Microsatellite instability and its correlation with clinicopathological features in a series of thyroid tumors prevalent in iodine deficient areas

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Abbreviations: AJCC, American Joint Committee on Cancer; AMES, Age, distant metastases, extra thyroidal invasion, tumor size; BA, Biallelic alteration; FNABC, Fine needle aspiration biopsy cytology; FTC, Follicular thyroid carcinoma; IDAs, Iodine deficient areas; ISAs, Iodine sufficient areas; LOH, loss of heterozygosity; MNG, Multinodular goiter; MSI-L, MSI-Low; MSI-H, MSI-High; MSI/MIN, Microsatellite instability; MSS, Microsatellite stable; MTC, Medullary thyroid carcinoma; PCR, Polymerase chain reaction; PTDC, Poorly differentiated thyroid carcinoma; PTC, Papillary thyroid carcinoma; RER+, Replication error positive; UI, Urinary Iodine

Abstract

Thyroid tumors display diverse spectrum of histopathological groups with geographic variation in its prevalence. Influence of iodine deficiency (a major causative factor) in its etiology, prevalence, or aggressiveness is debatable which reflects the existence of various genetic events in pathogenesis. The present study was undertaken to study the role of Microsatellite instability (MSI) or LOH (loss of heterozygosity), an indicator of defective mismatch repair system as a genetic change and to explore it as a prognostic marker in thyroid tumors. Tumor tissues from total thyroidectomy surgical specimens and blood (matched control) of 36 patients from iodine deficient areas (10 benign; 26 malignant) were obtained after their consent. Urinary iodine analysis was done by alkali ash method for which 10 ml of urine was collected from 18 patients before surgery. Genomic DNA,

isolated from tumor tissue and blood was amplified by polymerase chain reaction (PCR) using mono and dinucleotide markers - BAT-26, BAT-40, TGF(RII, IGFIR, hMSH3, BAX, D2S123, D9S283, D9S851 and D18S58. PCR products were analysed on 8% denaturing polyacrylamide gel followed by autoradiography. Of total, 66.6% of tumors [70% (7/10) benign and 65.4% malignant cases (17/26)] showed MSI/LOH. Strong association of MSI/LOH with low iodine ($P = 0.01$) and with AMES risk groups *i.e.* age ($P = 0.02$), tumor size ($P = 0.04$) and metastases ($P = 0.002$) in thyroid tumors was observed. This may help in predicting the biological behaviour and strengthening the hypothesis that iodine deficiency has influence on MSI in thyroid tumors. Our results further substantiate the risk group classification and help in deciding the treatment modality in particular patient.

Keywords: Iodine deficiency; Microsatellite markers; Risk group; Thyroid cancer

Introduction

Thyroid tumors are the most frequent neoplasms of the endocrine system. The present therapy is based on patient age, tumor size and histology that decide the risk potential (Goretzki *et al.*, 1998). Though total thyroidectomy is recommended for all cases of differentiated thyroid carcinoma as a primary treatment, sometimes hemithyroidectomy in low risk patients is advocated (Gemsenjager *et al.*, 1997). Geographic variation in the biological behaviour has been observed. In the endemic iodine deficient region, total thyroidectomy has been recommended by some investigators irrespective of risk group even in occult papillary thyroid cancer (Mishra *et al.*, 2002a). This discrepancy in deciding the tumor behaviour and treatment protocols encourages the development of molecular prognostic markers which could provide possible answer in predicting the biological behaviour of thyroid tumors.

Diverse spectrum of tumors originating from thyroid follicular cell, exhibiting different biological behaviour is suggestive of alternative pathways of thyroid tumorigenesis (Farid *et al.*, 1994). Faulty mismatch repair mechanism can be demonstrated by frequent

somatic variation in the size of microsatellites called as microsatellite instability (MSI/ MIN) or loss of one allele known as loss of heterozygosity (LOH) in tumor DNA as compared with matching normal DNA (Ionov *et al.*, 1993). It reflects a defect in DNA replication or repair in damaged DNA and therefore, also known as replication error positive (RER+) phenotype which persists throughout the lifetime of the tumor (Shibata *et al.*, 1994).

Lower incidence of MSI in thyroid tumors is because mutations in mismatch repair genes have been identified only in minority of cases. In a series of nine cases no incidence of MSI was reported (Vermiglio *et al.*, 1995) whereas limited incidence of MSI was observed in larger series of tumors (Soares *et al.*, 1997). PCR based MSI analysis in thyroid tumors may allow identification of MMR status that can be further correlated with the risk groups that could help in deciding the extent of treatment in a particular patient. This study was undertaken to evaluate the status of MSI/LOH in thyroid tumors using mono and dinucleotide microsatellite markers situated on various chromosomes and to identify its association with high and low risk groups thereby exploring its role as a prognostic marker in thyroid cancer.

Iodine deficiency has been considered as a major factor in the higher incidence of follicular and anaplastic carcinoma in iodine deficient areas (IDAs) as compared to papillary thyroid carcinoma in iodine sufficient areas (ISAs) with controversies (Franceschi, 1998). The relationship of endemic goiter and thyroid carcinoma including, prevalence, distribution and aggressiveness is debatable. Although iodine deficiency has been studied as a major environmental factor that leads to thyroid tumorigenesis, the wide variation in response to iodine deficiency as measured by the prevalence of follicular and papillary carcinoma is also reported. Since the role of iodine deficiency on MSI/LOH, which reflects the defective MMR system, is not known, the present work was undertaken to study the influence of iodine deficiency on the response to DNA damage in thyroid tumors.

Materials and Methods

Patient selection

Clinical assessment of thyroid tumors was based on history of goiter. Fine needle aspiration biopsy cytology (FNABC) was performed for the diagnosis of thyroid tumors. Total thyroidectomy was proposed in all cases. Following total thyroidectomy whole body radioiodine scan was performed to detect metastases. After radioactive iodine ablation of residual and metastatic deposits thyroid suppression therapy was ins-

tituted in all cases. All available clinical data was combined with pathologic study of surgically resected specimen for assessment of risk group. Tissue specimens of thirty six cases of sporadic thyroid tumors from unrelated patients hailing from iodine deficient regions were obtained from total thyroidectomy surgical specimens during the last two years at endocrine surgery operation theatre in our institute. From the same group of patients, 5 ml of blood was taken in 200 μ l of 0.5 M EDTA as control after taking their consent. The samples were immediately snap frozen in liquid nitrogen and stored at -70°C till DNA extraction. The present study material comprised of histopathology of series of thyroid tumors such as single case of multinodular goiter with oncocyctic carcinoma, adenomatous goiter, Hashimoto thyroiditis, oncocyctic adenoma, microfollicular adenoma, two cases of multinodular goiter (MNG), poorly differentiated thyroid carcinoma (PDTC), three cases of diffuse hyperplasia and medullary thyroid carcinoma (MTC), nine cases of follicular thyroid carcinoma (FTC) and twelve cases of papillary thyroid carcinoma (PTC). A total of ten benign and twenty-six malignant cases were taken for the study. Thyroid tumors were diagnosed, classified and staged according to American Joint Committee on Cancer (AJCC) criteria (Beahrs *et al.*, 1988). The clinicopathological features of 36 cases taken for the present study are demonstrated in Table 1.

For analysis of urinary iodine, 10 ml of urine of eighteen patients out of 36 could be collected before surgery. The subset (18 patients) included six cases of PTC, four cases of FTC, two cases of PDTC and three cases of MTC and one case of Hashimoto's thyroiditis, oncocyctic adenoma and multinodular goiter with oncocyctic carcinoma.

DNA extraction from blood and tissue specimen

DNA was isolated from tumor tissue as well as blood using phenol-chloroform extraction method (Blin and Stafford, 1976).

Microsatellite analysis

Table 2 shows characteristics of six mono - and four dinucleotide microsatellite markers, which were studied to examine MSI/LOH in thyroid tumors. The primer sequences for markers were searched from human genomic database. Polymerase Chain Reaction (PCR) amplification of DNA was done with primer pairs of concentration of 6 pmol, 200 μ M dNTPs, 10 mM Tris - Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl_2 , 0.25 U of *Taq* polymerase (MSI, Fermentas), 100 ng DNA and 2 μ Ci [α - ^{32}P] dCTP (specific activity: 4,000 Ci/mmol) (BRIT, India) in a total volume of 25 μ l. PCR conditions involved an initial denaturation at 95°C for 3 min followed by 30 cycles (95°C for 1 min,

50°C to 60°C for 2 min and 72°C for 3 min) and a final extension at 72°C for 8 min. PCR products were mixed with equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% Xylene cyanol), denatured for 3 min at 95°C and loaded onto an 8% polyacrylamide gel containing 7 M urea. Gels were run at 55 W for 2 hours, transferred onto a Whatman sheet, dried under vacuum and autoradiography was done.

The tumor was called unstable if its PCR product had altered band pattern when compared to alleles in corresponding matched blood DNA (Zhou *et al.*, 1997). The changes could be mono-allelic (insertion/deletion), bi-allelic (biallelic alteration) and LOH. MSI/LOH were categorized as MSI-High (MSI-H), if change was observed at or more than 30% loci studied and MSI-Low (MSI-L) if it was observed at less than 30% of loci examined.

Determination of Urinary Iodine

Urinary iodine was analyzed using alkali ash method (Karmarkar *et al.*, 1986) with slight modifications. Urine sample was mixed to evenly suspend any sediment. Appropriate dilutions were prepared from a stock iodine solution (168.5 mg in 100 ml of water) to give standards covering a range of 0-20 mg/L and volume was made up to 100 µl. To 100 µl of urine sample, 300 µl of potassium carbonate (0.00063 N) was added to each tube and mixed. After drying the

samples at 80°C to 100°C for overnight, ashing was done by placing the samples into the muffle furnace at 600°C for six h. After cooling, 3 ml of sodium meta-arsenite (0.035 N) was added and centrifuged at 2,500 g for seven min. The supernatant was incubated for five min at 56°C in a water bath followed by addition of 3 ml of ceric ammonium sulfate (0.005 N) at 30 s intervals to each tube, mixed, and replaced in a water bath. Twenty min after the addition of the ceric ammonium sulfate, transmittance was read at 420 mµ in a spectrophotometer against water (blank) to calculate the concentration (mg/L) of urinary iodine.

Statistical analysis

Non-parametric tests including Fisher's exact probability test (two tailed) and 2×2 contingency table with Yates correction were applied to study the association between MSI/LOH and clinicopathological features in thyroid tumors. Karl Pearson's correlation coefficient test was used to examine the correlation of iodine deficiency with MSI/LOH.

Results

Microsatellite analysis

Thirty-six cases paired to their controls (blood), were examined for MSI at six mononucleotide and four

Figure 1. Abnormal electrophoretic migration at mono and di-nucleotide repetitive regions, identified in tumor DNA (T) when compared to blood DNA (control, N). (A) BAT-26: Insertion; (B) BAT-40: Insertion; (C) D2S123: Deletion; (D) D9S283: Loss of Heterozygosity; (E) D9S1851: Insertion; (F) D18S58: Loss of heterozygosity. Changes are indicated by an arrow.

Figure 2. No change in allelic pattern at mononucleotide microsatellite markers observed in tumor DNA (T) as compared to germline DNA (N). (A) BAX: (lanes 1-8); (B) hMSH3: (lanes 1-4); (C) TGFβRII: (lanes 1-4); (D) IGFIR: (lanes 1-4).

Table 1. Clinicopathological features of thyroid tumors.

Case no.	Gender* /Age**	Diagnosis	TNM	Tumor size (cm)
1	F/24	Diffuse hyperplasia	na	na
2	F/46	PTC	T3NoMx	5
3	F/66	FTC	T3NoM1	6
4	F/46	FTC	T4bNoM1	5
5	F/44	PTC	T3N1Mx	8
6	M/31	Diffuse hyperplasia	na	na
7	F/44	MNG	na	na
8	M/38	Microfollicular adenoma	na	na
9	F/50	Diffuse hyperplasia	na	na
10	F/60	Adenomatous goitre	na	na
11	F/50	PDTC	T4NoM1	11×9
12	F/19	MNG	na	10×8
13	M/40	PTC	T4N1bM1	6
14	M/65	MTC	T4bNoMx	25×20
15	M/36	MTC	T3N1Mx	2
16	F/56	FTC	T3NoM1	10
17	F/68	Hashimoto's thyroiditis MNG with oncocytic	na	8
18	F/60	Carcinoma	na	na
19	M/67	FTC	T4NoM1	8
20	F/27	PTC	TxN1M1	14.5
21	F/55	FTC	T4NoM1	10
22	F/43	PTC	T3N1Mx	6
23	F/51	Oncocytic adenoma	na	7
24	M/55	MTC	T3N1Mx	7
25	M/55	FTC	T4NoMx	7
26	F/37	PTC	T2NoMx	4.5
27	F/65	FTC	T2NoM1	4
28	F/33	PTC	T2NoMx	4
29	F/48	PDTC	T4N1Mo	10
30	M/62	PTC	TxN1Mx	>5
31	F/26	PTC	T2bN1bMx	3.5
32	M/27	PTC	T4N1Mo	4
33	M/44	FTC	T2NoMx	6
34	M/57	PTC	T3N1Mo	7
35	M/38	FTC	TxN1Mo	2×1.5
36	M/38	PTC	T4NoM1	<5

*M = Male; F = Female; **Age in years; TNM (Tumor Node Metastases) staging according to AJCC criteria (Beahrs *et al.*, 1988); PTC (Papillary thyroid carcinoma); FTC (Follicular thyroid carcinoma); PDTC (Poor differentiated thyroid carcinoma); MTC (Medullary thyroid carcinoma) and MNG (Multinodular goiter); na = not available.

Table 2. Characteristic features of microsatellite markers examined in thyroid tumors or tumor like lesions.

Name of marker	Repeat pattern	Chromosomal location	~PCR product size
BAT 26	(A) ₂₆	5 th intron of hMSH2, 2p	117-130 bp
BAT 40	(A) ₄₀	2 nd intron of β hydroxy steroid dehydrogenase	94-112 bp
BAX (38-41)	(G) ₈	19q13.3-q13.4	94 bp
TGF β RII (665-737)	(A) ₁₀	3p22	73 bp
IGFIIR (4030-4140)	(G) ₈	6q26-27	110 bp
hMSH3 (381-383)	(A) ₈	5q	150 bp
D2S123	(CA) ₁₃ TA (CA) ₁₅ (T/GA) ₇	2p16	197-227 bp
D9S283	(CA) _n	9q13-q22	178-203 bp
D9S1851	(CA) _n	9q22.3	143-159 bp
D18S58	(GC) ₅ GA(CA) ₁₇	18q22.3	144-160 bp

dinucleotide microsatellite loci. Of total, 66.6% tumors showed MSI/LOH, which includes 70% (7/10) benign and 65.4% malignant cases (17/26) as evident by electrophoretic mobility shifts in tumor DNA as compared to blood DNA. These changes were monitored as mono allelic (deletion, insertion), biallelic allelic [bi-allelic alteration (BA)] and LOH (Figure 1). However, no change has been observed at TGF β RII, IGFIIR, BAX and hMSH3 loci in tumor DNA (Figure 2).

Out of the total benign tumors analyzed for MSI/LOH, six cases were unstable at BAT - 40, while a single case of diffuse hyperplasia was unstable at BAT - 26 and D9S1851. Rest of them were microsatellite stable (MSS).

Of malignant cases, PTC (66.6%) (8/12) and FTC (77.7%) (7/9) exhibited instability at BAT - 26, BAT - 40, D2S123, D9S283, D9S1851 and D18S58. Out of the two cases of PDTC examined, single case was unstable at D9S283 and another one was observed as stable. Chromosomes 2, 9 and 18 exhibited allelic changes at D2S123, D9S283 and D18S58 respectively in a single case of FTC. Two out of three (66.6%) MTC exhibited alteration at BAT - 40 and D2S123 while the remaining one did not harbor any change.

Microsatellite alterations and risk assessment

Twenty-six cases of malignant thyroid tumors were evaluated for their association between the risk groups by studying independent variables like patient age, tumor size and metastases vs instability or stability at microsatellites. The risk assessment was done according to AMES (Age, distant metastases, extra thyroidal invasion, tumor size) risk classification (Kebebew and Clark, 2001). Men of age less than 40 years and women less than 50 years are at low risk if they do not have distant metastases. Older patients with intra-thyroidal tumor with minor capsular invasion, primary tumor of size less than 5 cm and no distant metastases are at low risk potential. MSI/LOH positive malignant tumors were found to be present in 81.2% (13/16), 83.37% (15/18) and 65% (13/20) of high-risk patients when compared with age, tumor size and metastases respectively. However, MSI/LOH was observed in 40% (4/10); 37.5% (3/8)

Table 3. Association of microsatellite changes with risk of developing thyroid carcinoma by studying independent variables.

Case no.	MSI/LOH and risk groups			
	Age	Tumor size	Metastases	MSI-L/MSI-H
2	Low	High	Low	+ve (MSI-L)
3	High	High	High	+ve (MSI-L)
4	High	High	High	+ve (MSI-L)
5	Low	High	High	-ve
11	High	High	High	-ve
13	High	High	High	+ve (MSI-L)
14	High	High	Low	+ve (MSI-L)
15	Low	Low	High	-ve
16	High	High	High	+ve (MSI-H)
19	High	High	High	+ve (MSI-L)
20	High	High	High	+ve (MSI-H)
21	High	High	High	+ve (MSI-H)
22	Low	High	High	-ve
24	High	High	High	+ve (MSI-L)
25	Low	High	Low	+ve (MSI-H)
26	Low	Low	Low	+ve (MSI-H)
27	High	Low	High	-ve
28	Low	Low	Low	-ve
29	Low	High	High	+ve (MSI-L)
30	High	High	High	+ve (MSI-L)
31	Low	Low	High	-ve
32	Low	Low	High	+ve (MSI-L)
33	High	High	Low	+ve (MSI-L)
34	High	High	High	+ve (MSI-L)
35	Low	Low	High	-ve
36	High	Low	High	+ve (MSI-H)

Age: Male <40 years; Female <50 years (Low risk). Tumor size: less than 5 cm (Low risk). Metastases (with nodal involvement): High risk (TNM classification on the basis of histopathology). MSI: -MSI-L (low); MSI-H (High); -ve (stable).

and 83.3% (5/6) low risk patients (Table 3). Significant association was observed between MSI/LOH and low risk groups [$P = 0.02$ for age, $P = 0.04$ for tumor size and $P = 0.002$ for metastases using Fisher's exact probability test (two tailed)].

MSI-L and MSI-H

In the present study only one benign case presented with diffuse hyperplasia (10%) (1/10) exhibited MSI-H. However, 33.3% cases of FTC (3/9) 25% cases of PTC (3/12) and no case of PDTC (0/2) manifested MSI-H. None of the MTC (0/3) studied showed MSI - H. MSI-L was found to be frequent in the low and

Table 4. Evaluation of MSI/LOH with different microsatellite markers and urinary iodine in thyroid tumors and tumor like lesions.

Case no.	Microsatellite changes	Urinary Iodine (mg/L)
1	*High (BAT-26, D9S1851)	na
2	**Low (BAT-26)	10.5
3	Low (D9S283)	< 1.25
4	Low (BAT-26)	11.25
5	***MSS	> 20
6	Low (BAT -40)	na
7	Low (BAT-40)	na
8	MSS	na
9	Low (BAT-40)	na
10	MSS	na
11	MSS	< 1.25
12	Low (BAT-40)	na
13	Low (BAT-40)	na
14	Low (D2S123)	< 5.0
15	MSS	> 20
16	High (BAT-40, D18S58)	10.5
17	Low (BAT-40)	11
18	MSS	5.75
19	Low (BAT-26)	< 1.25
20	High (BAT-40, D9S283, D9S1851)	< 1.25
21	High (D2S123, D9S1851)	na
22	MSS	< 5.0
23	Low (BAT-40)	> 20
24	Low (BAT-40)	> 20
25	High (D2S123, D9S283, D18S58)	na
26	High (D2S123, D9S283)	na
27	MSS	na
28	MSS	10
29	Low (D9S283)	7.5
30	Low (BAT-40)	na
31	MSS	na
32	High (D9S283, D9S1851, D18S58)	< 1.25
33	Low (D9S283)	na
34	Low (D9S283)	na
35	MSS	na
36	High (BAT-40)	na

*MSI-High (MSI-H); **MSI-low (MSI-L); ***Microsatellite Stable (MSS); na = not available.

high-risk groups but a strong association of MSI-H was seen in low risk group (tumor size) [Fisher's exact probability test (two tailed), $P = 0.17$ for age; $P = 0.1$ for metastases and $P = 0.002$ for tumor size].

MSI/LOH and tumor stage

Clinical stages reflecting the behaviour of the tumors were studied with MSI/LOH. Fourteen out of eighteen malignant tumors (78%) with clinical stage T3 or T4 demonstrated MSI (Fisher's exact probability test [two tailed], $P = 0.006$). However, only 40% tumors with T2 stage showed MSI/LOH.

Evaluation of Urinary Iodine and its Association with MSI/LOH

Urinary iodine (UI) values in mg/L were calculated in 15/26 thyroid carcinomas and 3/10 benign tumors (Table 4). The range between 0-20 mg/L has been considered as low while normal value lies above 20 mg/L.

Out of the total twelve cases of carcinoma excluding MTC, examined for UI, eleven had low UI. Out of eleven, eight were microsatellite unstable and three were MSS (72.3% vs 27.3%) [Pearson's correlation coefficient test ($P = 0.01$)].

Out of three cases of MTC, two showed MSI and each had low and normal UI value. However, the remaining case was MSS and had normal UI. Very weak association could be established between low UI and MSI ($P = 0.2$) using 2X2 contingency table with Yates correction.

Discussion

Carcinoma of the thyroid is an infrequent tumor with geographic variation in its prevalence (Farid *et al.*, 1994). Environmental factor like iodine deficiency plays a crucial role in promoting the development of thyroid carcinoma. Higher incidence of follicular and anaplastic carcinoma is seen in IDAs as compared to ISAs (Lawal *et al.*, 2001). Higher incidence of metastases in IDAs is observed where the majority of cases had synchronous, symptomatic skeletal metastases (Mishra *et al.*, 2002b). Diverse spectrum of tumors and tumor like lesions originating from thyroid follicular cell, exhibiting different biological behaviour is suggestive of alternative pathways of thyroid tumorigenesis (Farid *et al.*, 1994). Occult thyroid cancers are rarely (0.9%) present as distant metastases (Lind *et al.*, 1996) and commonly regarded as being of no clinical relevance. Recently a study on patients (most of them hailed from IDAs) with differentiated thyroid cancers (4/156), who presented initially with distant metastases and no gross signs of thyroid dis-

ease, described the aggressiveness of the tumors (Mishra *et al.*, 2002a). This is in consistence with the existence of various genetic events influenced by the iodine deficiency, involved in predisposing to thyroid carcinoma originating from follicular cells, which provides useful model to study its molecular genetics and may provide a rationale approach in deciding clinical treatment.

Microsatellite instability/LOH, an indicator of faulty DNA mismatch repair system is believed to be associated with the genetic defects, including the activation or inactivation of specific genes and promote tumorigenesis.

Recently attention has been centered on PCR based analysis of MSI/LOH in tumor tissues as compared to corresponding matched normal, has been proved to be helpful in understanding the genetic basis of thyroid tumorigenesis that allows the identification and characterization of number of molecular events underlying the multistep process of transformation of normal thyrocytes into differentiated thyroid adenomas to carcinomas to poorly differentiated carcinomas.

Up to now studies on the occurrence and role of MSI/LOH in thyroid tumors are fragmentary. A study based on small series of cases and limited number of microsatellite markers examined could not show any evidence of MSI (Farid *et al.*, 1994; Alves *et al.*, 2000) whereas other reports have been suggestive of limited role of MSI in both benign and malignant tumors using the different set of microsatellite markers (Soares *et al.*, 1997; Zhou *et al.*, 1997; Tomita *et al.*, 1999). In our present study, 66.6% of thyroid tumors exhibited MSI/LOH on examining six mono and four dinucleotide microsatellite markers, including all the benign tumors. This present work is in continuation of the previous published work (Vaish *et al.*, 2003) where all the ten benign tumors were analyzed for the presence of MSI at BAT-26, BAT-40, TGF(RII, IGFIIR, hMSH3 and BAX. The initial results encouraged examining the role of MSI/LOH in malignant tumors as well with more number of markers to establish MSI as a prognostic marker.

The equivalent incidence of MSI (70%) in benign and (65.4%) in malignant thyroid cancers supports the hypothesis that MSI/LOH, which causes genomic instability is responsible for thyroid tumorigenesis at the early stage as well. Higher incidence of MSI was observed using different panel of markers in malignant vs benign tumors (Stoler *et al.*, 2002). Our present data is in concordance with the previous study using a different subset of markers (Soares *et al.*, 1997). Another study described overall frequency of 21.5% in fifty-one thyroid tumors (Lazzereschi *et al.*, 1999), which differs from ours. High frequency of MSI at BAT - 40 and BAT - 26 in present study sug-

gests that the biochemical defects governing the spectrum of MSI in thyroid and colorectal cancer share some similarity that contradicts the previous finding (Onda *et al.*, 2001).

Relatively higher frequency of MSI/LOH in FTC followed by PTC and PDTC in our study explains the impaired efficiency of DNA repair mechanisms that promotes carcinogenesis as well as contributes to tumor progression. All the mono-allelic, bi-allelic changes and LOH were observed in FTC and PTC at different microsatellites located on 2, 9 and 18 chromosomes in contrast to MTC. This study indicates different molecular mechanisms playing role in the pathogenesis of PTC or FTC and MTC however; the number of MTC cases is too low to draw such conclusion.

We report the presence of deletion as the most frequent microsatellite alteration. Deletion at chromosome 1, 2, 9 and 18 may hypothesize the presence of tumor suppressor gene flanking the respective markers. Absence of instability at TGF(RII, IGFIR, BAX, hMSH3 and D2S123 loci rules out the possibility of any genetic defect at these loci.

According to AMES risk classification, the three independent risk factors viz. age, tumor size and metastases were studied with the MSI/LOH status. Association of MSI/ LOH with low risk group may help in further defining the treatment protocols where the conservative approach is followed. MSI - H was found to be frequently associated with high-risk group cases. This finding is contradictory to the previous one where no associations between MSI status, patient's age, gender and tumor size were observed (Lazzereschi *et al.*, 1999).

We report higher prevalence of MSI-L in tumors behaving aggressively with advanced clinical stage (T3 or more). However, no significant association has been observed with MSI - H and clinical stage.

Many studies from IDAs report a preponderance of advanced lesions at presentation, which as such contribute to poor prognosis (Sarda and Kapur, 1990). We see relatively higher number of FTC and PDTC compared to ISAs. Some reports suggest that iodine deficiency may act as promoter rather than carcinogens in rats (Ohshima and Ward, 1984). This indicates that iodine deficiency somehow alters the molecular pathway that further leads to tumorigenesis. Correlation of urinary iodine values in a subset of patients was carried out with MSI/LOH results. To our surprise, it was observed that all the patients, diagnosed with FTC, PTC or PDTC exhibiting MSI/LOH were found to have low urinary iodine value. To the best of our knowledge this is the first report of its kind. However, no relation between benign tumors and low urinary iodine could be established. Iodine deficiency is involved in thyroid tumorigenesis, influ-

encing its genotypic and phenotypic features (Tallini, 2001). Our observations may suggest that iodine deficiency influences MSI/LOH, an indicator of defective MMR functions in thyroid tumorigenesis. It may be possible that thyroid carcinoma arising from follicular cell in IDAs has unique ways to respond to DNA damage that should be substantiated by further studies.

These findings confer that MSI/LOH not only acts as an early event in thyroid tumorigenesis but also is involved in progression of thyroid carcinoma, thereby having a great impact on the biology of thyroid tumors. Higher prevalence of MSI/LOH and its frequent association with advanced clinical stage could help in predicting biological behaviour of thyroid tumors. Examination of MSI/LOH with a panel of microsatellite markers in clinical subjects may further substantiate risk group classification in thyroid carcinoma patients and thereby defining the treatment protocols in a particular patient.

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