Sequential Deletions in both Arms of Chromosome 9 are Associated with the Development of Head and Neck Squamous Cell Carcinoma in Indian Patients

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In the deletion mapping of chromosome (chr) 9 in head and neck lesions of the Indian patient population by microsatellite markers, we have identified four discrete areas (D1-D4) with high loss of heterozygosities (LOHs) viz. 9p24-p23 (D1), 9p22-p21 (D2), 9q11-q13 (D3) and 9q22.3 (D4) regions. The deletions in D2 and D4 regions were suggested to be essential for the development of dysplastic lesions of head and neck, whereas the deletions in D1 and D3 regions were responsible for progression of the dysplastic lesions to early invasive head and neck squamous cell carcinoma (HNSCC). The microsatellite size alterations (MAs) were observed in the chromosomal 9pter-p23, 9p22-p21(D2), 9q13 and 9q21.1-q21.2 regions with gradual increase during progression of the tumor. Additional chromosomal alterations like loss of normal copy of chr.9 and biallelic alterations were also seen in our samples. There is a correlation between HPV infection with TNM stages, histopathological grades and LOHs at D1 and D4 regions. Whereas tobacco habit is associated with the occurrence of LOHs at D1 and LOHs / MAs at D2 region.

Key Words: Head and neck squamous cell carcinoma, Chromosome 9, Tumor suppressor genes, Loss of heterozygosity, Microsatellite size alteration, Human papilloma virus

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide (1) and it accounts for 30-40% of all cancer types in Indian subcontinent (2). The epidemiological studies have identified different etiological factors (e.g. tobacco, betel nut leaf quid, alcohol), some environmental factors and infection with oncogenic genotypes of human papillomavirus (HPV), responsible for the development of HNSCC (3,4). Among the HPVs, the high risk HPV-16 and 18 are most prevalent in the HNSCC tumors (5). But, the role of these etiological factors in the initiation and progression of this tumor is not clear.

The allelotyping studies (using microsatellite markers) of HNSCC have identified >30% loss of heterozygosities (LOHs) in several chromosomal regions e.g. 3p, 5q, 6p, 8p, 9p, 9q, 11p etc. (6). Among these deleted chromosomal regions, the loss in chr.9p21 region has been proposed to be necessary for the development of benign or precursor squamous hyperplasia of head and neck lesions (7,8). Various other laboratories have also reported similar high frequency of LOHs in the chr.9p21-22 region of this tumor along with microsatellite size alterations (MAs) in chr.9 (3,9). A candidate TSG, p16 (cdk4/6 inhibitor), has been identified in the chr.9p21 region and the inactivation of this gene either by mutation, deletion or hypermethylation has been seen in HNSCC tumors (10). But Nagai (8) suggested that the inactivation of P16 might be necessary for the development of carcinoma-in-situ from dysplastic lesions. This indicates that there might be another tumor suppressor gene (TSG) present in this region, required for the development of hyperplastic lesions from normal mucosa. On the other hand, the importance of chr.9q deletion in the progression of HNSCC has not yet been studied in detail though El-Naggar et al (11) have suggested that the LOHs at certain loci of chr.9q might be associated with the development of HNSCC. However, no one has detected any deletion in other regions of chr.9 in HNSCC, though some of the regions e.g. 9p23-24 (12), 9q11-12 (13), 9q22.3 (14) and 9q32 (15), have been shown to be necessary for the development of other tumors. This might be due to the focussed analysis in certain regions of chr.9 in HNSCC tumors.

Table I - Clinicopathological features and HPV status of head and neck lesions

Clinical Features	Patient No.	Median Age Years	Age Range Years	HPV -16/-18 Positivity HPV+	HPV-	Percentage	P value
PRIMARY SITE							
Oral cavity	79	49	8-76	46	33	58.0	
Larynx	8	61	48-75	07	01	87.5	0.174
Orofacial	11	44	22-70	08	03	72.7	
TNM STAGE							
Dysplasia	24	48	32-70	09	15	37.5	
Stage I	6	54	45-65	03	03	50.0	
Stage II	28	49	8-76	16	12	57.0	0.0001
Stage III	28	51	30-70	21	07	75.0	
Stage IV	12	51	26-70	12	00	100.0	
GENDER							
Male	79	48	8-76	51	28	64.5	0.251
Female	18	46	26-62	09	09	50.0	
TUMOR DIFFERENTIATION*							
Well	41	52	22-76	25	16	61.0	
Moderate	26	48	8-70	20	06	77.0	0.024
Poor	7	50	32-75	07	00	100.0	
LYMPH NODE							
Positive	36	48	26-70	25	11	69.4	0.879
Negative	38*	50	8-76	27	11	71.0	
TOBACCO							
Tobacco +	53	44	22-76	35	18	66.0	0.4005
Tobacco -	45	49	8-75	26	19	58.0	

**Excluding dysplasia*

Thus, in the present study attempts have been made to delineate the deletions in different regions of chr.9 (seen in HNSCC and other tumors) necessary for the development of HNSCC. We have done this deletion mapping at 22 microsatellite marker loci from both arms of chr.9 in 24 primary dysplastic lesions, 34 stage-I/II and 40 stage-III/IV tumors from head and neck regions of Indian patient population. Also, the probable association of these changes with different clinicopathological parameter including tobacco intake, HPV-16/18 has been investigated.

Materials and Methods

Sample collection and clinical data. Ninety-eight freshly operated specimens from head and neck region of 97 patients were collected along with their corresponding normal tissue or peripheral blood leukocytes (PBL) from Chittaranjan National Cancer Institute, and Cancer Center & Welfare Home, Calcutta. The detailed clinical history of the patients is presented in Table I. The primary tumor (A) of one patient (# 5090) at inner side of the cheek was collected along with his

tumor of secondary recurrence (B) on the tooth gum. The secondary tumor formation took place after 6 months of operation of the primary tumor. Out of 98 collected specimens, 2 (# 5090B and 333) were tumors of secondary recurrence. All the samples were collected after obtaining consent from the patients as well as hospital authorities. Among the orofacial samples, 4 were from maxilla, 3 were from mandible and nasal cavity each and 1 was collected from the cheek. The samples were frozen immediately after collection and stored at -80°C until use. The tumors were graded and staged according to the UICC TNM classification. The patients were considered as tobacco habituated if they consumed at least 10-15 cigarettes/bidis or equivalent amount of chewable tobacco per day for at least 10 years.

Microdissection and DNA extraction. The microdissection procedure was used to remove the normal cells present as contaminant in the specimens (16). After microdissection, samples containing >60% dysplastic epithelium / tumor cells were taken for DNA extraction. DNA was extracted by proteinase-K digestion followed by phenol: chloroform extraction (16). When the adjoining normal tissue of the lesion was contaminated with infiltrating tumor cells, then PBL was taken for normal DNA extraction.

Microsatellite analysis. The location of the microsatellite markers of chr. 9 and the genetic distances among them are mentioned in Table II. The markers were selected from the different deleted regions seen in HNSCC and other tumors (7,12-15).

Table II - Overall patterns of LOHs and MAs of the microsatellite markers in dysplastic head and neck lesions and invasive HNSCC samples

Microsatellite Markers	Cytogenetic Position	Genetic Distance cM	Dysplastic Lesions		Invasive HNSCC		
			LOH % (No)	MA % (No)	LOH % (No)	MA % (No)	
D9S54	9pter-24	*	0 (0/5)	27 (3/11)	15 (6/39)	24 (13/54)	
D9S1810	9p24-23	5.88	0 (0/16)	4.5 (1/11)	42.8 (21/49)	25 (12/48)	D1
D9S1849	9p24-23	2.55	7 (1/14)	5 (1/18)	47 (24/51)	25 (14/55)	D1
D9S168	9p23	6.55	10 (2/19)	4.7 (1/21)	36 (17/47)	23.6(13/55)	D1
D9S269	9p23	2.19	0 (0/6)	8 (1/12)	16 (6/37)	10 (6/59)	
D9S157	9p22-21	8.17	33 (3/9)	48 (10/21)	31 (15/48)	20.8(10/48)	D2
D9S942	9p21	5.49	21 (4/19)	19 (4/21)	23 (13/56)	25 (15/60)	D2
D9S171	9p21	5	0 (0/13)	0 (0/21)	21 (9/34)	12 (8/66)	
D9S169	9p21	6.47	0 (0/10)	0 (0/11)	19 (9/47)	18 (11/60)	
D9S104	9p21-13	2.61	18 (3/16)	12 (3/24)	21 (7/33)	16 (8/48)	
D9S163	9q11-13	12	0 (0/15)	0 (0/23)	36.8 (14/38)	15.8(10/66)	D3
D9S15	9q13	4.12	5 (1/17)	4.3 (1/23)	14.8 (7/47)	23.3(14/60)	
D9S153	9q13	11.1	10 (1/10)	0 (0/10)	21 (10/41)	16.6(10/60)	
D9S257	9q21.1-21.3	12.8	5 (1/19)	9 (2/22)	22.6 (7/31)	25.9(14/54)	
D9S12	9q22.2-22.3	8	0 (0/16)	10 (2/20)	25.7 (9/35)	18.3(9/49)	
D9S197	9q22.2-22.3	1.02	32 (6/19)	0 (0/17)	36 (14/39)	20 (12/59)	D4
D9S280	9q22.2-22.3	0.38	31 (5/16)	0 (0/17)	29 (12/42)	9.5(4/42)	D4
D9S1851	9q22.2-22.3	1.43	26 (5/19)	5.8 (1/17)	30 (14/47)	14 (7/49)	D4
D9S287	9q22.2-22.3	0	0 (0/6)	0 (0/22)	27 (10/37)	14.8(8/54)	
D9S180	9q22.2-22.3	1.6	0 (0/6)	0 (0/11)	21.6 (8/37)	14.8(8/54)	
KM9.2	9q32	20.5	0 (0/10)	0 (0/11)	19 (9/47)	19 (11/56)	
D9S103	9q34	6.57	0 (0/7)	0 (0/12)	21 (8/37)	6.2 (4/64)	

** D9S54 is located approximately 6.9 cM from the chr.9p telomere. D1-4: Highly deleted chr.9 regions.*

Primer sequences of these markers were obtained from Genome Database except the marker KM9.2 (15). The PCR was performed in a 20 µl reaction volume containing 67 mM Tris (pH 8.7), 16.6 mM $(NH_4)_2SO_4$, 1-2 mM $MgCl_2$, 0.01% Tween-20, 4 pmol of each primer, 0.2 mM of each dNTPs, 50-100ng of template DNA and 0.5 units of Taq-DNA polymerase (Gibco-BRL, USA) as described by Dasgupta et al, (16). One of the paired primers in the reaction mixture was end labeled with [γ32p] ATP (specific activity 3000ci/mmol, Amersham, U.K.) using T4-Polynuclotide kinase (Gibco-BRL, USA). The PCR conditions were 95°C for 5 min, then 30 cycles at 95°C for 1 min, annealing at appropriate temperature (50-62°C) for 1 min and extension at 72°C for 2 min followed by final extension at 72°C for 7 min. The labeled PCR products were electrophoresed in 7% polyacrylamide gel containing 8M urea and autoradiographed as described by Dasgupta et al (16).

In our analysis, the microsatellite markers could detect LOHs and MAs in the presence of 50% and 10-30% tumor DNA, respectively (16). The LOHs were interpreted for all informative (heterozygous) cases by densitometric scanning (Shimadzu, CS-900) of the autoradiographs. The allelic loss was recorded if there was a complete absence of one allele or if the relative band intensity of one allele was reduced at least 50% in the tumor in comparison to the homologous allele in the corresponding normal DNA (16,17). The MAs were detected by a shift in the mobility of one (MA1) or both (MA2) alleles in comparison to the same alleles in the corresponding normal DNA (18). The samples showing loss of one allele and size alteration of the other allele (LOH+MA) at the same locus was considered for calculating both LOH and MA. The calculations of LOHs and MAs as well as proper pairing of the samples were done according to Dasgupta et al (16).

Detection of HPV-16 and 18. The presence of HPV in the head and neck lesions was detected by performing PCR using primers (MY09 and MY11) from the consensus L1 region (19). The typing of HPV-16/ 18 in the L1 positive samples was done by PCR using specific primers from the HPV-16 E6 (20) and HPV-18 E7 (21) region. The PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide for visualisation under UV-light and photographing. For final confirmation of the HPV types, the PCR products after gel electrophoresis were transferred to the nylon membrane for southern hybridization with 32p-labelled HPV type specific probes (5). As a positive control for HPVs, the DNAs from SiHa (for HPV-16) and HeLa (for HPV-18) cell lines and the HPV type specific plasmids were used.

Statistical analysis of the clinical data. To determine the association between high LOH/MA regions and different clinicopathological features (tobacco habit, nodes at pathology, tumor stages and HPV infection) of the tumors the Chi-square analysis was performed. Probability values of $P<0.05$ was regarded as statistically significant.

Results

Analysis of allelic alterations (LOHs/MAs) in the samples. In this analysis we have seen different types of allelic alterations i.e., LOHs, MA1, MA2 and LOH+MA (loss of one allele and size alteration of the other allele) in the samples (Figs 1 & 2). The allelic alterations (LOHs and / or MAs) have been seen at chr.9p and 9q in 70% (69/98) and 59% (58/98) of the samples respectively, indicating the importance of both arms of chr.9 in the development of HNSCC.

In the 74 HNSCC samples analysed, the highest frequencies of LOHs of 36-47%, 23-31%, 37% and 29-36% were seen in the 9p24-23 (D9S1810, D9S1849 and D9S168): D1, 9p22-21 (D9S157, D9S942): D2,

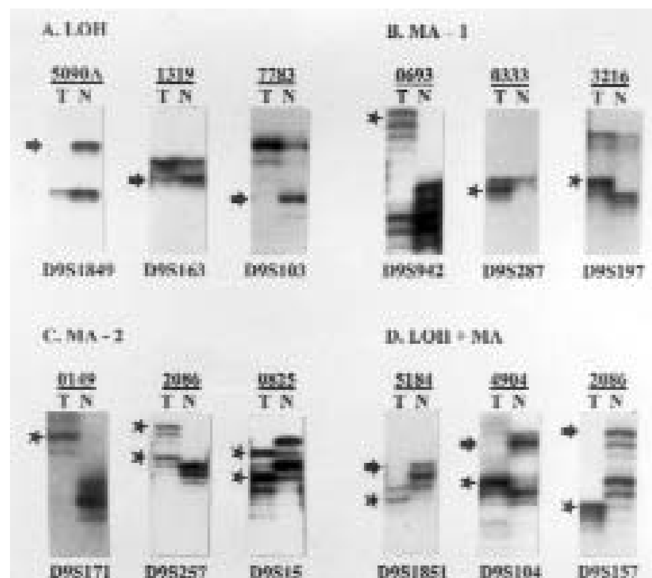

Fig. 1 - Representative photograph showing LOHs and MAs at different marker loci on chr. 9 in different head and neck samples. T: DNA of the dysplastic/ tumor cells after microdissection; N: corresponding normal tissue or PBL.
➔ Indicates loss of the corresponding allele;
✱ indicates size alteration of one or both alleles.

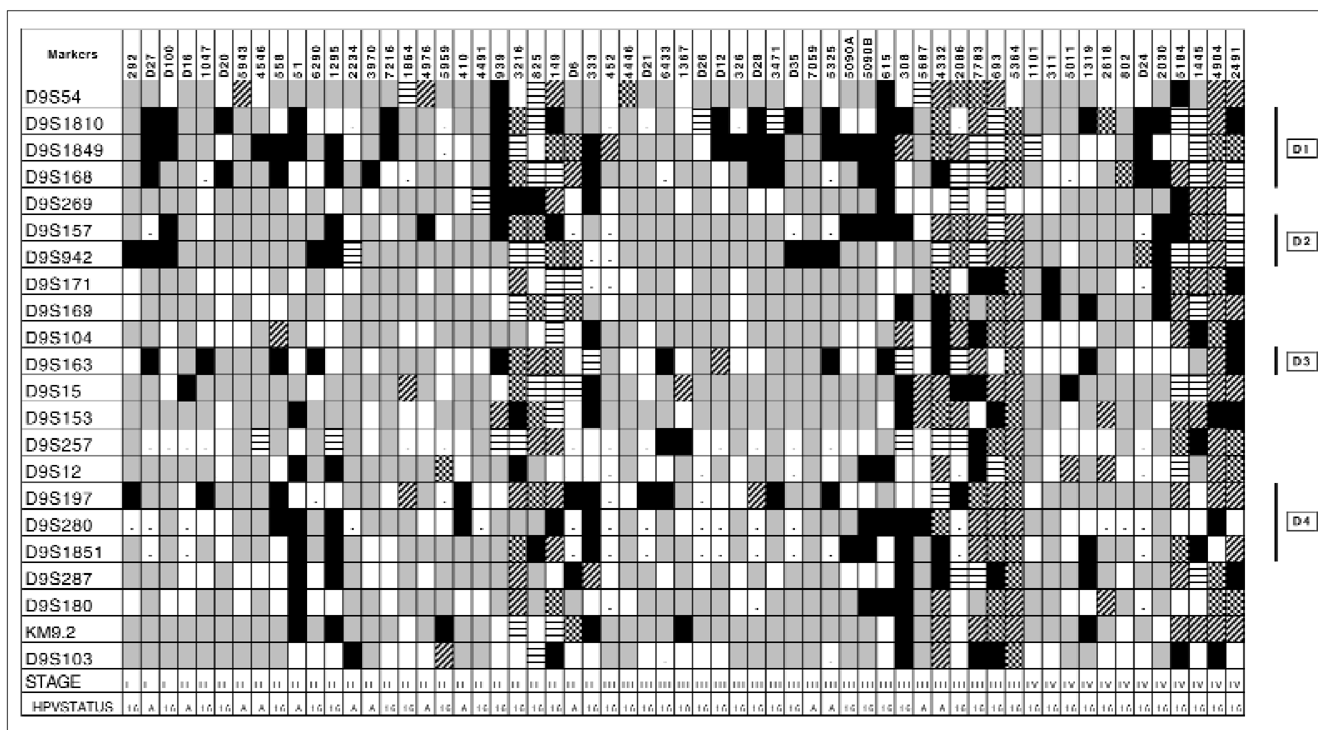

Fig. 2 - Allele status of the chr.9 markers in the HNSCC samples that showed alteration.

LOH , MA-1 , MA-2 , LOH+MA , RH , NI , ND

LOH: Loss of heterozygosity, MA-1: Microsatellite size alteration of one allele, MA-2: Microsatellite size alterations of both alleles, LOH+MA: Loss of one allele and size alteration of the other allele, RH: Retention of heterozygosity, NI: Noninformative, ND: Not done, A: HPV absent, 16: HPV-16 present, 18: HPV-18 present. D1-4: Highly deleted regions.

9q11-13 (D9S163): D3 and 9q22.3 (D9S197, D9S280 and D9S1851): D4 regions, respectively (Fig.2 & Table II). On the other hand, in the dysplastic lesions highest frequency of LOHs of 21-33% and 26-32% were seen only in the D2 and D4 regions, respectively. Whereas, only 7-10% of LOHs were detected in the D1 region and without any LOHs in the D3 region in dysplastic samples.

In the HNSCC samples, the highest frequency of MAs (20-26%) were seen in the 9p23-24, 9p21-22, 9q13, 9q21.1-21.3 and 9q22.3 regions (Table II). Interestingly most of the high MA regions were coincident in and around the high deleted regions except D3. Seven tumors at stage-III/IV and 1 tumor at stage-II showed allelic alterations (LOHs and/or MAs) in all the markers studied indicating probable absence of normal copy of chr.9 in these tumors (Fig.2). In the analysis of primary (A) and recurrent (B) tumors of patient #5090, it was seen that both the samples showed same pattern of allelic alterations along with additional LOHs at four loci in the recurrent tumor (Fig.2). However, unlike HNSCC tumors, very low MAs were seen in D1, D3 and D4 regions in the dysplastic lesions except in the D2 region where 19-48% MAs were observed.

Consistent homozygous loss at any marker loci is supposed to be the location of putative TSG(s). In this study, we did not find any homozygous deletion i.e. loss of both alleles, but in 41 samples some markers showed either MAs of both alleles (MA-2) or MA of one allele and loss of the other allele (LOH+MA) (Figs.1&2). In chr.9p, the biallelic alterations were found to be concentrated in the D1 and D2 regions whereas in chr.9q the high biallelic alteration was seen in the 9q21.1-21.3 (D9S257) region.

HPV-16/18 infection in the head and neck lesions. The prevalence of HPV was detected in 62% (61/98) of the samples (Table I and Fig.2). However, majority of the HPV positive samples (60/61) was found to be infected with HPV16 and only one sample (#1367) seemed to be infected with HPV18 (Fig.2). The HPV infection was significantly associated with tumor stages and grades (Table I).

Table III - Clinicopathological correlation of the regions that showed high LOHs and MAs

(a): Correlation of the four high LOH regions

Characteristics	Chr 9p24 23(D 1)		Total	P value	Chr 9p22 21(D 2)		Total	P value	Chr 9q11 13(D 3)		Total	P value	Chr 9q22.3(D 4)		Total	P value
	LOH+	LOH-			LOH+	LOH-			LOH+	LOH-			LOH+	LOH-		
Tobacco+	24	28	52	0.039	22	28	50	0.006	07	45	52	0.769	24	27	51	0.130
Tobacco-	12	34	46		08	37	45		07	38	45		14	30	44	
HPV+	27	34	61	0.047	22	36	58	0.095	11	50	61	0.189	28	31	59	0.057
HPV-	09	28	37		08	29	37		03	33	36		10	26	36	
Node+	19	17	36	0.012	10	25	35	0.630	07	29	36	0.280	14	21	35	1.000
Node	17	45	62		20	40	60		07	54	61		24	36	60	
Dysplasia	04	21	23	0.018	07	17	24	0.738	0	23	23	0.020	09	14	23	0.590
Stage I/II	14	20	34	0.580	11	22	33	0.874	07	27	34	0.735	11	23	34	0.190
StageIII/IV	19	21	40		12	26	38		07	33	40		18	20	38	

Table III (b): Correlation of the four high MA regions

Characteristics	Chr 9pter-p23		Total	P value	Chr 9p22-21		Total	P value	Chr 9q13		Total	P value	Chr 9q21.1-21.2		Total	P value
	MA+	MA-			MA+	MA-			MA+	MA-			MA+	MA-		
Tobacco+	16	36	52	0.97	19	31	50	0.029	07	48	55	0.208	08	33	41	0.817
Tobacco-	14	32	46		08	37	45		08	27	35		08	29	37	
HPV+	22	39	61	0.132	18	40	58	0.475	12	46	58	0.167	12	35	47	0.176
HPV-	08	29	37		09	28	37		03	29	32		04	27	31	
Node+	13	23	36	0.368	08	27	35	0.35	06	26	32	0.693	05	18	23	0.862
Node-	17	45	62		19	41	60		09	49	58		11	44	55	
Dysplasia	05	19	24	0.98	12	12	24	0.004	01	23	24	0.123	02	20	22	0.195
Stage I/II	07	27	34	0.026	05	28	33	0.25	05	23	28	0.567	06	20	26	0.75
StageIII/IV	18	22	40		10	28	38		09	29	38		08	22	30	

Clinicopathological correlation of the allelic alterations. It was evident from Fig. 3 that the high incidences of LOHs at D2 and D4 regions were present in the dysplastic lesions and remain more or less constant (in case of D2)or increased (in case of D4) in the later stages of the tumor. Thus, it indicated that the deletions in these regions were necessary for the development of dysplastic head and neck lesions. But the LOHs in the D1 and D3 regions were seen to be significantly associated with the progression of the dysplastic lesions to the stage-I/II HNSCC tumors (P value: 0.018-0.02) (Fig. 3). The D1 region was associated with the nodal involvement of the tumor, HPV positivity and tobacco habits (P value: 0.012-0.047)(Table III). Whereas, the D2 and D4 regions were associated with tobacco habits and HPV positivity of the samples, respectively. On the other hand the MAs in the D1 and D2 regions were seen to be associated with the progression of the tumor (P value: 0.026) and the tobacco habits (P value: 0.029), respectively.

Discussion

Among the four highly deleted regions, the D2 region (Fig.2 and Table II) overlapped with the regions reported by other investigators in HNSCC tumors, lung carcinoma, melanoma, etc. (7,22). Whereas the dele-

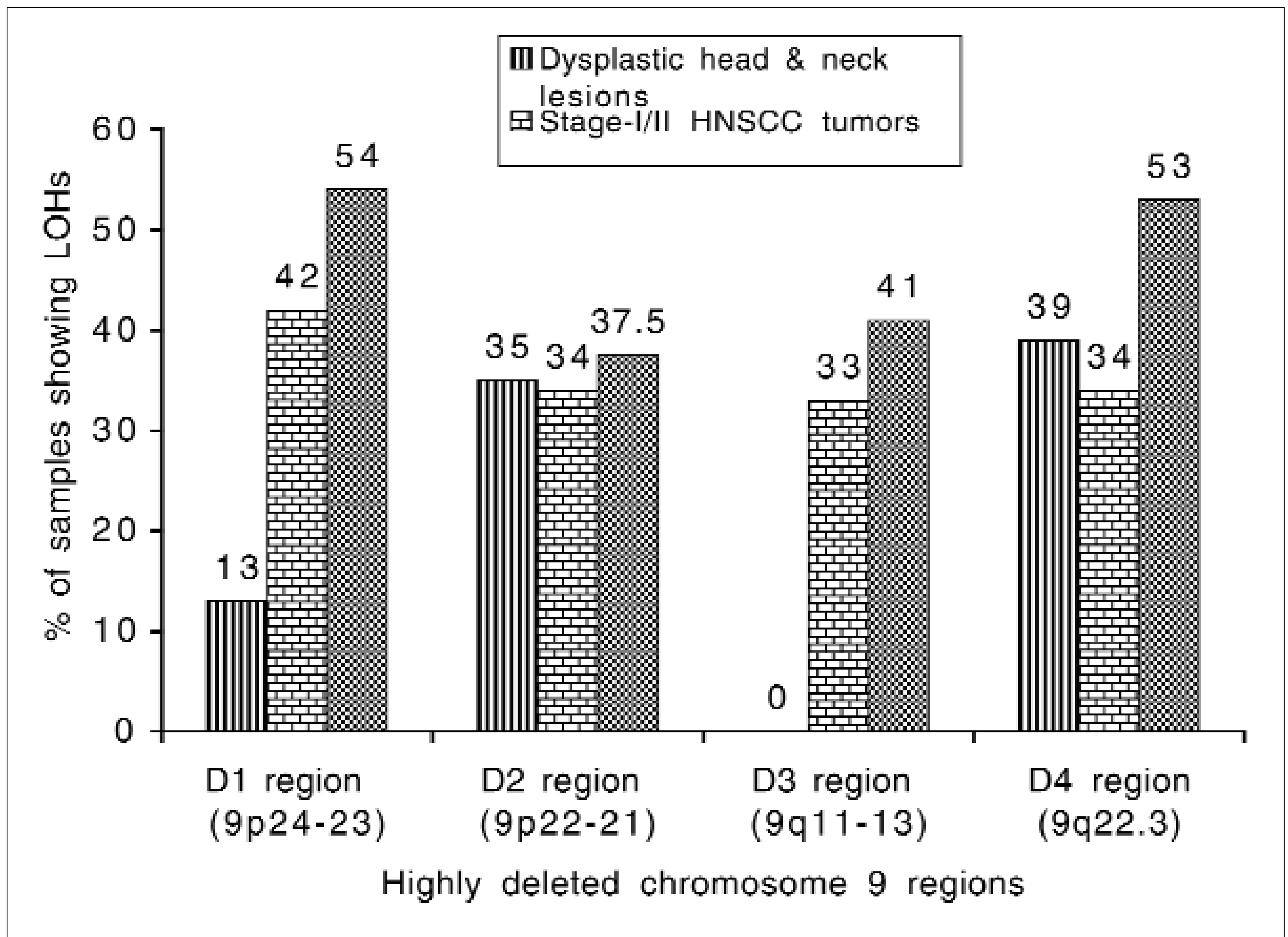

Fig. 3 - Pattern of deletion in the D1-D4 regions of Chr. 9 during HNSCC development. The lesions showing MA and NI at all the markers of a particular deleted region were not considered for LOH calculation of that region.

tions in D1, D3, D4 regions (Fig. 2 and Table II) were coincident with the deleted regions seen in the carcinomas of breast, bladder, and basal cell etc. (12-14). The identical frequencies of deletions in the D2 and D4 regions in both dysplastic lesions and HNSCC tumors have indicated that deletions in these regions were associated with the development of dysplastic lesions (Fig.3). Others have also reported the involvement of D2 region in the early stages of head and neck cancer (7,9). We have narrowed down this deleted region within 5.5 cM (D9S157-D9S942) (Fig.2). Between the D9S157 and D9S942 markers, comparatively high LOHs were observed in the D9S157 locus though D9S942 marker was 5 kb upstream of the putative tumor suppressor gene, *P16*. Thus, it seems that the candidate TSG(s) responsible for the pre-malignant lesion may be located near the D9S157 locus. Similarly, using microcell hybrid system Parris et al (23) suggested the location of a new TSG in 9p21 region, telomeric to *P16* gene. However, to rule out the possibility of *P16* involvement in the development of premalignant head and neck lesions, the other mechanisms of *P16* inactivation i.e. mutation, methylation etc., should be studied in same set of samples along with the deletion mapping. We have localized the D4 region within 1.81 cM of 9q22.2-22.3 (D9S197-D9S1851). Among the three markers in the D4 region, the D9S280 and D9S1851 markers were in close vicinity of two important genes e.g. *FACC* (Fanconi's anaemia complementation group C) and *PTC* (candidate TSG for bladder cancer and nevoid basal cell carcinoma). But the importance of these genes in the development of HNSCC has not yet been studied.

The significant increase of LOHs in the D1 and D3

regions in stage-I/II tumors than the dysplastic lesions has suggested that the deletions at these sites are associated with the progression of dysplastic lesions to early invasive tumors (Table III, Fig.3). We have narrowed down the D1 region within 9.1 cM of 9p23-24 region. Though a novel oncogene *GASC1* (Gene amplified in squamous cell carcinoma) responsible for esophageal cancer (24) and an important signal transduction gene, *JAK2* (Janus kinase2), were present in this region, yet no candidate TSG has been identified from this region. On the other hand, we have used only one marker (D9S163) from the D3 region. For exact localization of TSG loci more markers should be studied from this region.

Similar to our results, differential losses at different regions of chr.3 was reported to be essential for the development of lung carcinoma (18) and HNSCC (our unpublished data). Also, Huebner K proposed that for the development of lung, kidney and other cancers compound mutations at different TSGs located in different regions of chr.3p were necessary (25).

Recently, two candidate TSGs e.g. *GAS1* (Growth arrest specific gene1) at chr.9q21.3-22 (26) and *DEC1* (Deleted in esophageal cancer1) at chr.9q32 (27) were shown to be responsible for the development of bladder carcinoma and esophageal carcinoma, respectively. Also, El-Naggar et al (11) showed total 35% LOHs in chr.9q using three markers from the 9q13-21, 9q31-33 and 9q34-ter regions in HNSCC samples. But we have not seen high LOHs in these regions.

In contrast to the HNSCC tumors, dysplastic lesions showed high MAs only in the D2 region particularly in the D9S157 marker (Fig.2 & Table II). Thus, the MAs may not be the early event in this tumorigenesis. Other investigators in their analysis of molecular abnormalities (LOHs and MAs) also reported similar phenomenon on chr.3 in the HNSCC (16,28). The presence of comparatively high biallelic alterations in and around the high molecular abnormality regions (except D4) indicates that the LOHs or MAs in one allele of these regions may impose selective pressure on the other allele of the same locus for deletion or size alteration (Fig.2). Also probable loss of normal copy of chr.9 mainly in late stages of the tumor may provide some growth advantage in this tumor progression. Similar phenomena have been reported in the analysis of other chromosomes in HNSCC tumors (16,29). Thus, during progression of the tumor a) the gradual increase of MAs, b) loss of normal copy of chr.9, c) interstitial alterations (LOHs and MAs) and d) the occurrence of LOHs at additional loci in the recurrent tumor (#5090), indicate that additional genetic alterations are necessary for clonal outgrowth of a subpopulation of cells for subsequent tumor progression (Fig.2). The etiological factors like tobacco intake and HPV positivity might have some role in this tumorigenesis due to the significant association of these factors with some deleted regions. Also, the association of deletion at D1 region with the nodal involvement of the tumor has indicated its necessity in this tumor progression.

Thus, it can be concluded from our analysis that differential deletions in four highly deleted regions (D1-D4) of chr.9 in head and neck lesions are needed for the development of specific stages of HNSCC progression. The loss of function of TSGs located at these regions may have sequential cumulative effect in this tumor progression. Along with these deletions, other molecular changes in the chromosome, like MAs, interstitial alterations and loss of normal copy of chr.9 may have some role in this tumor progression by imposing some selective pressure for growth advantage of the tumor.

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