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Cytogenetic Damage in the Peripheral Blood Lymphocytes of Thyroid Cancer Patients during Pre and Post ^{131}I Therapy

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ABSTRACT

Aim: *^{131}I therapy is widely used in patients of differentiated thyroid carcinoma for the ablation of remnant thyroid tissue post thyroidectomy as well as metastasis. These patients are in hypothyroid state with consequent reduction in renal clearance of ^{131}I thereby increasing the extent of whole body exposure. Present study was carried out to assess the DNA damage due to the presence of thyroid carcinoma as well as post-therapeutic ^{131}I exposure at 72h by measuring micronuclei (MN) frequency in peripheral blood lymphocytes (PBLs) in patients of differentiated thyroid cancer.*

Methods: *The study group consisted of 25 differentiated thyroid carcinoma patients and 35 healthy donors. The blood samples of the patients were collected pre and post ^{131}I therapy (1.6-9.3 GBq) and processed for PBL MN frequency by cytokinesis-block MN assay.*

Results: *The mean basal MN frequency was significantly high in thyroid cancer patients as compared to the healthy donors ($p< 0.001$). ^{131}I exposed patients demonstrated significant increase in the MN frequency at 72h after therapeutic ^{131}I exposure compared to their basal MN index ($p<0.001$). However the percent increase in MN frequency post ^{131}I therapy did not show any correlation with administered dose in thyroid cancer patients.*

Conclusion: *The study has revealed significant DNA damage in PBLs as indicated by increased PBL MN frequency post ^{131}I therapy. However marked individual variations were observed in the DNA damage response to ^{131}I therapy.*

Keywords: *Micronuclei, thyroid cancer, DNA damage, ^{131}I, internal radiation.*

INTRODUCTION

Internal administration of ^{131}I therapy to the patients of differentiated thyroid carcinoma (DTC) has been a standard mode of practice for the ablation of the remnant thyroid tissue. As these patients are in hypothyroid state due to the withdrawal of thyroxine hormone treatment, renal clearance of the ^{131}I is reduced and it's likely that it will get retained in the body for a longer while which may increase the extent of whole body ^{131}I exposure.(1) ^{131}I is a reactor produced beta gamma emitting radioisotope with physical half life of 8.03 days. (2) It may be assumed that some of the ^{131}I which gets bound to the plasma proteins and even inorganic iodide dissolved in plasma can impart dose to the PBLs .(3) Ionizing radiation is a strong physical mutagen and clastogenic agent causing single strand and double stranded breaks. (4) MN formation is one of the cytological consequences of chromosomal damage caused by radiation exposure.(3) MN are chromosomal fragments or whole chromosomes which do not get included in the nucleus during cell division but are present in the cytoplasm of daughter cells as small additional nuclei. (5) Various workers in past have observed increase of the MN frequency in PBLs of DTC patients treated with ^{131}I.(6-8) In the present study we have aimed at measuring the basal cytogenetic damage in the blood samples of patients with DTC and intended to compare the change in PBL MN frequency pre and post ^{131}I therapeutic exposure in presence and absence of metastasis. As change in PBL MN frequency has been reported to be dose dependent, we have also tried to correlate it with the administered dose in individual patients.

MATERIALS AND METHODS

The study group consisted of total 60 individuals. Out of which 25 (M=14, F=11) were patients of differentiated thyroid carcinoma visiting the Centre for ^{131}I therapy post- thyroidectomy. Amongst them 16 were patients of papillary carcinoma, 7 follicular carcinoma and 2 were suffering from follicular variant of papillary carcinoma. Presence of metastasis was found in 16 patients whereas 9 patients did not exhibit metastasis. All 25 patients received ^{131}I therapy ranging from 1.6 to 9.3 GBq (Table 1). The remaining 35 (M=20, F=15) were normal healthy volunteers of age ranging from 22 to 61 yrs and were considered as controls. Blood samples of the patients were collected pre and 72h post ^{131}I therapy and processed by cytokinesis-blocked MN assay procedure along with the blood samples of controls.

Micronucleus assay

0.5ml of blood sample was incubated with phytohemaglutinin (40µg/ml) in Iscove's Modified Dulbecco's Medium to which cytochalasin-B(6 µg/ml) was added at 44 hrs. The culture was further incubated and terminated at 72 hrs. The whole blood culture was treated with hypotonic 0.8% KCL solution. The separated blood lymphocytes were then washed in Carnoy's Fixative (Metanol: Acetic acid 3:1) and fixed in methanol. The slides were made and air dried at room temperature for 24h and were stained with Giemsa stain, observed under oil immersion objecttive and scored for minimum 1000 binucleate cells. (9)

STATISTICAL ANALYSIS

All the results were expressed as Mean ± SD. MN frequency of the healthy individuals was compared with cancer patients using unpaired students 't' test. The MN frequency of individual cancer patients pre and post ^{131}I therapy was analyzed using paired't' test. $p < 0.05$ was considered to be significant.

RESULTS

Table 1 describes the clinical characteristics of the total study group consisting of 60 individuals including 34 males and 26 females between age range of 14-68 yrs. The group was divided into two categories as control and thyroid cancer patients. In Table 2 distribution of PBL MN frequency in the group is described. The mean basal MN frequency of total study group ranged

from 2.0 MN/1000 BN cells to 111.2 MN/1000 BN cells. The PBL MN frequency in 35 healthy volunteers (M=20, F=15) serving as a control group was 8.9 ± 5.8 MN/1000 BN cells.(Table 2) When the mean basal MN frequency of patient group was compared with control, significant increase was noted in the patients of thyroid cancer group. (46.16 ± 26.4 MN/1000 BN cells vs 8.9 ± 5.8 MN/1000 BN cells, ($p< 0.001$, Table 2).

Effect of age, sex and presence of disease on the MN frequency in PBLs of the total study population

The mean PBL MN frequency did not vary with gender and age in the total study group. (Table 2) We further analyzed the MN frequency of the thyroid cancer patients according to their clinical status. Out of total 25 patients 16 patients were showing presence of metastasis involving one or more sites such as lung, lymph node and bone and 9 patients did not show metastasis during the study period. Presence of metastasis did not significantly alter the MN frequency (Metastasis: 45.5 ± 28.3 MN/1000 BN cells, No metastasis: 47 ± 24.4 MN/1000 BN cells) (Table 2).

Table 3 shows the individual data for administered ^{131}I dose, MN frequency pre and post ^{131}I exposure along with percent increase in the MN frequency post ^{131}I exposure in each of the 25 thyroid cancer patients.

Statistically significant but varied increase was observed in the MN frequency of the all 25 thyroid cancer patients 72 h after receiving the therapeutic ^{131}I dose as compared to their basal MN frequency before ^{131}I therapy ($p<0.001$, Table 3), (Fig 1). There was no correlation between the administered dose of ^{131}I and the increase in MN frequency (Fig 2).The percent increase in the MN frequency post ^{131}I therapy was slightly more in patients with metastasis as compared to those with no metastasis. Metastasis positive: 158 ± 102 MN/1000 BN cells, Metastasis negative: 139 ± 96 MN/1000 BN cells). However difference was not significant statistically.

Table 1 Clinical characteristics of the study group

Parameters	**Control**	**Thyroid cancer**
Number	35	25
Age (yrs)	22 - 61	14 - 68
Sex a) Male b) Female	 20 15	 14 11
Type of thyroid cancer a)Follicular b)Papillary c)Follicular variant of papillary		 7 16 2
Metastasis a)Present b)Absent	-	 16 9
Radiation dose (GBq)	-	1.6-9.3

Table 2. PBL MN frequency in the study group

Group	Total no of individuals	MN/1000BN cells	p value
Control	35	8.9 ± 5.8	
a)Gender			
Male	20	10.0 ± 7.1	NS
Female	15	7.4 ± 2.9	
b) Age(yr)			
=<36	19	8.8 ± 6.4	NS
>36	16	9.1 ± 5.2	
Patient	25	46.0 ± 26.4	<0.001 *
a)Gender			
Male	14	38.1 ± 23.6	NS
Female	11	56.2 ± 27.4	
b)Age (yr)			
=<36	12	43.6 ± 22.1	NS
>36	13	48.3 ± 30.7	
c)Metastasis			
Present	16	45.5 ± 28.3	NS
Absent	9	47.0 ± 24.4	
d) MN/1000BN cells			
Pre ^{131}I therapy	25	46.1 ± 26.4*	<0.001**
Post ^{131}I therapy	25	119.6 ± 84.8	

*vs Control, ** vs Post ^{131}I therapy

Table 3. Percent increase in PBL MN frequency in individual thyroid cancer patients Pre and_post therapeutic ^{131}I administration .

Table 3S No	Therapeutic Radiation^{131}I dose (GBq)	Pre ^{131}I MN/1000BN cells	Post ^{131}I MN/1000BN cells	% increase in MN/1000BNcells post ^{131}I therapy
1	7.4	38.3	69.1	80.4
2	7.1	26.2	68.7	162.2
3	6.8	34.8	82.7	137.6
4	7.4	29	105.5	263.8
5	5.1	92.7	184.1	98.6
6	7.1	102.7	226	120.0
7	6.9	111.2	310.4	179
8	7.4	68.1	306.8	350.5
9	7.0	48.5	146.4	201.8
10	6.8	58	190.8	228.9
11	3.3	50	214	328
12	7.6	57.3	214	273
13	7.8	62.6	121.4	93.9
14	6.3	46.3	74.6	61.1
15	2.1	14.8	33.2	124.6
16	3.7	39.6	47.8	20.4
17	9.3	36.5	155.5	326.3
18	1.8	23.8	40.9	71.3
19	7.9	36.4	67.8	86.3
20	3.2	23.5	42.1	79.3
21	7.7	29.7	98.1	230.4
22	1.6	25.4	32.3	27.2
23	7.4	13.4	23.7	76.9
24	9.3	66.3	98.3	48.3
25	5.6	16.7	36.2	116

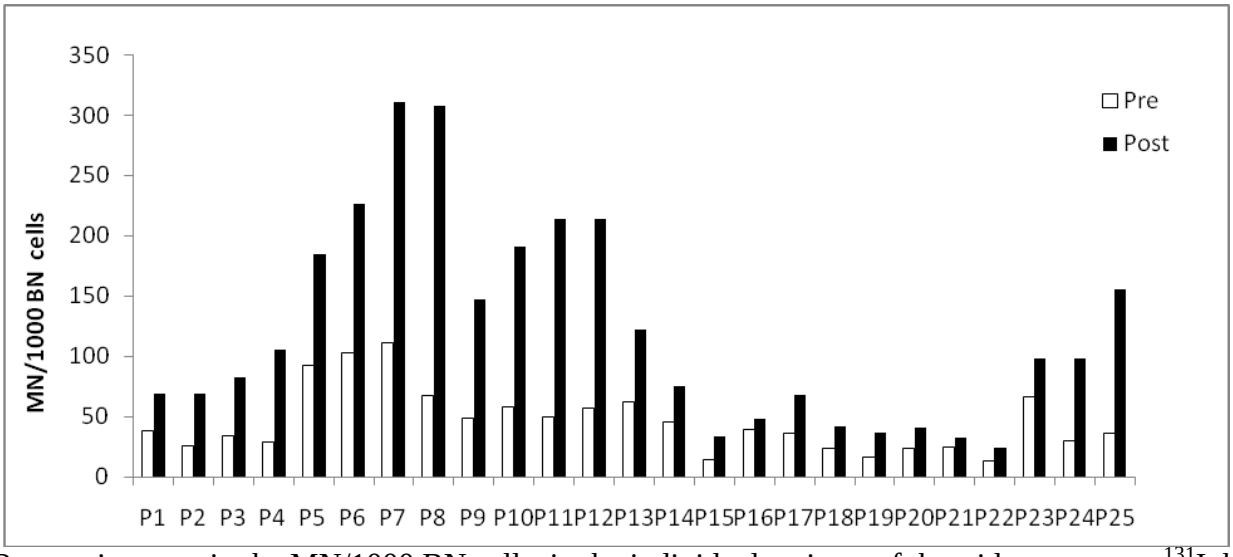

Fig 1 Percent increase in the MN/1000 BN cells in the individual patients of thyroid cancer post ^{131}I therapy

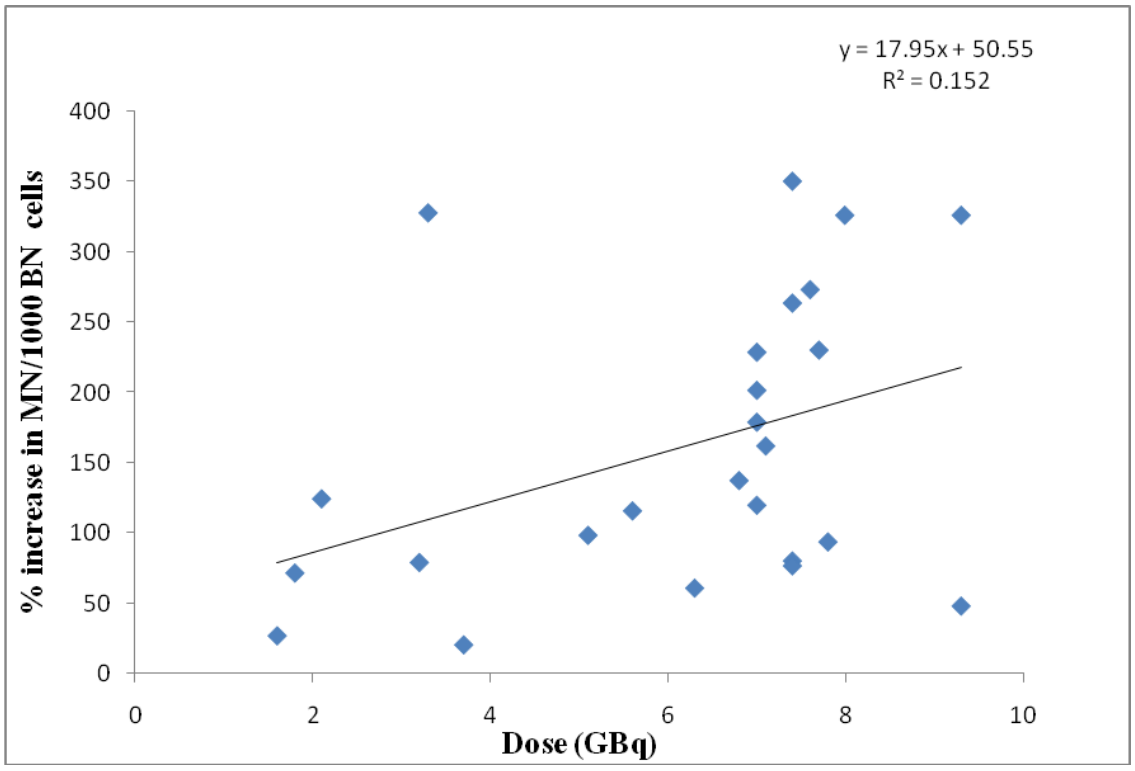

Fig 2. Correlation of the administered ^{131} I dose with percent increase in peripheral blood lymphocyte MN frequency in patients with thyroid cancer.

DISCUSSION

Many reports are available regarding the clastogenic effect of external radiation exposure but relatively less work is done for radioisotopes causing internal radiation exposure.[12-14] In the present study we have noticed significantly high PBL MN frequency in thyroid cancer patients as compared to the healthy volunteers even before their ^{131}I exposure, ($p<0.001$). Presence of carcinogenesis has been associated with increase in various chromosomal aberrations including MN. [15,16] Currently numerous studies have reported very strong correlation between the presence of cancer and increase in the MN frequency.[17,18] However equivocal reports are available regarding the relation of MN frequency

and thyroid cancer. [9,10,19,20] The present study is in agreement with reports exhibiting increased MN frequency in presence of thyroid cancer. [9,10,20] In a large cohort study of different types of cancer Bonassi *et al* (2007) [18] have attributed the increased PBL MN frequency to the defects in DNA repair and chromosome segregation which may result in generation of daughter cells with altered gene expression or number.[18] Use of molecular techniques such as FISH for identifying the chromosomal origin of the PBL MN may be useful in understanding the mechanism. Reports regarding the effect of age as well as gender on the MN frequency are not consistent and are contradictory.[21-23] However in our present study group no significant alteration was observed in the MN frequency based on the age and gender.

Many reports are available regarding the clastogenic effect of external radiation exposure but relatively less work is done for radioisotopes causing internal radiation exposure. [12-14] Differentiated thyroid carcinoma patients are often treated with oral ^{131}I which is a beta gamma emitter for ablation of remnant thyroid tissue or metastasis. Exposure to ^{131}I has been associated with chromosomal damage by using various cytogenetic markers.[4,8] Our study has exhibited a significant rise in the MN frequency at 72 h post ^{131}I therapy in all the thyroid cancer patients indicating additional cytogenetic damage due to ^{131}I exposure. Watanabe *et a l* [6] in their study have also reported peaking in PBL MN frequency after 3 days of ^{131}I therapy. Our observation is consistent with that of various other investigators who have also observed the significant increment in the cytogenetic damage as reflected by MN frequency after 72h of ^{131}I exposure. [1,8,11]

MN frequency is known to show a dose dependent increase with radiation exposure.[26] Vrndic *et a l* [3] have reported increase in PBL MN frequency post ^{131}I exposure which was correlated positively with the accumulation of the ^{131}I at 72h in the thyroid region and not with metastasis indicating importance of presence of ^{131}I concentrating thyroidal tissue in estimating the whole body exposure [3]. In our study we have not estimated the accumulation of ^{131}I *in vivo* and not observed any effect of metastasis and PBL MN frequency . Simillarly Sundaram *et al* [2] in their study did not observe any correlation of administered ^{131}I with increase in PBL MN frequency in hyperthyroid patients. [2] Administered ^{131}I dose may not give the idea about the actual retention of ^{131}I *in vivo* as no correlation was observed between the MN frequency and administered radioactivity. [2] In our present patient group similar findings were noted.

It has been observed that increase in PBL MN frequency is not only dependent on the radiation dose received, but also is a function of radiosensitivity of the cells, ability of the metastatic tumor to concentrate the ^{131}I and rate of renal clearance of the ^{131}I [1,3] . The differences existing in individual cellular radiosensitivity is attributed to many factors such as previous exposure to radiations, presence of disease and individual genetic makeup. [24,25] Ban *et al* [24]. (2004) in their study have reported differences in the radio sensitivity of PBLs in different types of cancer. Individual differences in the renal clearance of the ^{131}I may affect the level of radiation exposure to their PBLs. [24]

Patients of differentiated thyroid carcinoma receive at least one therapeutic dose of ^{131}I for ablation of the remnant thyroidal tissue and may get further exposure repetitively depending on the recurrence of the disease. In the instances of repetitive exposure ensuring the safety of dose along with the efficacy of the treatment is of great importance. Conventional physical dosimetry in such cases gives the information about the exposure received by calculating the absorbed dose but may not give idea about the biological effect of the same in individual patients [26]. Use of cytogenetic biomarkers in these cases can provide the additional information about the radiotoxicity of the therapeutic dose of ^{131}I. Currently use of various chromosomal aberrations including dicentrics, translocations, sister chrom-atid exchanges and other biological markers such

as gamma -H2AX ,glutamine, glycophorein A are being used for assessing the DNA damage post radiation exposure.(26) Amongst all these evaluation of PBL MN frequency is relatively simple and economical and hence was selected as a cytogenetic marker in present study.

This study has revealed increase in PBL MN frequency in patients of thyroid cancer which was further elevated after therapeutic ^{131}I exposure at 72h. It will be very useful in understanding the radio sensitivity of individual as the increase did not show any dose dependency, thereby indicating individual variation in DNA damage in response to *in vivo* ^{131}I exposure. Further work in larger patient population along with additional molecular markers for DNA damage can give detailed information about the origin and the mechanism of MN generation in individual patients. This will be helpful in improving management of the thyroid cancer patients in future.

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