MICROSATELLITE INSTABILITY IN SQUAMOUS CELL CARCINOMA OF HEAD AND NECK FROM THE INDIAN PATIENT POPULATION

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Genomic instability in simple repeated sequences has been observed in several human cancers. We have analyzed 50 squamous cell carcinomas of the head and neck (SCCHN) and 5 pre-malignant severe dysplastic tissues from Indian patient populations for microsatellite instability in 18 different loci spread over eight different chromosomes. Among the tumors analyzed, 45% exhibited instability at two or more loci, and 15% exhibited instability at 40% of the markers tested. Similar analysis of SCCHN tumors from other populations (British, American and French) showed much less frequency of instability. SCCHN tumors in the present study did not show any instability in the mononucleotide repeat sequences. There is also a clear distinction in the nature of the instability in these tumors in comparison with colorectal tumors. These results suggest that the underlying mechanism generating this type of instability is different from those reported for colorectal tumors.

Key words: *microsatellite instability; head and neck tumor; Indian patient population*

Squamous cell carcinoma of the head and neck (SCCHN) is one of the leading cancers in India. It is estimated that in the year 2001 the total number of new cases will be 137,000.[1] Cancer prevalence shows a geographical pattern attributable to lifestyle. Differences in lifestyle factors in determining regional variations of cancer prevalence may be crucial for a country like India with a wide ethnic and lifestyle diversity. It has been speculated that both ethnic background and lifestyle differences may contribute to the differences in predisposing factors to cancer development.[1]

A crucial molecular change measured in various types of cancers is genome-wide alteration in simple repeat sequences, which was observed initially in hereditary nonpolyposis colon cancer (HNPCC) as well as in sporadic colon cancer[2–4] and subsequently in many other tumors.[5] This type of instability, also known as microsatellite instability (MIN), can be detected as a change in the length of microsatellite sequences in tumor DNA compared with normal DNA from the same individual. Such alterations reflect a defect in replication or repair known as the replication error (RER) phenotype, which persists throughout the lifetime of the tumor.[6,7]

In HNPCC, MIN is apparently due to inherited and somatic mutations in any of the five mismatch repair (MMR) genes such as *hMSH2*,[8,9] *hMLH1*,[10,11] *hPMS1* and *hPMS2*[12] and *hMSH6/GTBP*,[13] located on human chromosomes 2p22-p21, 3p21.3, 2q31-q33, 7p22 and 2p16, respectively. Somatic mutations in MMR genes were also identified in sporadic colorectal primary tumors and cell lines demonstrating the RER^+ phenotype.[14,15] Colorectal cell lines with defective MMR genes exhibited at least a 10- to 100-fold increased rate of spontaneous mutation within expressed genes.[16,17] This observation provides a mechanism linking the RER^+ phenotype to the accelerated development of cancer in HNPCC kindred. The link between DNA repair defects with a specific pathway of tumor progression has been made from the observations that some human colon cancer cell lines and primary tumors with high frequency of MIN harbor mutations within small repeated sequences in the *TGF-β type II receptor* gene,[18] the *insulin-like growth factor II receptor* gene[19] and the *Bax* gene.[20]

The MIN detected in different tumors varies considerably. For example, 55% to 86% of the HNPCC tumors and other tumors arising in HNPCC kindred exhibited MIN, but only 15% of the sporadic colorectal tumors were observed to have such instability.[5] Similarly, the incidence of MIN in other sporadic tumors varies from as high as 67% in pancreatic tumors to as low as 3% in breast, brain, bladder and liver cancers.[5] The available data suggest that not all sporadic cancers showing the RER^+ phenotype can actually be accounted for by somatic mutations in known MMR genes.[21–24] It has therefore been suggested that MIN, occurring in tumors other than HNPCC, is attributable to defects in genes distinct from those responsible for colorectal cancer.[21]

Incidences of RER^+ type MIN reported for SCCHN in British and American patients are 28% and 30%, respectively.[25,26] In a recent French study, an even lower incidence of MIN (12.5%) has been reported.[27] In this report we show that the frequency of RER^+ type MIN in SCCHN tumors is much higher in the Indian patient population than that reported for the other populations. We also report that several features of the MIN in these tumors are distinctly different from that observed in colorectal tumors.

MATERIAL AND METHODS

Tumor samples

Fifty freshly operated primary SCCHN tumors and 5 samples of severely dysplastic tissue, along with their corresponding normal tissues or peripheral blood leukocytes (PBLs), were collected from the patients prior to treatment. Samples were immediately frozen and stored at −80°C. All the tumors were histopathologically diagnosed as squamous cell carcinoma and graded and staged according to the UIC TNM classification. The detailed history of the 50 patients' primary SCCHN tumors is presented in Table I.

Among the 50 patients, 35 were male and 15 female. The mean age of patients was 49.5 years. Patients were grouped into three

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TABLE I – HISTORY OF PATIENTS WITH SCCHN TUMORS USED IN THE STUDY[1]

Tumor	Age (yr)/sex	Site	Stage	Histopathology	Lymph node
A. Oral cavity SCCHN tumors					
0125	35/F	BM	—	SEVE DYS	—
2024	52/M	BM	—	SEVE DYS	—
3037	55/M	BM	—	SEVE DYS	—
0751	25/M	BM	—	SEVE DYS	—
0710	42/M	BM	—	SEVE DYS	—
4119	60/M	BM	I	WDSCC	—
1491	58/M	BM	I	WDSCC	—
D27	62/F	TNG	I	WDSCC	+
4976	45/F	AL	II	WDSCC	—
0939	50/M	BM	II	MDSCC	—
4546	35/F	BM	II	WDSCC	—
6085	62/M	BM	II	WDSCC	—
5943	60/M	HP	II	WDSCC	+
0825	32/M	P	II	PDSCC	—
0826	50/M	P	II	PDSCC	—
0222	60/F	TNG	II	WDSCC	—
7524	34/F	TNS	II	WDSCC	—
4491	34/M	TNS	II	MDSCC	+
D12	35/M	AL	III	WDSCC	+
D30	38/M	AL	III	WDSCC	+
3915	50/M	AL	III	WDSCC	+
2086	55/F	BM	III	WDSCC	+
4446	50/M	BM	III	WDSCC	+
4332	54/M	BM	III	WDSCC	+
0452	42/M	L	III	PDSCC	—
D25	53/M	TNG	III	WDSCC	+
D28	42/F	TNG	III	WDSCC	—
3471	52/M	TNG	III	WDSCC	+
1367	60/M	TNS	III	MDSCC	—
7783	45/F	TNS	III	WDSCC	+
2705	65/F	BM	IV	MDSCC	+
D24	70/M	TNG	IV	WDSCC	+
4274	35/F	TNG	IV	WDSCC	+
0802	50/M	TNS	IV	MDSCC	+
B. Laryngeal SCCHN tumors					
6290	62/M	Larynx	II	PDSCC	—
0693	50/M	Larynx	III	MDSCC	—
1295	75/M	Larynx	III	PDSCC	—
1445	70/M	Larynx	IV	WDSCC	—
4708	56/M	Larynx	IV	MDSCC	+
2878	69/M	Larynx	IV	WDSCC	—
C. Orofacial SCCHN tumors					
2234	22/M	MD	II	WDSCC	—
3970	46/M	MD	II	WDSCC	—
0149	38/M	MD	II	WDSCC	—
0051	76/M	MX	II	WDSCC	—
3216	38/M	NC	II	MDSCC	—
5364	37/M	MX	III	MDSCC	+
D21	30/F	MX	III	WDSCC	+
2618	42/F	MX	IV	PDSCC	+
D29	45/F	MX	IV	PDSCC	+
4904	40/F	NC	IV	PDSCC	+

[1]AL, alveolus; BM, buccal mucosa; HP, hypopharynx; L, lip; MD, mandible; MX, maxilla; NC, nasal cavity; P, palate; TNG, tongue; TNS, tonsil; SCC, squamous cell carcinoma; SEVE DYS, severe dysplasia; WDSCC, well-differentiated SCC; MDSCC, moderately differentiated SCC; PDSCC, poorly differentiated SCC.

categories, as follows: 1) oral cavity, 2) larynx and 3) orofacial, on the basis of affected primary sites. Thirty-four tumors were found in the oral cavity (4 alveolus, 14 buccal mucosa, 7 tongue, 5 tonsil, 2 palate and 1 each hypopharynx and lip), 6 tumors were from the larynx and 10 tumors were categorized as orofacial tumors (5 maxilla, 3 mandible and 2 nasal cavity). Severe dysplastic changes were observed in five tissues. Histopathologically the tumors were classified as stage I (3 tumors), stage II (16 tumors), stage III (16 tumors) and stage 4 (10 tumors). Among these 50 tumors, 22 were lymph node positive, and 28 were diagnosed as free from any lymph node involvement. For five patients (D6, 377, 333, 4717 and D35), no history was available except that they were from the head and neck region.

Microdissection and DNA isolation

The normal cells present as contaminants in the primary tumor tissues were removed by a microdissection procedure.[28] More than 50 to 60 serial tumor sections (10 to 20 μm) were dissected and placed on glass slides using cryostat (model CM 1800, Leica, Heidelberg, Germany). The representative 5 μm tumor sections from different regions of the tumor (beginning, middle and end) were stained with hematoxylin and eosin for diagnosis as well as for marking the tumor-rich regions. The normal tissues present in the tumor sections were then removed by microdissection. The adjacent normal tissues of the primary tumor were similarly dissected for the presence of tumor cell infiltration. For those cases in which the "normal" tissue contained any tumor cell infiltration,

TABLE II – FREQUENCY OF INSTABILITY OF MICROSATELLITE MARKERS IN SCCHN TUMORS[1]

Loci	Chromosomal location	Type of repeat	Percentage of tumors showing instability
D3S1276	3p12	$(CA)_n$	16 (8/50)
D3S1611	3p21.3	$(CA)_n$	14.8 (8/54)
D3S1293	3p24-25	$(CA)_n$	9.4 (5/53)
D3S1307	3p26	$(CA)_n$	14.8 (8/54)
D9S104	9p11-12	$(CA)_n$	20 (7/35)
D9S171	9p21	$(CA)_n$	18.5 (5/27)
D9S15	9p21	$(CA)_n$	27 (10/37)
D9S168	9p21	$(CA)_n$	30.8 (12/39)
D9S54	9p23	$(CA)_n$	31.4 (11/35)
HD	4p16.3	$(CAG)_n$	35.5 (11/31)
SAT	6p21-23	$(CAG)_n$	18.4 (9/49)
DRPLA	12p13.31	$(CAG)_n$	19.2 (10/52)
DM	19q13.1-q26.3	$(CTG)_n$	29.7 (11/37)
AR	Xq11-12	$(CAG)_nCAA$	27.3 (12/44)
DHFRP2	6	$(AAAC)_n$	13.5 (7/52)
vWF	12p12-12p ter	$(TCTA)_n$	11.5 (3/26)
ACPP	3.3q21-qter	$(TAAA)_n$	10.2 (5/49)
BAT26	2p26	A_{26}	4 (1/25)[2]

[1]Numbers in parentheses in the fourth column indicate tumors showing instability out of total tumors analyzed.–[2]Alteration in BAT26 (mononucleotide repeat) was examined only in those tumors that showed instability in two or more markers.

PBLs of the corresponding patient were taken as normal. The microdissected tumor samples containing <60% tumor cells were not taken for further analysis. High molecular weight DNA from the tissues was extracted by proteinase K digestion followed by phenol-chloroform extraction.[29]

Polymerase chain reaction

Microsatellite loci (Table II) were amplified by PCR in a 25 µl reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 10% DMSO, 0.2 mM of each dNTPs, 4 pmol of each primer, an optimal concentration of $MgCl_2$ (1.5 to 3 mM) determined separately for each primer set, and 50 to 100 ng of template genomic DNA. Reaction mixtures were heated to 95°C for 3 min, cooled to annealing temperature (50° to 65°C) to add 0.5 U Taq DNA Polymerase (GIBCO BRL, Gaithersburg, MD) and cycled for 30 times in GeneAmp 9600 (Perkin-Elmer, Oak Brook, IL) PCR machine. Each cycle was 10 sec at 95°C, 5 sec at the appropriate annealing temperature (50° to 60°C) and 10 sec at 72°C. Final extension was carried out at 72°C for 7 min. Samples without DNA for each primer pair were included as negative controls. Prior to PCR, the forward primer in the reaction mixture was end-labeled with [γ^{32}P]dATP (specific activity 3,000 Ci/mM, Amersham, Buckinghamshire, UK) using T4 polynucleotide kinase (GIBCO BRL). Two microliters of the amplified DNA was electrophoresed on a denaturing 6% polyacrylamide gel at 45 to 60 W for 2 to 4 hours. After electrophoresis the gels were exposed to X-ray films (KODAK X-Omat) for autoradiography.

Definition of microsatellite instability and loss of heterozygosity

The status of MIN was scored as positive if one or both alleles in the tumor exhibited size variation due to either expansion or contraction of the repeat sequences in comparison with paired normal tissue from the same individual. Loss of heterozygosity (LOH) was determined by densitometric scanning (Shimadzu, Singapore, Singapore CS-900) of the autoradiographs. A given locus was scored as having LOH if the relative band intensity of one allele was at least 50% decreased in the tumor samples compared with normal samples. The value was calculated as the ratio of the band intensities of the larger to the smaller bands in the tumor DNA samples divided by the same ratio in the normal DNA samples. An LOH index of >1.5 (loss of the smaller allele) or <0.67 (loss of the larger allele) corresponds to at least a 50% reduction in relative band intensities.

TABLE III – MICROSATELLITE INSTABILITY (MIN) IN SCCHN TUMORS SHOWING ALTERATIONS IN ≥2 MARKERS

Tumor	% of MIN for dinucleotide repeat	% of MIN for trinucleotide repeat	% of MIN for tetranucleotide repeat	Overall % of MIN
D06	100 (4/4)	100 (5/5)	0 (0/1)	90 (9/10)
4708	50 (2/4)	75 (3/4)	100 (3/3)	73 (8/11)
4904	78 (7/9)	80 (4/5)	33 (1/3)	71 (12/17)
4332	56 (5/9)	100 (4/4)	50 (1/2)	67 (10/15)
2086	50 (4/8)	100 (3/3)	50 (1/2)	62 (8/13)
0693	57 (4/7)	80 (4/5)	0 (0/3)	53 (8/15)
0825	37 (3/8)	80 (4/5)	33 (1/3)	50 (8/16)
1445	44 (4/9)	80 (4/5)	0 (0/2)	50 (8/16)
3970	0 (0/9)	80 (4/5)	66 (2/3)	35 (6/17)
0149	56 (5/9)	20 (1/5)	0 (0/2)	38 (6/16)
0710	0 (0/4)	60 (3/5)	33 (1/3)	33 (4/12)
3216	44 (4/9)	0 (0/4)	33 (1/3)	31 (5/16)
5364	44 (4/9)	20 (1/5)	0 (0/2)	31 (5/16)
5943	37 (3/8)	25 (1/4)	0 (0/3)	26 (4/15)
D30	25 (1/4)	50 (1/2)	0 (0/2)	25 (2/8)
0751	0 (0/4)	60 (3/5)	0 (0/3)	25 (3/12)
4446	37 (3/8)	0 (0/5)	0 (0/2)	20 (3/15)
1491	25 (2/8)	20 (1/5)	0 (0/3)	19 (3/16)
D21	25 (1/4)	20 (1/5)	0 (0/2)	18 (2/11)
0802	22 (2/9)	20 (1/5)	0 (0/3)	18 (3/17)
7783	33 (3/9)	0 (0/5)	0 (0/3)	18 (3/17)
6085	11 (1/9)	100 (1/1)	0 (0/2)	17 (2/12)
1295	0 (0/9)	50 (1/2)	33 (1/3)	14 (2/14)
0377	11 (1/8)	20 (1/5)	0 (0/2)	13 (2/15)
1367	22 (2/9)	0 (0/5)	0 (0/2)	13 (2/16)

[1]Values in parentheses indicate number of markers observed to have instability out of total markers analyzed for that tumor.

RESULTS

Frequency of MIN in SCCHN tumors

Fifty SCCHN tumors and five severely dysplastic tissues were examined for genomic instability at 18 microsatellite loci (1 mono-, 9 di-, 5 tri- and 3 tetranucleotide repeats) spread over eight different chromosomes (Table II). Three of these markers (HD, DM and DRPLA) are associated with different neurological disorders and show germline expansion. Since chromosomes 3 and 9 show frequent LOH in SCCHN tumors, among 18 markers selected, 4 were from chromosome 3 and five from chromosome 9. Also, five mononucleotide repeat sequences from different growth-related and repair genes such as *BAX*, *IGFIIR*, *TGFβRII*, *hMSH3* and *hMSH6* were analyzed[18–20,30] only in those tumors that exhibited instability in two or more of the loci listed above (Table II). Each microsatellite locus was amplified in paired normal and tumor DNA samples. Fifty-one of 55 tumors were analyzed for at least 10 markers, and the frequency of instability for each marker was determined (Table II). Differences in DNA band patterns between tumor and normal cell pairs were observed to be 1) at least in one locus in the case of 60% tumors (33/55) and 2) in two or more loci in the case of 45% tumors (25/55).

Relatively recently it has been proposed that in certain tumors larger repeats are more commonly altered than smaller repeats.[31] This prompted us to determine MIN levels separately for di-, tri- and tetranucleotide repeats for each tumor that exhibited instability in more than two markers (Table III). Among 25 tumors examined, 21 exhibited instability for both di- and trinucleotide repeats, but only 9 tumors were observed to be unstable for tetranucleotide repeats. Thus, previously reported "elevated microsatellite alterations at selected tetranucleotide" (EMAST) repeats for non-HNPCC tumors including head and neck cancers[31] have not been observed in the present study using a limited number (three) of tetranucleotide repeats. We therefore based our classification of the 25 tumors showing instability at two or more loci on overall MIN observed and compared with the data obtained in studies on SCCHN tumors in other populations.

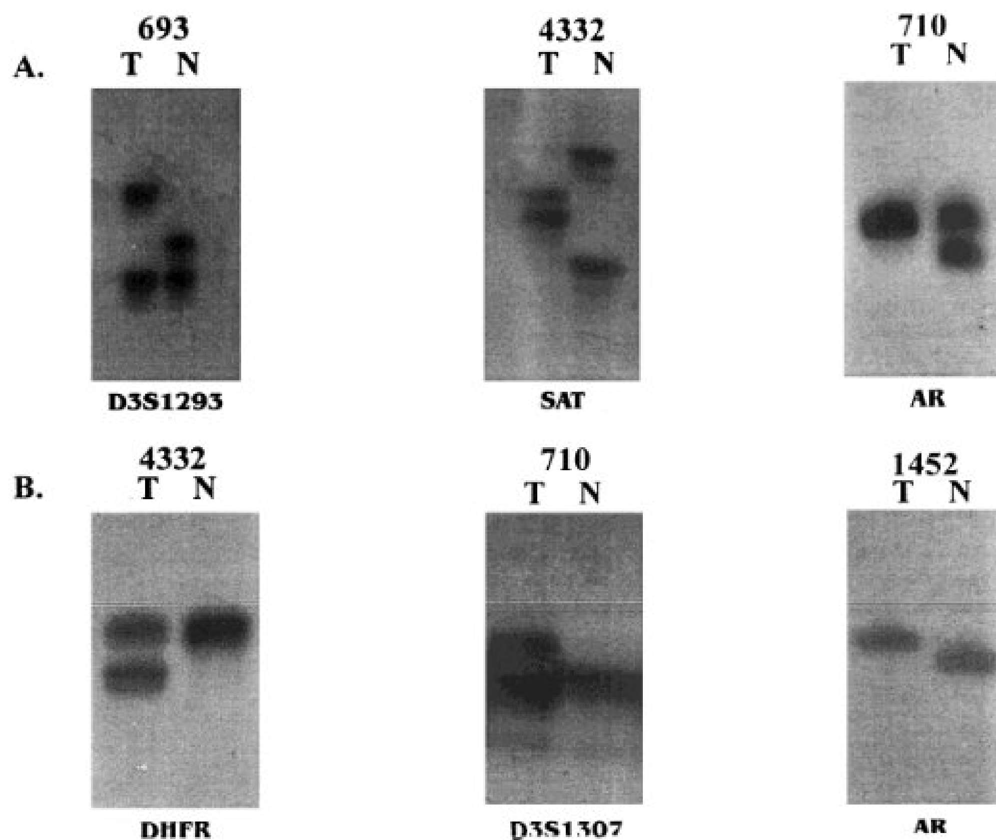

FIGURE 1 – Patterns of allelic alteration in microsatellite unstable SCCHN tumours. The markers (shown at the bottom of each panel) were amplified from paired tumor (T) and normal (N) tissues taken from selected tumor samples (shown at the top of each panel), analyzed by denaturing polyacrylamide gel (6%) and autoradiographed. Patient samples are shown in which the tested markers are heterozygous (*a*) and homozygous (*b*) in normal tissues.

Eight tumors (15% of the total 55 tumors) show instability at >40% of the markers and 17 tumors (30% of 55) show instability at 10% to 40% of the markers tested (Table III). Among 17 tumors in the low MIN group (10% to 40% instability), four (#3970, #0710, #0751 and #1295) exhibited instability for tri- and/or tetranucleotide markers only. The remaining 30 tumors exhibited MIN for <10% of the markers tested; these may be considered as microsatellite stable tumors (data not shown). Interestingly, seven of eight tumors that exhibited instability at >40% of the markers tested were observed to have MIN in the D3S1611 marker, located within *hMLH1* gene. Five of these tumors exhibited LOH at D3S1611 (data not shown), suggesting that these tumors probably have a defective *hMLH1* gene.

Types of MIN observed in SCCHN tumors

Six different types of alterations in the microsatellite sequences were observed in the present study (Fig. 1). Those markers that were heterozygous in normal tissues were observed to have three different types of alterations in tumors: 1) alteration in length of one allele (Fig. 1*a*, tumor 0693), 2) alteration in length of both alleles (Fig. 1*a*, tumor 4332) and 3) loss of one allele with increase in band intensity for the other allele (Fig. 1*a*, tumor 710). Similarly, those markers that were homozygous in normal tissues also exhibited three different types of alterations in tumors: 1) generation of a new allele in addition to the existing one (Fig. 1*b*, tumor 4332), 2) replacement of the existing allele with two new alleles (Fig. 1*b*, tumor 710) and 3) in a few cases replacement of the existing allele with one new allele (Fig. 1*b*, tumor 1452).

LOH was observed in 38 different genotypes. Among these 38 genotypes, in four cases one allele was observed to have altered length while the other allele was lost (data not shown). None of the SCCHN tumors analyzed were observed to have the type of MIN commonly found in colorectal tumors (*i.e.*, new alleles observed in addition to those detected in normal cells). The SCCHN tumors with MIN were always observed to have no more than two alleles—either or both having an altered size compared with the marker in normal tissue, implying that these tumor tissues are composed of a homogeneous cell population with respect to the marker tested. The possibility that the bi-allelic alterations observed in our study might be due to sample mixup has been ruled out by genotyping the paired normal and tumor samples with four different RFLP markers (data not shown), which clearly distinguished between the paired samples. Relative frequencies of mono- and bi-allelic alterations as well as LOH observed in these tumors are shown in Figure 2. The results show that most of the instabilities are mono-allelic in nature. However, one significant observation is that the bi-allelic and LOH types of alterations were observed in equal frequencies. For 5% of the genotypes, the pattern was ambiguous and the result could not be ascertained with confidence.

Lack of instability of mononucleotide repeat in SCCHN tumors

A striking observation in the present study is the absence of any noticeable instability at mononucleotide markers in SCCHN tumors. The mononucleotide marker BAT 26, which amplifies a repeat of 26 deoxyadenosine, located in the fifth intron of the

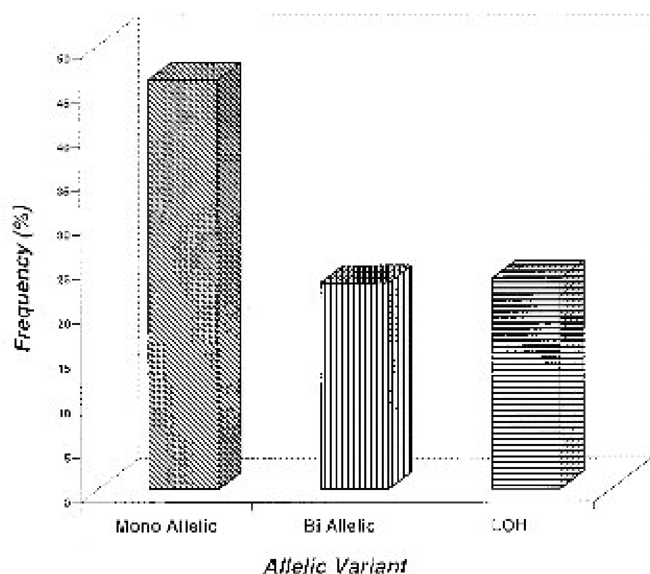

FIGURE 2 – Frequency of various allelic alterations in SCCHN tumors. Variations in frequency of monoallelic (one allele varied from paired normal), biallelic (both alleles varied from paired normal) and LOH (one allele lost in tumor tissue) in tumor tissues are shown.

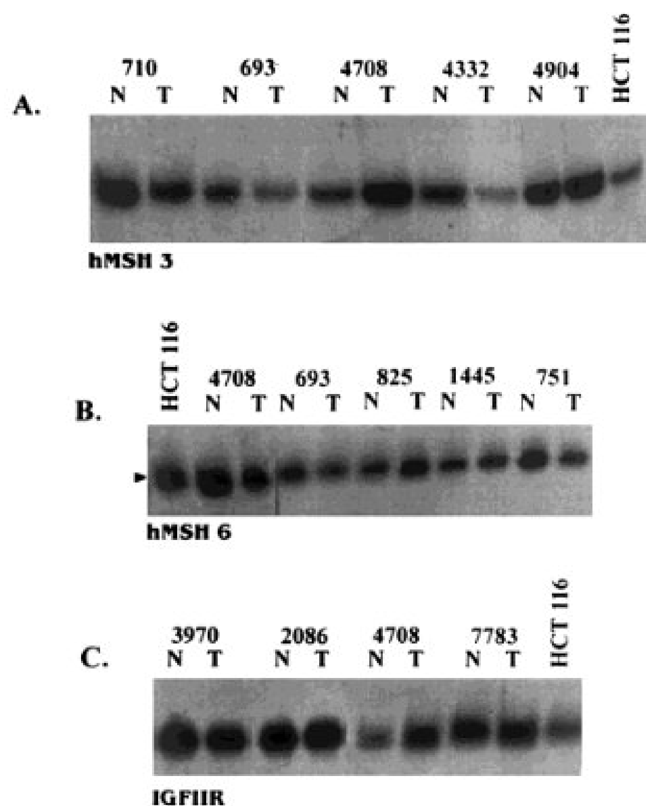

FIGURE 3 – Absence of instability in mononucleotide repeat in SCCHN tumors. The mononucleotide repeats present in the coding sequence of specific genes (*a*, *hMSH3*; *b*, *hMSH6*; *c*, *IGFIIR*) were amplified from paired normal (N) and tumour (T) tissues taken from selected tumour samples (shown at the top of each paired sample), analyzed by denaturing polyacrylamide gel (6%) and autoradiographed. HCT116 is an RER$^+$ colorectal tumour cell line known to have defects in the *hMLH1* gene; it was included as a positive control. Solid arrowhead indicates shifted allele.

hMSH2 gene, is quasi-monomorphic in the general population but shows considerable instability in tumors and is considered to be the best indicator of MIN.[32] However, this marker was completely monomorphic among all tumors tested (24/25) except for one (#4904), in which only one nucleotide difference was detected by sequencing (data not shown). Colorectal tumors with MIN have been reported to show high frequency of instability in mononucleotide runs in coding regions of genes known to be involved in growth regulation.[18–20,30] We therefore studied instability in the mononucleotide runs of *BAX*, *IGFIIR*, *TGFβR2*, *hHSH3* and *hMSH6* genes in SCCHN tumors (Fig. 3 and data not shown). However, none of the tumors tested exhibited instability in these mononucleotide runs. The observed lack of instability in the tested mononucleotide repeats due to experimental error was tested by inclusion of control in the PCR and gel electrophoresis. Thus, instability in *hMSH6* locus was observed in case of both RER$^+$ colorectal cell lines HCT116 (Fig. 3*b*) and DLD1 (data not shown) used as controls.

Comparison of MIN in different clinicopathological stages of SCCHN tumors

The frequency of MIN in 50 SCCHN tumors was compared using different clinicopathological parameters (including age, site, histological differentiation, TNM staging and positive node at pathology), and no correlation was observed (Table IV). Similar observations have also been made for SCCHN tumors from other ethnic populations.[25] Tumors from all the sites examined, except the tongue, exhibited MIN. We had only one sample from the lip, which did not show instability. Tumors from TNM stages I and II exhibited a high incidence of MIN (Table IV), indicating that this phenomenon is common in the earliest stage of the disease. This argument is further strengthened from the observation that two severely dysplastic tissues (#0751 and #0710) also exhibited the MIN phenotype. Examination of the age of diagnosis of SCCHN tumors reveals that patients with early age of onset exhibit a high incidence of MIN in their tumors (Table IV).

DISCUSSION

The result of this investigation indicates that a subset of SCCHN tumors from Indian patients shows microsatellite instability in various short tandem repeat sequences. Various investigators used different criteria for defining a tumor as microsatellite unstable.[33,34] In some studies, a tumor with instability in as few as one locus has been classified as a microsatellite unstable tumor,[33] while others used more stringent criteria such as instability in 40% of the loci tested.[34] In the present study 45% of the tumors studied show instability at more than two loci, and 15% of the tumors exhibited instability at 40% of the markers tested. The incidence of microsatellite instability reported for SCCHN tumors in the American patient population is 29% (at one or more loci) and that of British patient populations is 28% (at two or more loci).[25] Also, in the British study only 3 of 56 tumors exhibited instability at more than 40% of the markers tested.[25] Another study reported only 7% SCCHN with MIN; all of them exhibited instability at 40% or more loci.[35] In a French study only 1 tumor exhibited high MIN type, and 6 tumors were classified as low MIN type out of 56 tumors analyzed.[27] Thus SCCHN tumors from Indian patients show much higher MIN frequency than patients from other populations.

In contrast to HNPCC and sporadic gastric and endometrial tumors, which show instability mostly at the smaller (mono- and dinucleotide) repeats, elevated levels of instability in tri- and tetranucleotide repeats were reported for other sporadic tumors.[31] It has been recommended that this different type of MIN be termed *elevated microsatellite alterations at selected tetranucleotide (EMAST) repeats* to achieve uniformity in the classification of

TABLE IV – COMPARISON OF MICROSATELLITE INSTABILITY AND CLINICOPATHOLOGICAL PARAMETERS IN SCCHN[1]

Clinical parameter	Tumors with MIN in ≥2 markers		p-value[2]
	%	No./total	
Age (yr)			
20–40	57	(8/14)	
41–60	39	(11/28)	NS
>61	33	(3/9)	
Site			
Oral cavity	38	(13/34)	
Larynx	66	(4/6)	NS
Orofacial	60	(6/10)	
TNM stage			
I	33	(1/3)	
II	37	(6/16)	NS
III	62	(10/16)	
IV	40	(4/10)	
Pathology			
Seve Dys	40	(2/5)	
WDSCC	43	(12/28)	NS
MDSCC	66	(6/9)	
PDSCC	37	(3/8)	
Lymph node			
Positive	47	(10/21)	NS
Negative	42	(12/28)	

[1]NS, not significant. See footnotes of Table I for other abbreviations.–[2]Chi-square test.

tumors exhibiting MIN.[31] Using three tetranucleotide (AAAC, TCTA and TAAA) repeat markers, we observed no EMAST-like activity; on the contrary, the instability of tetranucleotide markers was lower (9/25) compared with the observed instability (21/25) of di- and trinucleotide repeat markers. However, the number of tetranucleotide repeats used in this study was limited and did not include $(AAAG)_n$, which is reported to be particularly susceptible to a higher level of instability.[36]

In the present study, MIN observed in SCCHN tumors has two distinctive features. First, complete absence of instability at mononucleotide repeat sequences was observed. This observation suggests that genes that are defective in these tumors may be different from that of RER^+ colorectal tumors. Second, the appearance of one or two new alleles with concomitant loss of one or both constitutional alleles was noted. This observation is in sharp contrast to the type of MIN commonly found in colorectal tumors, which would include new alleles in addition to the constitutive allele(s) present in the normal tissue. However, careful analysis of published data from various tumors including SCCHN reveals that similar results were also obtained by some workers.[27,28,33] One plausible explanation for this observation might be that a transient form of MMR deficiency occurred in the early stage of tumor development and subsequently defective cells were selected that exhibited no further instability. Alternatively, it has been argued that such an observation could be made due to clonal expansion of an already altered cell from heterogeneous normal tissue.[37]

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