays were carried out with lymphocytes of healthy donors for the construction of dose-response curves at different radioactive concentrations of ^{131}I (0.074 to 0.740 MBq/mL) for 24h, thus comparing chromosome damages *in vivo* and *in vitro* in order to estimate the absorbed dose through Monte Carlo program. Radioiodine therapy induced a higher total CA rate in hypothyroid compared with euthyroid patients, except 24h after administration of ^{131}I probably due to higher uptake induced by TSH, although without statistical significance ($p > 0.05$). The frequency of dicentrics obtained in lymphocytes of all patients 24h after treatment, was equivalent to that induced *in vitro* in the radioactive concentrations range of 0.07 to 0.50 MBq/mL, corresponding to an absorbed dose of up 1.0 Gy. The data obtained suggest that the use of rhTSH-aided thyroid remnant ablation is efficacy and secure for the patients with DTC. Cytogenetic analysis after one year are planned in order to evaluate late effects.

1. INTRODUCTION

Among various kinds of tumors, thyroid gland cancer is the most common malignant neoplasia of the endocrine system, affecting relatively young patients between 25 to 65 years old, with a predominance for females. Differentiated thyroid cancer (DTC) represents approximately 95% of thyroid malignancies: papillary thyroid cancer accounts for 85% and follicular thyroid cancer for 10% of DTC [1]. Total or partial thyroidectomy is the initial

treatment for the majority of patients with DTC [2]. Postoperative remnant ablation with ^{131}I is indicated for all patients with stage 3 and 4 of the disease [3]. Remnant ablation requires TSH stimulation. This may be accomplished by withdrawing thyroid hormone treatment or by using recombinant human thyroid-stimulating hormone (rhTSH). RhTSH is a heterodimeric glycoprotein produced by recombinant DNA technology that has the ability to stimulate thyroglobulin production and radioiodine uptake by thyroid cells [4].

Nowadays, stimulation by recombinant human thyroid-stimulating hormone (rhTSH) has gained wide acceptance as an alternative to thyroid hormone withdrawal in the management of patients with DTC. RhTSH has the advantage to avoid both the clinical consequences of hypothyroidism, with a positive impact on the quality of life and work productivity [2]. Other authors demonstrated that the use of rhTSH is associated with a significant decrease in whole-body irradiation, which may be relevant [5] taking into account the collateral adverse effects of ^{131}I ablation therapy, where health tissues are also exposured to radiation. Many studies have also shown that clearance of ^{131}I from the body is faster in euthyroid status [5, 6], minimizing stochastic genetic risks. Another important advantage of rhTSH-aided cancer treatment is that it dismisses prolonged periods of high endogenous TSH stimulation, which may stimulate tumor growth. Several studies have found that the two methods are equally effective in preparing patients for ^{131}I remnant ablation, with a greater quality of life achieved when rhTSH is used [5, 7, 8].

It is known that ^{131}I is a reactor-produced radioisotope with a physical half-life of approximately 8 days which accumulates preferably in thyroid tissue [9]. It is a beta particles (E_{max} = 0.61 MeV and E_{avg} = 0.19 MeV) and gamma rays (E = 0.36 MeV) emitter, with about 90% of the secondary effects of its radiation being produced by beta particles, whose track length is relatively short (~0.8 mm) in soft tissue [10]. Because of its favorable physical and radiochemical characteristics, ^{131}I has been widely applied for therapeutic and diagnostic benefit for more than five decades.

In spite of the recognized therapeutic efficacy of r-hTSH for patients with DTC, the data regarding the impact of the rhTSH-aided cancer treatment associate with ^{131}I are still scarce at the cellular level and, consequently at the level of individuals in relation to conventional treatment. Only two studies have been carried out, as far as we know, on the cytogenetic effects of ^{131}I associated to rhTSH. One was initially carried out at our laboratory [11], utilizing an animal model which was studied through chromosome aberration technique. Another study compared the frequency of chromosome translocations between DTC patients off levothyroxine and those receiving rhTSH, measured before and 45 days after radioiodine treatment [12]. In this case, however, the authors analyzed only one type of chromosome aberration (translocation) that was assessed only 45 days after exposure to ^{131}I.

The present study was therefore proposed to evaluate the cytogenetic effects of the therapeutic exposure to radioiodine used for remnant ablation, in peripheral lymphocytes of DTC patients with either rhTSH or thyroid hormone withdrawal, using the chromosome aberration technique. The chromosome damage induced by treatment of euthyroid patients pretreated with rhTSH (group A) and of hypothyroid patients after prolonged levothyroxine

withdrawal (group B), were compared using blood samples collected 24h, 7 and 30 days after administration of ^{131}I. The degree of cytogenetic damage obtained after *in vivo* exposure was compared with that obtained *in vitro* for 24h, through of the construction of dose-response curve to different radioactive concentrations of ^{131}I (0.074 – 0.740 MBq/mL) to blood samples of healthy donors (group C). The estimate of absorbed dose of ^{131}I as a function of administered activity was carried out utilizing Monte Carlo calculation.

2. MATERIALS AND METHODS

2.1. Patients

The study was performed in 17 patients who had undergone near-total thyrodectomy for DTC. None of the patients had been previously treated with external radiotherapy or ^{131}I. The patients were randomly divided into two groups: group A (n = 8 ; seven females and one male; mean age 43.2 yr) was submitted to rhTSH stimulation (0.9 mg im per 2 consecutive days) while receiving LT4 therapy, whereas group B (n = 9; eight females and one male; mean age 47.2 yr) received radioiodine treatment following the standard protocol of LT4 withdrawal. A thyroid remnant ablation dose of 4995 to 7030 MBq ^{131}I (135 – 190 mCi) was administered. All patients provided written informed consent to the study protocol, which was approved by the Ethics Research Commission of the University Hospital of the University of São Paulo (FMUSP) on 23/06/2010 (n^0 349/10). The blood samples (peripheral lymphocytes) of each patient were collected for cytogenetic evaluation before administration of rhTSH and ^{131}I (basal), 24h, 7 and 30 days after radioiodine administration. All blood samples were supplied by FMUSP.

2.2. Healthy donors

In order to elaborate the dose-response curves, blood samples from 5 healthy donors from both sexes (2 males and 3 females), age range from 29 to 48 y, with an average of 37.4 years, with no irradiation history, non-smokers and with no drug treatment at the time of blood sampling, were analyzed. Thus, 1 mL of whole blood from each donor, in 3 mL of culture medium, was exposed to different radioactive concentrations of $Na^{131}I$, in order to obtain ^{131}I activities corresponding to 0.074 – 0.740 MBq/mL, equivalent to radioiodine activities of 370 – 3700 MBq (10 – 100 mCi) in human body, and maintained 24h at 37^0C. This period of time was chosen taking into account the maximum 24-h ^{131}I thyroid uptake in human [13]. The radioactivity range of ^{131}I was choosen considering a reference man of 70 kg body weight, with 5 L of blood receiving an oral activity of 3.7 GBq ^{131}I. The solutions of $Na^{131}I$ used in the assays were provided by the Center of Radiopharmacy of IPEN-CNEN (São Paulo, Brazil).

2.3. Cytogenetic assay

For chromosome damage evaluation, a culture with 1 mL of whole blood from each donor (patients or healthy donors), 3 mL RPMI medium (Cultilab) with 10 % foetal calf serum (Sigma), 100 μL of phytohaemaglutinin (5 μg/mL, Gibco MRL., USA) and 60 μL of BrdU (Sigma, St. Loius, USA) was incubated at 37^0C for 48h. Nearly 2h before fixation, 40 μL of Colcemid (0.7 μg/mL) (Sigma) were added. At the end of incubation, cells were harvested by centrifugation, submitted to hypotonic treatment with 0.075 M potassium chloride and 1 % sodium citrate (Merk), and then fixed in a fresh fixative solution of methanol and acetic acid (Merk), 3:1. This cell suspension was transferred to microscope slides in a pre-heated humid atmosphere at 65^0C, then air-dried overnight at room temperature. The slides were stained with Hoechst 33258 (Sigma), covered with 0.5 mL of McIlvaine buffer, pH 8.0 , exposed to UV light (245 nm) for 20 min at 60^0C, rinsed with distilled water, air-dried and stained for 15 min with 5% Giemsa (Cultilab) in Sorensen phosphate buffer, pH 6.8.

For the identification of different types of structural chromosome aberration, the criteria adopted by IAEA [14] were used. Only the metaphases containing 44 or more chromosomes were scored. Three parameters were considered: incidence of affected cells (percentage of cells with aberrations), degree of intracellular damage (number of cells with aberrations) and the occurrence of dicentric chromosomes, a specific type of chromosome aberration The number of cells with structural chromosome aberrations, the types of structural alterations, and the number of cells in different cycles of mitotic division and the number of chromosomes for each metaphase were registered.

2.4. Measurement of ^{131}I absorbed dose

In order to estimate the absorbed dose via biological dosimetry, the chromosome aberrations (dicentrics) frequencies observed *in vivo* in DTC patients lymphocytes after 24h of ^{131}I therapy were plotted in the dose-response curve, elaborated with data of healthy donors lymphocytes obtained after 24h of ^{131}I exposure *in vitro*.

Monte Carlo N-Particle Transport Code, version 4.0 program was used [15] to correlate the administered activity of ^{131}I (MBq) with the corresponding absorbed dose (Gy). This software utilizes a statistical method in which the physical process of radiation interaction with matter is simulated, considering three main variables, i.e., types of radiation involved (β particles, excitation electrons and photons), their energy spectrum, final volume and the atomic composition of samples.

2.5. Statistical analysis

The statistical analysis was performed using GraphPad Prism program (version 5.0) for graph and table elaboration. The dose-response curves obtained by plotting radioactive concentrations of ^{131}I versus frequency of chromosome aberrations were fitted by a linear – quadratic model, according to the equation $Y = \alpha D + \beta D^2$, where Y is the frequency of chromosome aberrations, α and β are constants of the model and D is the absorbed dose in Gy. The data obtained were analyzed by an ANOVA test, an analysis of variance that permits to determine differences between group means and their associated procedures. Statistical significance was assigned for $p < 0.05$.

3. RESULTS AND DISCUSSION

In the present study, the incidence of CA induced by radioiodine remnant ablation in DCT patients and comparison between the euthyroid or hypothyroid status were investigated. The patients of the two groups, although randomly assigned, were comparable for gender and age, thus avoiding any possible differences in CA yield associated with such clinical parameters.
The cytogenetic analysis of lymphocytes of patients treated with ^{131}I with or without rhTSH showed many types of structural chromosome aberrations, among them dicentrics, double minute, acentric fragments, gaps and chromosome breaks (Table 1, Fig. 1). The dicentric chromosome was the most frequent and is considered the best indicator of damage induced by ionizing radiation.

Table 1: Frequencies of structural chromosome aberrations (CA) observed in peripheral lymphocytes from patients of group A (rhTSH + ^{131}I) and group B (^{131}I) analyzed before and after administration of radioiodine.

Group	Sample	Number of analyzed cells	Structural CA						Cells with aberration (%)	Number of aberration/cell	Frequency of dicentric/cell
			Dic.	Ring	Ace.	Dm.	Gap	Break			
	rhTSH	1600	6	0	4	5	9	5	1,8	0,018	0,0062
	Basal	1277	8	0	1	5	6	4	1,8	0,018	0,0071
Group A	24h	1376	36	1	12	15	18	9	6,0	0,063	0,0262
(n=8)	7 days	1600	25	1	9	14	10	4	3,7	0,039	0,0175
	30 days	1304	9	0	11	7	9	1	2,5	0,026	0,0088
	Basal	1800	10	0	6	5	8	5	1,9	0,019	0,0078
Group B	24h	1800	42	0	19	18	16	9	5,8	0,058	0,0256
(n=9)	7 days	1667	29	0	13	15	8	10	4,3	0,044	0,0200
	30 days	1900	24	0	10	6	13	7	3,1	0,033	0,0167

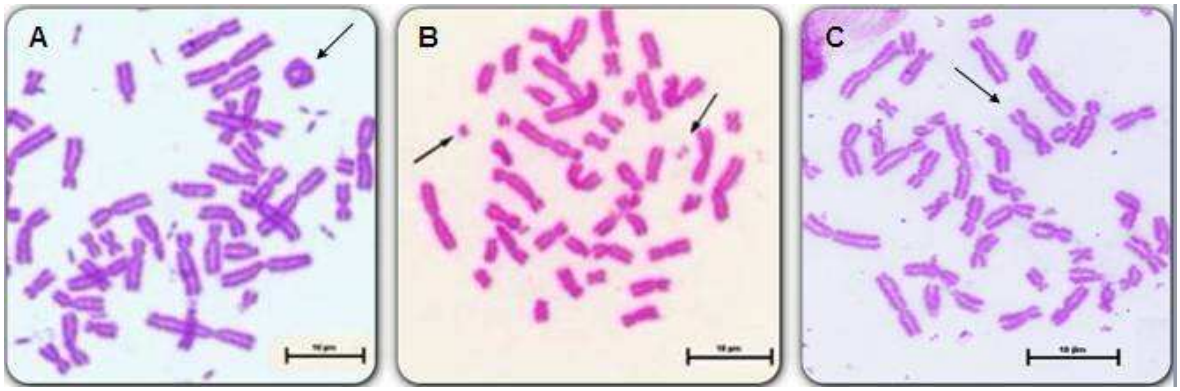

Figure 1: Photomicrographs showing structural chromosome aberrations observed in peripheral lymphocytes of differentiated thyroid cancer patients treated with ^{131}I. A) centric ring; B) double minute; C) dicentric.

Fig 2 shows the relation between the frequency of chromosome aberration obtained from patients (groups A and B) as a function of the time after radioiodine administration. An increase in the frequency of cells with chromosome aberrations was seen 24h after ^{131}I administration analyzed in relation to the basal values to both groups of patients, with a gradual decline in the three parameters, after 7 and 30 days. The high variability observed between patients can be due to the difference in the sensibility of each individual to the clastogenic action of radiation.

The modal chromosome number (2n=46) was higher than 90 % and no positive correlation was observed with ^{131}I or rhTSH administrations. In the same manner, the percentage of cells in the first mitotic division was higher than 90% in both basal and treated samples, suggesting that neither ^{131}I nor exogenous hTSH interfere in the cell cycle kinetics of human lymphocytes.

Fig. 3 shows the mean values of CA obtained from both groups of patients for each parameter considered as a function of time. Baseline CA did not differ in the patients from group A and B. A maximum value was observed 24h after treatment, while the mean value was higher for group A, probably as a consequence of the higher radioiodine uptake induced by TSH [16]. There was a tendency for a gradual decline after 7 and 30 days with higher values for group B, although not statistically significant ($p > 0.05$) for all parameters. The gradual decline with time of cells presenting genetic damage may be, in part, the result of various integrated mechanisms: selective death of lymphocytes damaged by radioiodine, normal turnover of cells and/or renal clearance of ^{131}I, among others. Some studies reported a faster clearance of ^{131}I (~50%) in patients submitted to rhTSH therapy in relation to radioiodine therapy [6], with consequent lower retention of whole-body ^{131}I, which can be in part, the result of a decline in the frequency of CA on circulating lymphocytes.

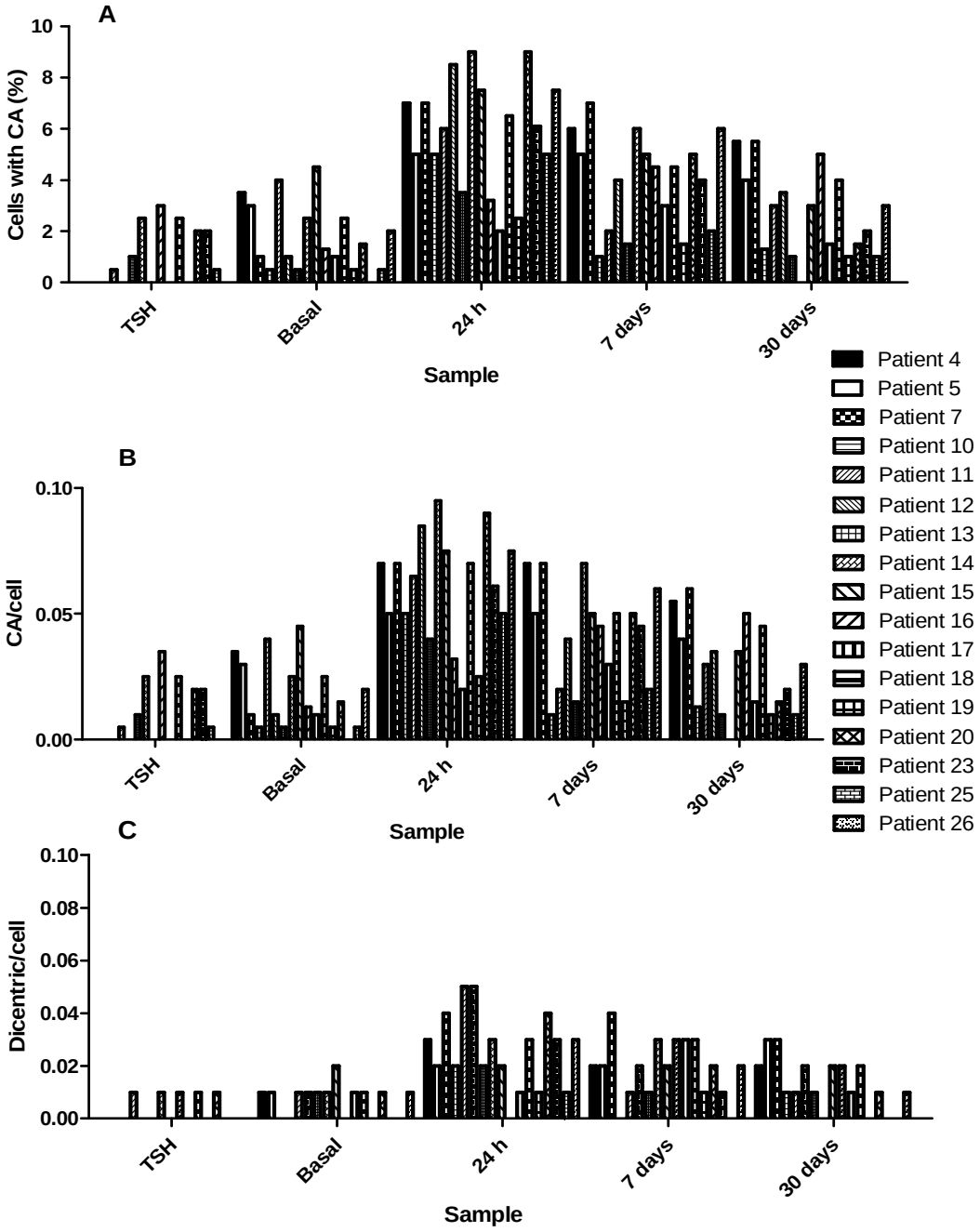

Figure 2: Frequencies of chromosome aberrations (CA) obtained in peripheral lymphocytes of patients (Groups A + B) as a function of time after administration of ^{131}I. A) Percentage of cells with CA; B) Number of CA/cell; C) Number of dicentrics/cell.

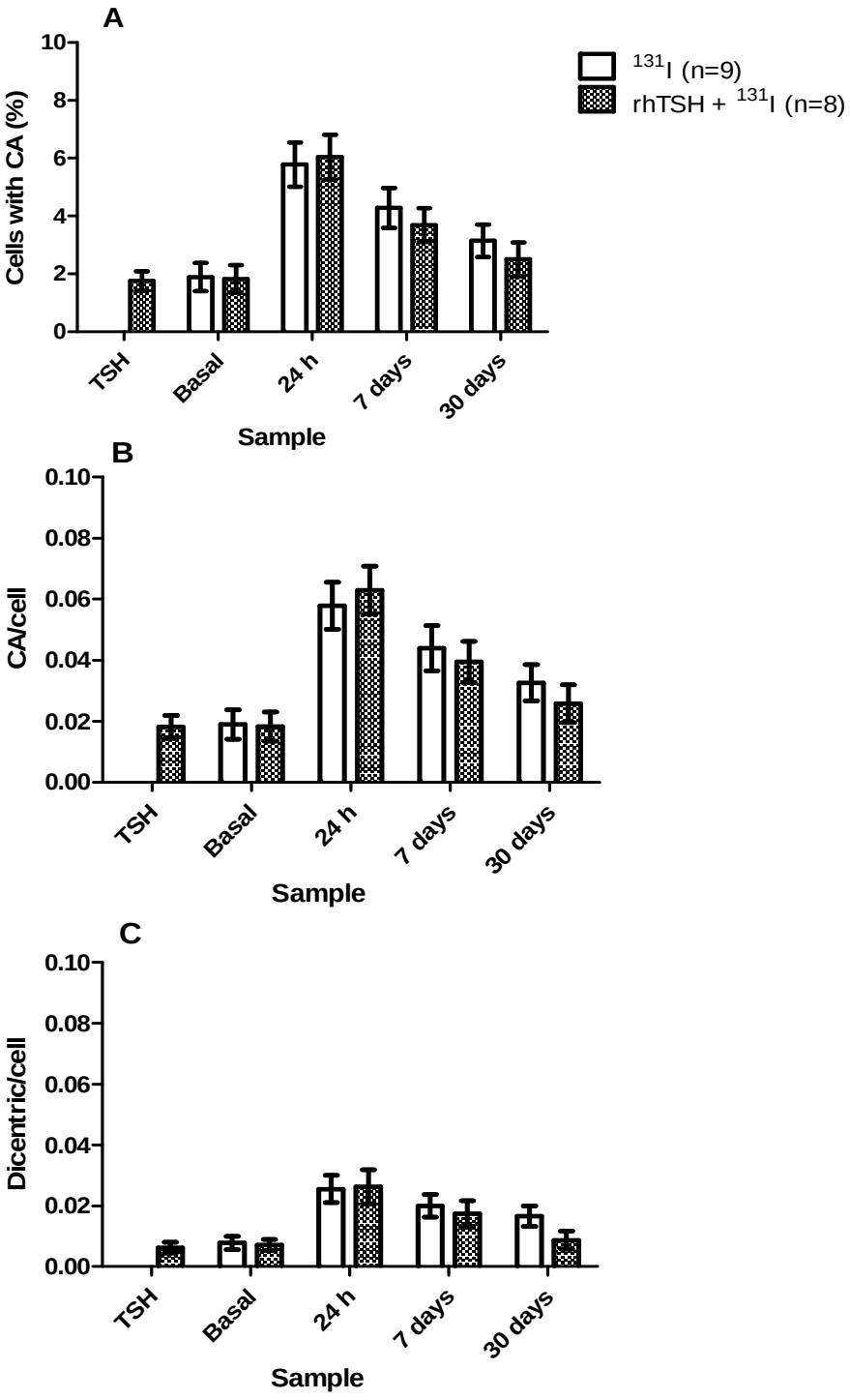

Figure 3: Mean of the frequencies of chromosome aberrations (CA) observed in peripheral lymphocytes in two groups of DTC patients (group A with rhTSH + ^{131}I and group B only with ^{131}I), before and after radioiodine treatment. A) Percentage of cells with CA; B) Number of CA/cell; C) Number of dicentric/cell.

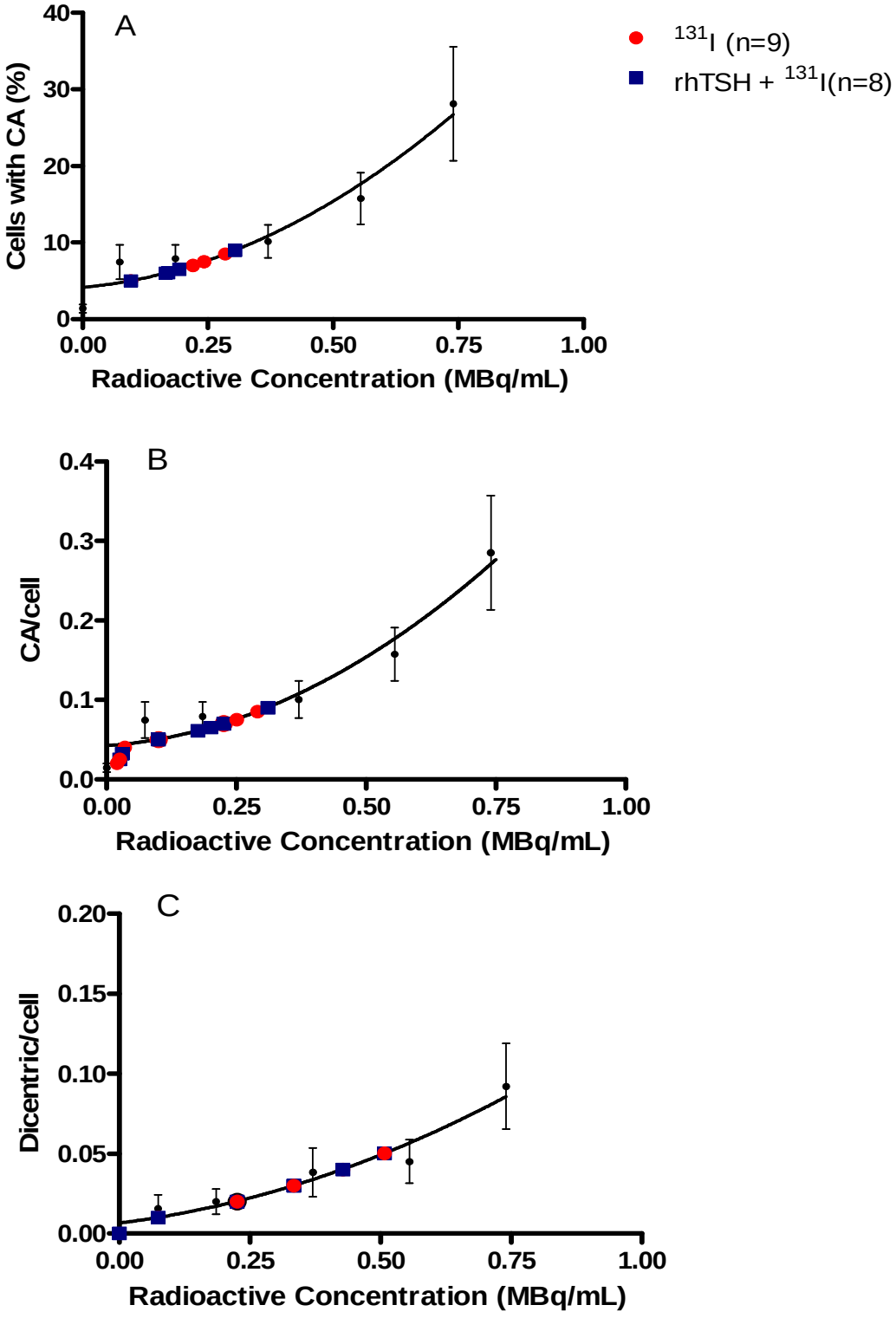

Figure 4: Dose-response curves of chromosome aberrations (CA) induction adjusted by linear-quadratic model ($Y = \alpha D + \beta D^2$) obtained from healthy donors, after exposure to different radioactivity concentrations of ^{131}I *in vitro*. The values obtained from DTC patients [■Group A (rhTSH + ^{131}I) and ●Group B (^{131}I)] were inserted into the curves. A) Percentage of cells with CA; B) Number of CA/cell; C) Number of dicentric/cell.

Fig. 4 shows the dose-response relationship for the induction of CA *in vitro*, as a function of the radioactive concentration of $Na^{131}I$, obtained from peripheral lymphocytes of healthy individuals. There was a trend to an increase in the frequency of cells with structural CA as a function of the concentration of ^{131}I. The data were best fitted to the linear-quadratic model, $Y=\alpha D + \beta D^2$, for the three parameters considered. The 17 patients`individual values (■ group A; ● group B) obtained 24h after administration of ^{131}I have plotted together with the dose-response curves. The cytogenetic damage, considering the number of dicentric per cell, obtained *in vivo* in the cells of the patients can be considered equivalent to the damage induced by the radioactive concentration of 0.07 – 0.55 MBq/mL of ^{131}I for 24h, *in vitro*. It is shown via Monte Carlo calculation that after rhTSH-aided administration of high ^{131}I activities, the estimated absorbed dose was up to ~1 Gy. A dose of less than 2 Gy has long been considered the safety threshold level for all radioiodine therapies [17].

The current report lies on the relatively small number of patients studied and the short time point of chromosome evaluation. Thus, cytogenetic analysis one year after ^{131}I administration is planned, in order to evaluate late cytogenetic effects due to the therapeutic exposure to radioiodine, as well as the renal clearance rate and the calculation of the absorbed dose in remnant thyroid and in whole-body for DTC patients off levothyroxine and for those receiving rhTSH.

4. CONCLUSION

The data obtained show that therapeutic exposure of ^{131}I associated or not with rhTSH induced chromosome damage in DCT patients with maximum values 24h after radioiodine administration with a gradual decline as function of time, higher in group A than in group B. Euthyroid patients pretreated with rhTSH showed, however, lower frequency of CA than hypothyroid patients after traditional prolonged levothyroxine withdrawal , 7 and 30 days after ^{131}I administration. Neither treatment, i.e., with ^{131}I only or associated with hTSH, resulted in an aneugenic effect or influenced the kinetics of cellular proliferation in blood lymphocytes. The data obtained also suggest that the use of rhTSH-aided thyroid remnant ablation is efficacy and secure for the patients with DTC. Further studies with a larger number of patients are needed, however, to confirm the current results.

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