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Original Research Article

Profiling of TOC-Microsatellite Loci at 17q25 in OSCC Patients with Therapeutic Validation

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Abstract

Abstract: Treatment strategies for oral cancer are still primarily based on tumor-node-metastasis classification. A variety of prognostic biological markers correlated to survival have been described over the years, but very few of these have been tested for prognostic accuracy. The advent of genome-wide screening methods such as comparative genomic hybridization, Microarray, microsatellites and SNPs have opened up new possibilities to catalogue chromosomal aberrations, point mutation for disease diagnosis as well as therapeutic validation. Aberration of the Tylosis Oesophageal Cancer (TOC) gene mapped to chromosome 17q25 has been recognized as a genetic marker for the development of Oesophageal, Breast and Ovarian cancer.

Material & Methods: A total of 26 Microsatellite markers at 17q25 were examined in 150 OSCC patients treated with cisplatin and capecitabine to establish genetic marker for early detection and therapeutic validation. A total of 150 primary tumour tissues, and corresponding blood samples from patients attending the and corresponding blood samples from patients attending the King George's Medical University, Lucknow, from 2010 to 2016, were collected. The tissue samples were obtained, either at the time of investigative biopsy or during the time of the surgical resection of the lesions.

Results: Overall incidence of LOH/MSI was 40%±20.84 with the frequency of LOH and MSI of individual markers ranged from 12-83%. LOH was relatively more frequently detected at five loci, namely D17S1817 (57.33%), D17S1864 (81.33%), D17S1603 (68.66%), D17S1602 (83.33%), D17S929 (64%). Low incidence of LOH and MSI was observed in D17S2238 (20.7%) and D17S926 (12.66%) and D17S2101 (18.7%).

Conclusion: To the best of our knowledge, this is the first report suggesting an association between allelic loss at D9S1602 & D17S1864 on TOC loci and recurrence in OSCC from India in patients treated with Cisplatin & Capecitabine.

Keywords: Tylosis Oesophageal Cancer (TOC), Microsatellite Markers, Chr.17q25.

Introduction

Oral squamous cell carcinoma (OSCC) is a major cause of morbidity and motility world wide, accounting for more than 275000 new cases and over 120, 00 deaths every year [1] The current treatment strategies for oral squamous cell carcinoma include a combination surgery, radiation therapy and chemotherapy. Concomitant chemotherapy and radiotherapy also show promise of an improved survival benefit (8%) relative to conventional treatment, i.e., surgery and radiotherapy in combination or as single modality treatment in SCCHN [2]. However, recurrence develops in approximately one third of the patients despite definitive treatment and is the most common cause of death for patients with oral cancer (OSCC) [3]. Treatment strategies for oral cancer are still primarily based on tumour-node-metastasis classification. It is well known facts that the biological features of the tumours play a key role in the clinical response to any given therapy. Optimizing treatment protocols by objective measurable methods according to the genetic and biological properties of individual tumours could eventually lead to a survival benefit and lower morbidity. Cisplatin (CDDP, cis-diamminedichloroplatinum II) is a commonly used chemotherapeutic agent, which is effective as a single agent or in combination with other drugs in the treatment of a wide variety of malignant solid tumours, including cancer of the testes, ovaries, bladder, oesophagus and head and neck squamous cell carcinoma. Resistance to cisplatin is a major obstacle to effective cancer therapy because clinically relevant levels of resistance emerge quickly after treatment. The two major forms of cisplatin resistance are intrinsic resistance, in which previously untreated tumour cells are inherently insensitive to the chemotherapeutic agent, and acquired resistance, in which treated tumour cells become insensitive after drug exposure [4]. It has been believed that, acquired cisplatin resistance is multifactorial and depends on host factors, genetic and epigenetic changes, and numerous molecular events. Resistance may be due to decreased drug accumulation, alteration of intracellular drug distribution, reduced drug-target interaction, an increased detoxification response, cell-cycle deregulation, increased damaged-DNA repair and a reduced apoptotic response [5].

Five novel genes LUM, PDE3B, PDGF-C, NRG1 and PKD2 have been identified as predicting genetic markers to predict the efficacy of cisplatin-based chemotherapy against OSCC [5]. The CCND1 has also been reported as a potential marker of response to cisplatin as the overexpression of cyclin D1 responded with the drug [6]. A panel of biomarkers that reflect the biological features of the solid tumour, including chemo sensitivity have also been documented in breast, prostate and in soft tissue sarcomas [7]. Squamous cell oesophageal cancer has been associated with focal non-epiderotyfic pamo plantar kerotoderma or tylosis and autonomic dominantly inherited disorder of the skin reported in UK, USA and German, families [8]. But the incidence of Tylosis with oesophageal cancer is low in the general population. Aberration of the tylosis oesophageal cancer (TOC) gene mapped to chromosome 17q25 has been recognized as a genetic marker for the development of oesophageal, Breast and ovarian cancer. Two polymorphic microsatellites-D17S1839 (48%) and D17S1603 (43%) of TOC locus at chromosome 17q25.1 had also been reported in primary breast cancer [9]. Hence, this Oesophageal cancer Susceptibility locus TOC could be targeted as a marker for OSCC as well as therapeutic validation to meet the clinical challenges as no reports have been advocated in OSCC with association of TOC locus so far.

Review of Literature

Genomic alteration may add prognostic information and indicate biological aggressiveness, thereby emphasizing the need for genetic profiling of oral cancer. Oral squamous cell carcinoma is reported to arise through the accumulation of numerous specific chromosomal alterations. Chromosomal arms 3q, 6q, 8q, 9p, 9q, 11p, 11q, 17q and 20p have suggested for putative oncogenes and tumour suppressor genes associated with oral cancer. Molecular Profiles of oral cancer vary throughout the world and are influenced by both aetiological factors and ethnicity. Apart from tobacco and alcohol, human papilloma virus infection is also known risk factors for OSCC [7].

The Epidermis is a stratified squamous epithelium that provides a physical barrier between the organism and its environments, preventing dehydration and protecting against chemical and mechanical assaults. To perform this function, keratinocytes express specific keratins, assemble a layer of in soluble cross-linked protein. The tylosis also formed oral lesion and shows complete penitence by the age of 20 [10]. Loss of heterozygosis (LOH) at microsatellite markers that are located to the TOC locus have also been reported for adenocarcinoma and breast cancer. In OSCC high frequencies of LOH have also been detected on chromosome arms like 3q, 9q, 11q. The LOH at D9S162 giving a high relative risk of a death/ recurrence and D9S161 for survival respectively irrespective of treatment modality employed ie.52.5 Gy/15,60Gy/20 and 66 Gy/33 [11]. Low bcl-xL expression as well as p53 overexpression, and tumor response to chemotherapy in pretreatment biopsy specimens from patients enrolled in a clinical trial have been reported [12,13]. Additionally, conflicting results have also been reported regarding TP53 mutations correlated with sensitivity and resistance to cisplatin [13-15]

The advent of genome-wide screening methods such as comparative genomic hybridization, Microarray, microsatellites and SNPs have opened up new possibilities to catalogue chromosomal aberrations, point mutation for disease diagnosis as well as therapeutic validation. In clinical practice the use of high-throughput microarray technology is not possible as it is cost effective and subject to availability. Therefore, early detection of oral cancer associated with high risk of relapse and predicting therapeutic validation remain challenging question in clinical practice. Thus, there is an urgent need to identify better way with low cost methods to predict which patients treated for OSCC with develops recurrence so that the high risk patients can be selected for more rigorous treatment and follow up. Microsatellite markers could be used in this regard.

The TOC gene is implicated in the etiology of a number of sporadic tumors that are an important cause of mortality worldwide. This Oesophageal cancer Susceptibility locus TOC could be targeted as a marker for OSCC as well as therapeutic validation.

In this study 26 Microsatellite markers at 17q were examined in 150 OSCC patients treated with cisplatin and capecitabine to establish genetic marker for early detection as well as the marker responsible for drugs sensitivity and resistance for accurate clinical practice.

Materials and Methods

Patients and samples

In this study, a total of 150 primary tumor tissues, and corresponding blood samples from patients attending the King George's Medical University, Lucknow, from 2010 to 2016, were collected. The tissue samples were obtained, either at the time of investigative biopsy or during the time of the surgical resection of the lesions. Written informed consent in accordance with

Institutional Review Board guidelines was taken from all patients. One portion of the biopsy was utilized for routine histopathological examination and the rest was frozen immediately and stored in liquid nitrogen. Peripheral blood lymphocytes from the patients, used to assess the normal genotype of the loci of our interest, were stored at -80C until DNA extraction. Histopathological diagnosis was also performed according to standard criteria. Clinicopathological staging was determined as per UICC TNM staging system version 6. Patients then received standard treatment based on tumor stage and individual clinical status (Table-4 for clinicopathological data.).

Mode of treatment

Treatment strategy for each patient was given on the basis of stages. Treatment modality was combination therapy (n = 150) cisplatin & capecitabine.

Preparation of DNA and PCR

DNA was extracted from blood and tumor tissue using a proteinase K digestion and phenol–chloroform method (27). Twenty six microsatellite markers - D17S579, D17S722, D17S751, D17S785, D17S801, D17S926, D17S929, D17S937, D17S939, D17S1544, D17S1602, D17S1603, D17S1817, D17S1839, D17S1864, D17S2192, D17S1988, D17S2101, D17S2238, D17S2239, D17S2240, D17S2242, D17S2243, D17S2244, D17S2245, D17S2246 on chromosome arm 17q were used in this study. Genetic map location, physical order and primer sequence of selected microsatellite markers on chromosome at 17q loci, were obtained from the National Center for Biotechnology Information (NCBI's UniSTS site, www.ncbi.nlm.nih.gov/genome/sts/) (Table-1). The microsatellite primers were purchased from Bangalore Genie.

PCR reaction:- The microsatellite PCR was performed in 25 µl reaction volume containing 50 ng genomic DNA, 15 pmole each primer, 1.5 µl dNTPs (25 mM), 3.3 µl 10X assay buffer with $MgCl_2$ (15 mM), 0.6 µl Taq polymerase (3U/µl) (Bangalore Genei). DNA was amplified by PTC

200 MJ Research Thermocycler programme to provide first denaturation at 94^0C for 5 min. followed by 35 cycles of 1 min. at 94^0C, 1min. at 57^0C and 2 at 72^0C and final extension at 72^0C for 7 min.

The PCR products were observed in 3% agarose gels (wide range A 2790 of Sigma).

Table 1: Twenty Six Microsatellite Loci of TOC at Chromosome 17q were Used in OSCC Patients

S. No	Locus Name	Chromosome No/Position	Primer Sequences Forward	Primer Sequences Reverse	Product Size (bp)	(Ave. Allele Nos	Anne. Temp ºC
1	D17S579	**17q21.31**	5'AGTCCTGTAGACA AAACCTG-3'	5'CAGTTTCATACCAAGTTCC T-3'	111-125	3	57
2	D17S722	17q	5'AGCTGGGGGAATA GAGTGAGATTC-3'	5'AATTGCATGTCTCTGGGGT ACGGA-3'	158	2	57
3	D17S751	**17q25.2**	5'TTATTCACTGCATG CAAGCATCTC-3'	5'CTCACCATAGCTTGTTTCC CAAG-3'	125	2	57
4	D17S785	**17q25.1**	5'ATCCCTGGAGAGT GAAAATG-3'	5'AAGGCCAACCTGAAAACT AA-3'	181-207	6	57
5	D17S801	**17q**	5'CCTCAAACCGGAC AACTATTT	5'CAGAGAGCAAGATCCTAC CTC-3'	258-340	5	57
6	D17S926	**17q**	5'CATCACAGCACTG TACTCCA-3'	5'CCTGTATCAAAGTGTATAC TGGC-3'	114	2	57
7	D17S929	**17q25.1**	5'GCATGTCCCAGGG TTAGTCT-3'	5'TTGCTGTTGACAAAGTTCC A-3'	217-229	4	57

8	D17S937	**17q25**	5'CATGGAGGGACTTGCG-3'	5'-TTCCCAGAACCCGGTTT-3'	125-149	5	57
9	D17S939	**17q25**	5'AGCTATAAGTAACCATGTTTNTGG-3'	5'TACAGTGCAAACTCCTACCG-3'	191-215	4	57
10	D17S1544	**17q25**	5'GGCAGTGGTAAGCACCATG-3'	5'CTGGTTACTAAGTTCTGCTGAAACC-3'	178-179	3	57
11	D17S1602	**17q25.1**	5'AGGGAGTCCCCTTACC-3'	5'AGGCGGATATGATTTCAG-3'	197-127	4	57
12	D17S1603	**17q25**	5'GCTTGACCTTGGAAGGC-3'	5'CAGGCAATCAGTTTTCCTGTAG-3'	222-240	3	57
13	D17S1817	**17q25.1**	5'GGGGTGATGAAAGCAATCTG-3'	5'CTTGAACCTGGGAAGTGGA-3'	167-209	3	57
14	D17S1839	**17q25.3**	5'GGGTTCAAGCGCCTCA-3'	5'AGCGAGCCCCTGCCTTAT-3'	205-259	5	57
15	D17S1864	**17q25.1**	5'CCCAGGGAATCTTGTGTG-3'	5'GAGTCTTGGAGCGTGGATAC-3'	101-133	3	57
16	D17S2192	**17q25.1**	5'CCAAGGTCACCCTGCTAGTA-3'	5'CATCTATCTGTCCACCTATCTGC-3'	188-210	3	57
17	D17S1988	**17q11.2-q12**	5'CCCCACAGCTTACAGACCAT-3'	5'CAAGAGGAAAAGCAATTTCCC-3'	333-334	4	57
18	D17S2101	**17q25**	5'ACACTGACAAACTCATCCTCTGC-3'	5'TTATAATTTTCCCCACCATCCTGAC-3'	222	1	57
19	D17S2238	**17q25.1**	5'CTGGGTGACAGACCCAGACT-3'	5'GCTGGCTTGGTGGAATA-3'	195	2	57
20	D17S2239	**17q25.1**	5'CTGGGCTGGAGAGAAAGAGA-3'	5'CCCTCAGTGACTCCTACCTG-3'	162	4	57
21	D17S2240	**17q25**	5'GCCTTTCACTTGGCTGTCTC-3'	5'CCACATTTTAAGTGCTCGGC-3'	103	3	57
22	D17S2242	**17q25**	5'CAAGACAGTGCCACTGCACT	5'AGAGCTGGCAGATGCAGTGC-3'	192	2	57
23	D17S2243	**17q25.1**	5'GGCTCAAGCGATCCTGTGGC-3'	5'GTGAGGAATTTGAGACCAGC-3'	186	3	57
24	D17S2244	**17q25**	5'TGACTCACCAAGGCAATCAG-3'	5'TTGTACCACTTCACTCCAGCC-3'	165	3	57
25	D17S2245	**17q25.1**	5'GTGTCCAGTGTGCTGTCAGG-3'	5'GAGTGAGACCCTGTCGGAAG-3'	156	4	57
26	D17S2246	**17q25**	5'GGTTGCAGTGAGCCTAAATTG-3'	5'CTTGGGCGACACAGAGAGAC-3'	143	2	57

Sample preparation and DNA fragment analysis

The gels were scanned and the raw data were analyzed using the Fragment Manager software (Pharmacia Biotech). Preparation of the size marker, assessment of microsatellite instability (MSI) and LOH were performed as reported previously [16]**.**

Allelic status (LOH) was assessed by comparing constitutionally heterozygous (informative) alleles with their corresponding tumor alleles. Microsatellite loci were regarded as having LOH if either the entire upper or lower tumor allele of informative cases were visually absent or exhibited a 50% or greater diminishment of the allele than the corresponding normal allele in DNA isolated from the lymphocytes of patients. Alterations were described as microsatellite instability (MSI) when any additional band which was not seen in normal DNA was seen in tumor DNA. Samples showing allelic loss were subjected to repeat analysis after a second independent amplification. The mathematic model of LOH determination used is the following: height of normal allele two + height of normal allele one/ height of tumor allele two + height of tumor allele one.

Results

One hundred fifty patients with OSCC were included in this study. They included 132 (88%) men and 18 (12%) women with mean age at diagnosis of 55.3 years (range 38–73 for women, years) which was slightly lower in women than in men (men 49; range 42–82) but the difference was not significant. Tables-3,4 and figure- 2 show the clinicopathological data. The tongue was the most frequent localization affected in 59[Male-54, Female-5 (39.33%)], death was 31 [Male-28(51.85%), female-3(60%) (52.54%)] subsequently tumor and nodal status were T4a-17/12(70.58% death) and N2-41/24(58.53% death) respectively. The buccal mucosa was affected in 47[male-37, female-10 including one EP (31.33%)], death 23[male-19(51.35%), Female-4(40%),(48.93%)] and subsequently tumor and nodal status were T3-23/14(60.86% death) and N2-24/15(62.5% death) respectively. Hard Palate was affected in 7 (4.66%),death 2(28.57%). The lip was affected in 19 (12.66%) and death was 5(26.31%). The Floor of mouth was most frequently affected in men 7 (4.66%), death was 2(28.57%) whereas in women 1 (0.66%). The solid type was more frequent in men (72%) than in females (59%), whereas the peripheral type was more frequent in females (28%) than in men (19%), but these differences were not significant. Tumor status and death was found significant in this study (P≥5) but there was no significant differences observe in nodal Vs death. The only unicystic case was observed in a male patient . Primary tumor size was measured to be on average 2.1 cm. Median size was 1.75 cm, with a range between 0.6 and 5.0 cm. The unicystic case displayed the largest size with a 2.5 cm diameter followed by solid and peripheral cases with a mean size of 2.1 and 2.0 cm, respectively. The mean follow-up was 24 months with a range of 5–36 months. All of the patients were not alive at the date of the last documented follow up. Sixteen patients underwent radical surgery (67%) while the remaining ones underwent a conservative surgery (33%). No relationship was observed between the type of surgery and other variables, such as gender, histotype, localization and recurrence . Sixty five (43.3%) patients recurred during the period of follow-up, including 58 men (43.93%) and 7 (38.88%) women (EP-11). Recurrent cases were all solid type tumors particularly in tongue and buccal mucosa with a mean death of 15 months (range 5-30). 28 of the 54 recurrences affected the tongue. The size of recurrences was higher(P≥0.01). Table-2 and Figure-1 show the results of microsatellites analysis and the distribution of LOH and MSI in 150 cases of OSCC. Overall incidence of LOH/MSI was 40%±20.84 with the frequcncy of LOH and MSI of individual markers ranged from 12-83%. LOH was relatively more frequently detected at five loci, namely D17S1817 (57.33%), D17S1864 (81.33%), D17S1603 (68.66%), D17S1602 (83.33%), D17S929 (64%).

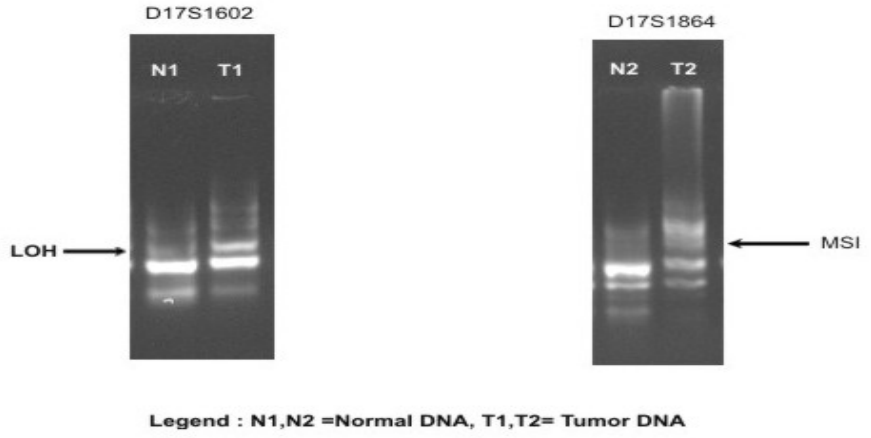
Low incidence of LOH and MSI was observed in D17S2238 (20.7%) and D17S926 (12.66%) and D17S2101 (18.7%). The incidence of LOH were highest in the tumors of tongue, buccal Mucosa and lip with multiple genetic loci range from 3to 8. Remaining two tmours with LOH at single or double loci.MSI were detected in all tumors of tongue, buccal Mucosa and lip with at list 5 loci. Furthermore, all tumors of tongue, buccal Mucosa and lip with LOH and MSI were from male patients whereas, 4% was observed in female.

Presence of LOH was having no association with the age, tumour size but tumour stage was significantly associated with LOH/MSI of the patients (P≥0.05). Mixed habit was also associated with LOH

Table 2: Analysis of 26 Microsatellite markers of TOC Locus examined in 150 OSCC Patients

SL. No	Locus	Allele No.	Allele frequencies	Ho (%)	He (%)	LOH (%)	MIS (%)
1	D17S579	7	0.570	137.33%	44.60%	55.33%/150 (83)	34.60%
2	D17S722	7	0.123	117.30%	17.30%	19%/150 (42)	12.60%
3	D17S751	8	0.089	132%	31.33%	21%/150 (21)	15.33%
4	D17S785	8	0.423	95%	37.33%	56%/150 (8)	39.30%
5	D17S801	7	0.532	90.66%	58.66%	55.33%/150 (7)	18%
6	D17S926	3	0.560	80.66%	42%	30.66%/150 (16)	12.66%
7	D17S929	8	0.432	81.33%	76.66%	64%/150 (8)	12.66%
8	D17S937	8	0.663	240%	36%	31.33%/150(47)	18.66%
9	D17S939	8	0.231	278%	42%	29.33%/150 (44)	17.33%
10	D17S1544	5	0.098	85.33%	11.33%	28%/150 (42)	13.6%
11	D17S1602	8	0.549	100.66%	60.66%	83.33%/150 (140	46.21%
12	D17S1603	7	0.378	71.33%	66%	68.66%/150 (103)	47.02%
13	D17S1817	8	0.501	128%	61.33%	57.33%/150 (86)	13.33%
14	D17S1839	8	0.765	153%	63%	54%/150 (81)	12.04%
15	D17S1864	8	0.798	124%	73.33%	81.33%/150 (122)	40.66%
16	D17S2192	7	0.543	86%	29.33%	36.66%/150 (55)	19.66%
17	D17S1988	6	0.045	92%	25.33%	34.66%/150 (52)	24.04%
18	D17S2101	1	0.098	92.66%	24%	28.66%/150 (28)	18.7%
19	D17S2238	4	0.085	22%	18.66%	30.66%/150 (31)	20.7%
20	D17S2239	6	0.439	63.33%	34.66%	36%/150 (54)	18.66%
21	D17S2240	5	0.564	67.33%	24%	25.33%/150 (38)	15.33%
22	D17S2242	3	0.503	54%	27.33%	25.33%/150 (38)	13.33%
23	D17S2243	2	0.296	66.66%	26%	26.66%/150 (40)	17%
24	D17S2244	3	0.327	48.66%	40.66%	42.66%/150 (64)	38%
25	D17S2245	5	0.405	52.66%	16%	26%/150 (39)	18.66%
26	D17S2246	2	0.097	86.66%	14.66%	30%/150 (45)	17.33%

Figure: 1



Legend : N1,N2 =Normal DNA, T1,T2= Tumor DNA

Figure 1

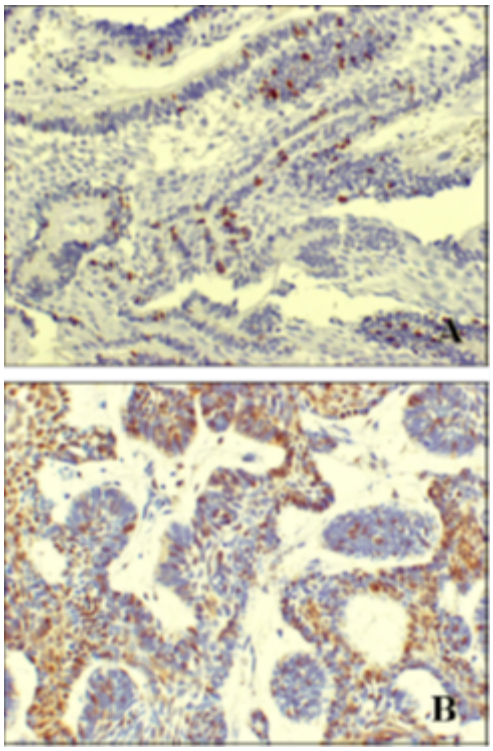


Figure 2: Histopathological findings Normal and tumor samples

Table 3: Clinicopathological Characteristics of OSCC patient Treated with Cisplatin & Capecitabine

Gender	
Male	132
Female	18
Body Weight	
Male	67.34 (43-79)
Female	55.3 (38-73)
Body Mass Index	
Male	25.81±3.02
Female	23.62± 4.03
Heamoglobin	
Male	10.5 ±1.02
Female	10.1 ±1.5
Habits	
Exclusive Chewers	76
Exclusive Smokers	25
Exclusive Drinkers	32
Mix Habitues	17
Pathological Grade	
Well Differentiated	24%

Moderate differentiated	49%
Poor differentiated	68%
Tumor/Nodal Location	
Tongue	M-54; (EP-2) ; F-5
Buccal Musoca	M-37; (EP-5); F-9, (EP-1)
Lip	M-19, (EP-1); F-Nil
Floor of Mouth	M-7; F-1
Alveolus	M-9(EP-1); F-2
Hard Palate	M-6, (EP-1); F-1
Pathological Stages	
T1	T-0/0; Bm-7/2, Li-0/0, Alv-1/0, Fm 0/0, Hp-1/0
T2	T-9/2; Bm-5/1, LI-13/5, Alv-2/0, Fm-3/0, Hp-1/0
T3	T-30/16, Bm-23/14, Li-6/0, Alv-6/2, Fm-3/0, Hp-4/2
T4	T-3/1, Bm-9/5, Li-0/0, Alv-0/0, Fm-0/0, Hp-0/0
T4a	T-17/12, Bm-3/1, Li-0/0, Alv-1/0, Fm-2/2, Hp-1/0
Nodal Status	
N0	T-0/0, Bm-2/1, Li-3/0, Alv-4/1
N1	T-13/6, Bm-19/6, Li-13/3, Alv-5/1
N2	T-41/24, Bm-24/15, Li-2/2, Alv-1/0
N3	T-5/1, Bm-2/1, Li-1/0, Alv-0/0
Treatment	
Surgery Only	5
Surgery + RT	12
Surgery + RT + CT	43
CT + RT	67
CT	23
Recurrence Status	
NO Recurrence	68
Relapse	58
Lost to follow up	14 + Relapse, EP-10
Clinical Outcome	
Alive without Disease	37 (24.66%)
Dead	65 (43.33%); M-43.93%; F-41.17%
Moth wise Mortality	5/1, 6/4, 7/1, 8/0, 9/6, 10/0, 11/4, 12/6, 13/5, 14/6, 15/3, 16/1, 17/6, 18/17, 19/4, 20/3, 23/1, 24/2, 26/3, 30/1
Alive with Disease (Month)	48 (38)

Table 4: Clinco pathological Co-relation in OSCC Patient Treated with Cisplatin and Capecitabine

	T1	T2	T3	T4	T4a	N0	N1	N2	N3	Male	Female
Tongue	0	9/2 (22.22%)	30/16 (53.33%)	3/1 (33.33%)	17/12 (70.58%)	0	13/6 (46.15%)	41/24 (58.53%)	5/1 (20%)	54/28 (51.85%)	5/3 (60%)
Buccal Mucosa	7/2 (28.57%)	5/1 (20.00%)	23/14 (60.86%	9/5 (55.55%)	3/1 (33.3)	2/1 (50%)	19/6 (31.57%)	24/15 (62.5%)	2/1 (50%)	37/19 (51.35%)	9/4 (40%)
lip	0 0	13/5 (38.46)	6/0 (0)	0	0	3/0 (%)	13/3 (23.07%)	2/2 (100%)	1 (0%)	19/5 (26.31%	0
floor of Mouth	0	3	3	0	2/2 (100%)	0/0	4 (0)	4/2 (50%)	0 (0)	7/2 (28.57%)	1 (0)
Alveolus	1 (0)	2 (0)	6/2 (33.33%)	0	1 (0)	4/1 (25%	5/1 (20%)	1 (0%)	0 (0%)	9/2 (22.22%)	2 (0)
Hard Palate	1 (0)	1 (0)	4/2 (50.00)	0	1 (0)	1/1 (100%)	2/0 (0%)	4/1 (25%)	0 (00%)	6/2 (33.3%)	1 (0)
Av Death per (%)	**22.22%**	**25**	**49.27%**	**50%**	**62.5**	**30%**	**28.57**	**57.89%**	**25%**	**43.93%**	**41.17%**

Table 5: OSCC Patients Showing Drugs response/Chemo Response Against TOC Microsatellite LOCI

SL NO	Lucus	CR	PR	NR
1	D17S579	0	1	0
2	D17S722	0	1	0
3	D17S751	0	0	0
4	D17S785	0	1	0
5	D17S801	0	0	0
6	D17S926	0	9	2
7	D17S929	14	4	0
8	D17S937	0	0	1
9	D17S939	0	1	0
10	D17S1544	0	2	1
11	D17S1602	25	4	0
12	D17S1603	11	4	0
13	D17S1817	14	4	0
14	D17S1839	0		0
15	D17S1864	19	5	0
16	D17S2192	0	1	0
17	D17S1988	0	1	
18	D17S2101	1	6	3
19	D17S2238	2	5	2
20	D17S2239	0	0	0
21	D17S2240	0	2	0
22	D17S2242	0	0	0
23	D17S2243	0	0	0
24	D17S2244	0	1	0
25	D17S2245	0	3	0
26	D17S2246	0	0	0

Discussion

Metastatic progression due to development or enrichment of therapy-resistant tumor cells is eventually lethal. Molecular characterization of such chemotherapy resistant tumor cell's DNA may identify markers responsible for malignant progression and potential targets for new treatment protocols. DNA copy number changes in selected tumor cells sampled from a chemotherapy-resistant bone marrow metastasis have been documented and subsequently gene analysis showed amplification of KRAS and ERBB2 oncogenes as possible molecular targets of therapy in tumor cells in gastroesophageal adenocarcinoma [17].

Two types of microsatellite alterations have been reported in human tumors: deletion, which causes loss of heterozygosity (LOH) at the corresponding loci and modification, also known as microsatellite instability (MSI). MSI was initially demonstrated in a subset of hereditary nonpolyposis colorectal cancers (HNPCC) and was associated with germline mutations in genes involved in DNA mismatch repair (MMR) mechanisms [11]. The results of this study were partially agree with the previous study and it may be due to HNPCC. Conflicting results have been reported on the frequency of MSI in many tumors and these discrepancies can be

attributed to different factors, including the source of tumor DNAs used for the analysis and the numbers and types of microsatellites included in each study. Thus, to standardize detection of MSI a panel of repeat loci (the Bethesda consensus panel) was established to define MSI. The panel only included mono- and dinucleotides repeats which are mainly affected by MMR deficiency. The present study panel was completely associated with the previous studty [17].

A second variety of instability has been more recently reported that appears to be not related to MMR deficiency and is best seen at tetranucleotide repeats. This form of instability has been termed EMAST (elevated microsatellite alterations at selected tetranucleotides) and appears to occur with high frequency in a variety of human tumors [12] and our study was also associated with the repeat frequency.

No direct data were available on the type and the frequency of microsatellite alterations in OSCC on TOC loci at 17q25, in spite of that our observations were associated with the documented reports of previous research, which involved in fifteen tumor suppressor genes in a series of 12 ameloblastomas were analyzed and found a high frequency (average 46%) of allelic loss [23]. It has also been also reported that a high frequency of allelic losses (between 33% and 50%) at the p53 locus whereas the TP53 locus, corresponding to the p53 tumor suppressor gene, and the loci D3S1312 and D3S1300, corresponding to the FHIT gene, were not altered in any of the tumors analysed [27,28].

It is possible that this deficiency contribute to tumorigenesis by increasing the mutation rate of genes directly involved in the process or that, on the other hand, it is simply an epiphenomenon of genetic and molecular alterations occurring in tumor cells. However, the finding that some of the loci were not altered in any of the tumors analyzed suggests that alterations at specific loci preferentially occur and/or are selected during OSCC development. We observed a significant association between the occurrence of microsatellite alterations, especially at specific loci, and a high tumor cell proliferation activity, that microsatellite alterations, in the form of instability or deletion, is a frequent event in OSCC suggesting a possible role in the process of tumor development. Moreover, it might have a prognostic significance being a predictor of high risk of recurrence, if the results of the present study will be confirmed on a large cohort of patients. Our findings are in agreement with the only available paper investigating allelic losses in OSCC.

LOH studies have implicated various regions on chromosomes 3p, 5q, 9p, 13q, 17p, 17q and 18q in the development of sporadic oesophageal cancer [8,11-14]. Recent studies have also linked sporadic oesophageal cancer with the TOC gene on chromosome 17q25.

The first LOH study for the TOC region in oesophageal cancer was done by von Brevern *et al*.[7] Their study investigated 35 sporadic squamous cell carcinomas of the oesophagus using six polymorphic markers. Twenty-four of the 35 informative cases (69%) revealed LOH at one or more loci. Deletions were most frequently observed with the marker D17S801 (64% LOH). Aberrations at these individual markers were as follows: D17S1864 (55%), D17S1839 (60%), D17S1817 (41%), D17S785 (42%), D17S1603 (52%) and D17S801 (64%).

Another study, by Iwaya *et al* [4], investigated LOH on chromosome 17q in 58 sporadic oesophageal squamous cell carcinomas. LOH was observed in 37 of 52 (71%) informative

cases at one or more loci, 89% (33/37). Their findings at the following loci were as follows: D17S1864 (64%), D17S1839 (70%),

D17S785 (40%), D17S1603 (52%), and D17S579 (34%).

A further study involving three different population groups showed LOH in the region of the TOC locus in sporadic squamous cell carcinoma of the oesophagus to be as follows: D17S1864 in France (50%), China (58%) and Japan (64%); D17S1603 in France (36%), China (64%) and Japan (52%); D17S1839 in France (40%), China (73%) and Japan (70%); D17S801 in France (57%), China (71%) and Japan (54%); D17S1817 in France (29%), China (54%) and Japan (N/A); and D17S785 in France (33%), China (47%) and Japan (40%).[5]

A novel gene, DMC1 located in the TOC gene locus (17q25.1), showed loss of gene expression in multiple human cancers. In addition, these findings showed LOH around the TOC gene, specifically at the D17S1839 and D17S1603 loci [6]. A recent study by Shahabi *et al* [8] investigated LOH in 60 sporadic oesophageal cancers from Iran. Forty-four of these 60 samples (73%) showed allelic imbalance at one or more loci within or adjacent to the TOC minimal region (70% of cases were informative). Aberrations at these individual markers were as follows: D17S1603 (71%), D17S1817 (61%), D17S2246 (69%) and D17S244 (64%). 15 Previous studies reported frequent LOH at chromosome 17q in both oesophageal and head and neck cancers. LOH was observed in 55% of these tumours. Losses were found most frequently at markers around the TOC locus. D17S1839 showed 48% LOH and D17S1603 43% LOH.

OSCC cancer is one of the 4 leading causes of mortality worldwide. In India it is the most common cancer in males, accounting for 19.3% of all cancers in this group of Patients. Our findings indicate that LOH ranged from 12% to 83%. LOH for the individual markers was as follows: D17S1817 (57.33%), D17S1864 (81.33%), D17S1603 (68.66%), D17S1602 (83.33%), D17S929 (64%).Low incidence of LOH and MSI was observed in D17S2238 (20.7%) and D17S926 (12.66%) and D17S2101 (18.7%).

Our figures for LOH at markers D17S926, D17S2101 and D17S2238 were lower when compared to studies conducted by von Brevern *et al*,[7] Iwaya *et al*,[4] Risk *et al* [5] and Shahabi *et al*.[8] However, LOH for D171602 and D17S929 was higher in our study compared to results from the study by Iwaya *et al*.[4]

Since these studies were conducted in different geographical settings it is possible that these differences could be attributed to regional genetic variation. Despite this, our findings are very similar to previous studies. This study lends support to the hypothesis that the TOC gene plays an important role in the development of oral squamous cell carcinoma. Further, since markers D17S929, D17S1602 and D17S1864 showed more than 60% LOH in our study, these loci are potential ‘‘hotspots’’ in the TOC gene. MSI, however, ranged from 12.66% to 47% for the 26 loci in the 17q region. MSI was found to be 12.6% for the markers D17S722. However, there were some interesting trends observed when comparing the molecular and clinicopathological parameters. Poorly differentiated tumours nodes were found to have high LOH for markers D17S1602 and D17S1864 (28.57% and 31.57%, respectively) in buccal mucosa. On the other hand, markers D17S926 and D17S751 show very low LOH and MSI in poorly differentiated tumours, indicating that these TOC loci are perhaps not important for the development of oral squamous cell carcinoma in our cohort of poorly differentiated cases. TNM staging showed that LOH occurred more frequently in late stage tumours for all markers.

These findings are similar to those of Iwaya *et al*.[4] A large proportion of patients who

died (17/12,70.58% for tongue;) showed LOH for markers D17S1602 and D17S1864. This would indicate that these markers may be markers of aggressive and/or late disease. Disease recurrence is a common and challenging problem in patients affected by OSCC. Identification of risk factors for local relapse after appropriate therapy with surgery, radiation or combination therapy still remains an active area of clinical research.[13]

Previous studies have observed loss of chromosomal region 9p21 in 70–80% of HNSCC, thus representing the most common genetic alteration in head and neck cancers.[8,14] The present study suggests that LOH at D17S1602 and D17S1864 on TOC at 17q25.1 is associated predominantly with recurrent disease and that this association is independent of T status, which is currently a predictor of outcome in OSCC. These indicate that LOH at D9S1602 and D17S1864 can serve as a prognostic marker and facilitate the prediction of the tendency of post-treatment recurrence in oral cancer patients.

To our knowledge, this is the first report suggesting an association between allelic loss at D9S1602 & D17S1864 on TOC loci and recurrence in OSCC from India in patients treated with Cisplatin & Capetabine. A previous study conducted in Britain [18] in oral dysplasia, also observed that individuals with LOH at 3p and/or 9p exhibited a 3.8-fold relative risk of developing cancer. Mao *et al*. have also shown losses of the 9p21 region to be related to early processes of tumorigenesis in HNSCC.[19]

Further, a study conducted by Lydiatt *et al*. at Nebraska, U.S.A, demonstrated that LOH of 9p correlates with recurrence of HNSCC.20 Also, microsatellite analysis of 9p21 and 3p14 has been shown to be simple tool for predicting second oral malignancy at previously treated oral cancer sites.[17] Many Labs have done immunohistochemical studies and shown a significant association between p16 expression with overall survival and disease free survival[11].

LOH at the TOC locus alone, whereas only 6 showed LOH at the BRCA1 locus alone. These results at least indicate that TOC and BRCA1 are independent targets of LOH, and that either gene may play a role in the genesis of some sporadic breast cancers. Although germline mutations of BRCA1 are common in familial breast cancers, somatic mutations of this gene are rarely seen in sporadic cases[26]. Taken together, these data suggest that TOC, once identified, could be assessed as a tumor suppressor whose inactivation contributes not only to esophageal cancers but also to a significant proportion of sporadic breast cancers.

The current study assumes significance because it offers a cost efficient option over existing RNA and protein profiling methods, as it can be carried out by PCR from a small amount of DNA from patient tumor as well as blood samples. Further, very few gene profiling studies have examined the utility of employing molecular profiling in predicting the prognosis and therapeutic outcomes of patients with OSCC on 3q,9q,119 but not in 17q of TOC[19-22].

Conclusion

To summarize, allelic losses at D9S1602 &D17S1864 on TOC at 17q25.1 can be utilized as a predictive marker for recurrence in patients with OSCC treated with cisplatin & capecitabine and may prove to be a useful tool in treatment planning. Currently, clinical and histological parameters are used as guidelines for the application of adjunctive therapy such as radiotherapy.

Our data strongly suggests that LOH at 17p25.1 can augment the predictive power of those clinical and histopathologic markers. Therefore, routine assessment of LOH at

D9S1602 and D17S1864 could be useful in predicting recurrence and guide therapeutic decision-making for subsequent follow up and management.

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