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Microsatellite Instability in Oral Squamous Carcinoma In Relation To Tumor Suppressor Gene P53

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Abstract: *Inactivation of tumor suppressor genes and activation of oncogenes have been considered to play an important role in the multistep carcinogenesis in humans. The objective of this study was to investigate the incidence of microsatellite alteration in relation to p53 tumor suppressor gene in oral squamous cell carcinoma. 37 patients who were histopathologically diagnosed with OSCC with no other superimposed premalignant and malignant conditions were considered for the study. Blood and tumour tissues were obtained from each patient and DNA from these were isolated*
.Microsatellite alteration in relation to p53 tumor suppressor gene was analyzed in blood and tissue samples using highly polymorphic dinucleotide marker D17S796 flanking the region and microsatellite instability and Loss of Heterozygosity were assessed. Microsatellite alteration in p53 gene with reference to marker D17S796 was observed in 80 % of OSCC patients which includes both MSI and LOH. The study also showed clinicopathological parameters were independent of Microsatellite alterations. A very high incidence of MSA in relation to marker D17S796 in p53 clearly indicates the early alteration and role of p53 gene Tumor Suppressor Gene in OSCC. The study also pointed towards the importance of early detection of MSA which aids in early diagnosis and treatment of the disease.

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I. Introduction

Squamous cell carcinoma arising from oral mucosal epithelium remains a lethal and deforming disease due to tumor invasion, orofacial destruction, cervical lymph node metastasis and ultimate blood borne dissemination.(1) Inactivation of tumour suppressor genes and activation of oncogenes have been considered to play important roles in the multistep process of human tumorigenesis. (2). Genomic instabilities as reflected by microsatellite alterations in specific target regions is an important feature of OSCC.(3) Microsatellites or simple sequence repeats or tandem repeats are repeating sequences of 1 to 6 base pairs of DNA. The appearance of abnormally long or short microsatellites in an individual's DNA is termed as Microsatellite Instability. Microsatellite alterations is characterised by MSI and LOH . In affected carcinomas the number of repeat units in the microsatellite increases or decreases, although the composition of the unit itself is unaffected. This instability is demonstrated by comparing DNA from the neoplasm to normal DNA from the same patient. MSI may thus be a marker of defective MMR mechanisms. [4,5] . p53 gene located on chromosome 17p13.1 is one of the most frequently mutated gene in human cancers. [6,7] Hence the detection of early microsatellite alterations in the same will help in early diagnosis of oral cancer at the molecular level.

Though extensive studies have been conducted in colorectal, breast and gastric carcinomas relating to microsatellite instability and tumor suppressor genes the studies relating the same in OSCC in Kerala population is very limited. In the present study we aim at detecting the early microsatellite alterations in the p53 tumor suppressor gene present in chromosome 17 using highly polymorphic dinucleotide marker D17s796 in Malayalam speaking south Indian population.

II. Materials And Methods:

A prospective case control design was conducted for duration of 1 year .A strict selection criteria was applied and 37 Malayalam speaking individuals native to Kerala who were diagnosed with OSCC without superimposed premalignant and other malignant conditions were recruited. A written proforma was designed to

record demographic details of patients like age, gender, habits, site of lesion, histopathological grading etc. The study was approved by the institutional ethical committee of Govt .Dental College,Trivandrum. 3 ml of peripheral blood sample was collected in EDTA vials and stored at -70 degrees till DNA isolation. After the biopsy half of the tumor tissue was fixed in formalin and sent for histopathology and remaining half was stored at -70 degrees for DNA studies. The tissue samples were included in the study after histopathological confirmation of OSCC. Both blood and tissue samples from all the 37 subjects were obtained and DNA was isolated from samples by conventional phenol chloroform method. The concentration of genomic DNA isolated from samples were checked by measuring its absorbance at 260 nm ,(A260/A280) was used to estimate the purity of DNA .A260/A280 between 1.7 and 1.8 was considered as good quality DNA. Primer for microsatellite marker D17S796 flanking the p53 gene was designed and synthesised with a 6 FAM labelled fluorescent dye for the forward primer. PCR was set with primer, Taq polymerase and ABI PRISM true allele PCR premix. To increase the sensitivity of microsatellite assay monoplex PCR was done. Amplification of D17 S796 was done with initial denaturation at 95 degrees for 12 minutes followed by 94 degrees for 30 minutes and final extension at 72 degrees. The amplified DNA fragments were then subjected to capillary electrophoresis using applied biosystems 3730 DNA analyser. The fluorescence data collected during fragment separation was analysed by Gene Mapper software V 4.0 (Applied Biosystems,Foster City,USA). The automatic allele calls were confirmed visually by examining the gene scan electropherograms. Graphic peak patterns of the electropherogram in each patient's blood and tissue DNA were compared for analysis of MSI and LOH. Micro satellites of blood were considered normal and microsatellite alterations in tissue MI was scored if one or both alleles at a given locus showed size variation, either increase or decrease or presence of novel peaks in comparison with normal tissue. Presence of stutter peaks was also considered in scoring. Tissue samples were classified as Microsatellite Stable (MSS) if no alteration was found. Statistical analysis was done using Chi-Square analysis or Fischer Exact Test wherever appropriate.LOH was scored if there was a complete loss of one allele or if the peak intensity of one allele was decreased by at least 50% in the tumor tissue compared to the same allele in the corresponding normal tissue.The value was calculated using a normalised allele ratio equation R=(A1)(N2)/(A2)(N1) where A1 and A2 are the heights of the allele from DNA of biopsy samples and N1 and N2 are heights of alleles from the DNA from blood. Cases in which LOH ratio was <0.67 or >1.5 were scored as LOH.For calculation of LOH samples showing homozygozity were not considered.

III. Results:

The study population comprised of 21 males(57%) and 16 females(43%) of which 26 subjects were above 60(70%) and 11 were below 60(30%).22 patients were with tobacco chewing habit (59%)11 patients with tobacco smoking habit(30%) and 4 patients were without tobacco habit(11%).6 patients had alcohol consuming habit.(16%).Buccal mucosa was the site of lesion in 14 cases(38%),tongue in 14 cases(38%),retro molar area and alveolus in 2 cases each(5% each),1 each in soft palate and hard palate and three in gingiva.14 tumor samples were well differentiated (38%),18 were moderately differentiated (49%) and 5 tumours were poorly differentiated(14%).Frequency of Microsatellite Alteration with reference to microsatellite marker D17S796 flanking p53 gene is 80%.(30 samples were informative in which 24 were Microsatellite Alteration positive and (80%) and 6 showed no alteration(20%).Frequency of MI in relation to marker D17S796 was 77%.7 cases showed no MI.(23%).Frequency distribution of LOH in relation to D17S796 was 23% and 77% of tumor samples showed no LOH.There was no significant correlation between Clinicopathological parameters like tumour differentiation,tumor location,age,habits etc.The marker D17S796 showed onoallelic lengthening in 11 cases,monoallelic shortening in 4 cases,biallelic shortening in 9 cases and biallelic lengthening in 2 cases.

IV. Discussion:

Neoplastic transformation in the normal human cells occurs as a result of series of genetic alterations, including the loss, gain or amplification of different chromosomes[8]. Genes that positively control cell cycle check points are the targets for oncogenic activation in cancer whereas negative regulators such as tumor suppressor genes are targeted for inactivation[9]. Carcinogenesis is a multistep process in which several oncogenes and tumour suppressor genes are considered to be involved. Putative TSGs, which may play an important role in oral squamous cell carcinogenesis are located on the arms of several chromosomes. [10] the existing model of carcinogenesis indicates that all human tum ours have an unstable genome and that allelic imbalances is a very useful tool in assessing the level of genetic damage in early stages of cancer. [11]

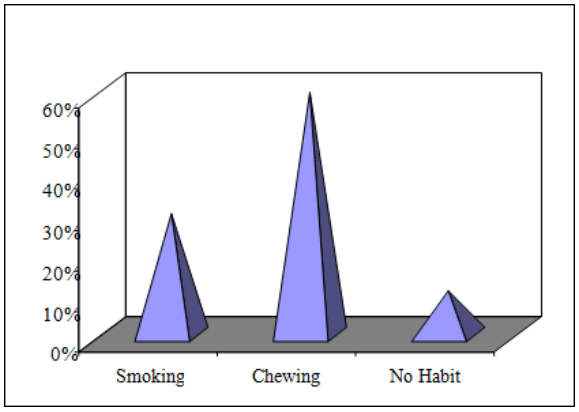


Fig 1.1: Distribution based on tobacco usage

Region	Frequency	Percent
Buccal Mucosa	14	37.8
Tongue	14	37.8
Retro Molar Area	2	5.4
Gingiva	3	8.1
Alveolus	2	5.4
Soft Palate	1	2.7
Hard Palate	1	2.7

Fig 1.2: Distribution based on region

In the present study we have analysed the microsatellite alteration in relation to tumor suppressor gene p53 (17p13.1) with highly polymorphic dinucleotide microsatellite marker D17S796 in 37 OSCC patients native to Kerala. The frequency of microsatellite alteration in relation to p53 gene was found to be 76.7 %.Frequency of LOH was found to be 23%.This data clearly shows the strong association of microsatellite alteration in p53 gene in OSCC. Majority of the previous studies agree with our study results. Various studies concluded a 42% to80% of LOH in relation to 17p. [(12), (13),(14),(15).]. Our results of higher degree of MS alteration in relation to p53 gene shows a strong association of this gene in oral carcinogenesis. p53 is the most frequently mutated gene in human cancers and more than 7000 mutations in various type of cancers have been reported.[(6),(7), (16).]An immunohistochemical analysis in relation to p53 gene products or gene sequencing could have given a clear picture of mutation status of the gene .However with our result of high degree of microsatellite alteration in relation to p53 gene ,we can see a close association of this gene in oral carcinogenesis. When compared with another genomic study using same DNA samples to assess MI in relation to mismatch repair genes ,it was clearly found that the degree of microsatellite alteration in relation to tumor suppressor genes are more when compared to mismatch repair genes.[(17)]. This finding totally agrees with study result of Glavac.D in which he analysed 125 OSCC with 22 markers and concluded that high frequency of alteration in tumor suppressor genes compared to mismatch repair genes indicates the dominant role of suppressor I comparison with the mutator pathway in head and neck squamous cell carcinogenesis.[(18).]

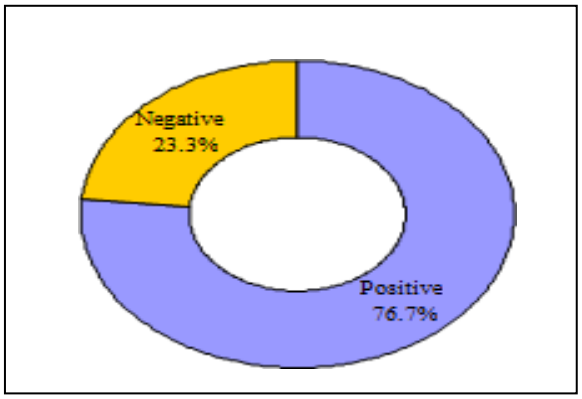


Fig 1.3: MSI in relation to D 17S796

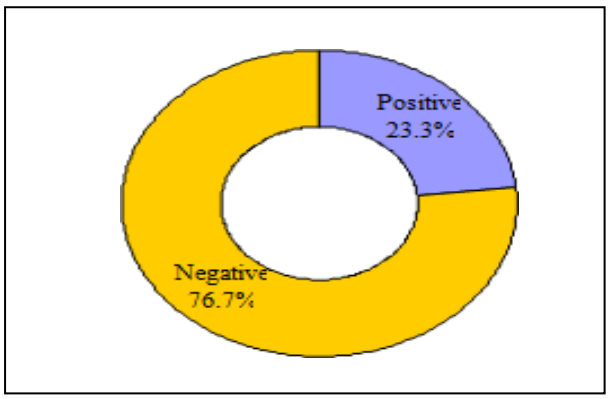
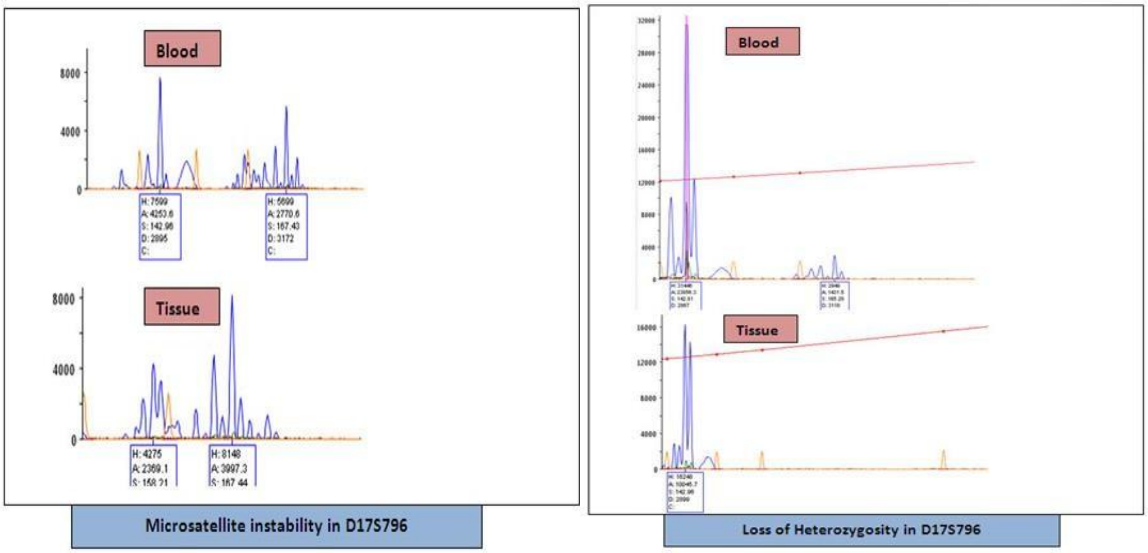


Fig 1.4: LOH in relation to D 17S796

In the present study the clinicopathological parameters like age, sex, gender, habits, site of lesion, histopathological differentiation of tumour. When assessed for microsatellite alteration no significant association was found. The same result has been reported by previous studies also[(19),(20),(21).]. The predominant allele alteration seen in the present study were mono allelic lengthening and and monoallelic shortening with no novel peaks. The presence of rare biallelic alteration in the epicentre of high molecular abnormality region indicates that LOH and MA in one allele of these regions might impose selective pressure on the other allele at

the same locus for deletion or size alteration.[22].



In this study with monoplex PCR used instead of multiplex PCR and fluorescence based MI and LOH analysis and capillary electrophoresis with automatic fragment analysis software it can be assumed that error is negligible. With the results of the present study it can be seen that MS alteration in relation to p53 Tumor suppressor gene is an early event in oral multistep carcinogenesis and the detection of the same in the early stages of the disease can have diagnostic and prognostic importance.

V. **Conclusion**:

The present study revealed an increased association of Microsatellite alteration which includes Microsatellite instability and Loss of heterozygosity in relation to p53 tumor suppressor gene clearly indicates that alteration in p53 gene is an early event in oral multistep carcinogenesis. Early detection of these events can have diagnostic and prognostic importance. Study also concludes that the clinical parameters and histopathological grading has no significant association with microsatellite alteration in relation to p53 tumor suppressor gene.

References:

[1]. Thomson PJ. Perspectives on oral squamous cell carcinoma prevention—proliferation, position, progression and prediction. Journal of Oral Pathology & Medicine. 2018 Oct;47(9):803-7.
[2]. Weinberg RA. Tumor suppressor genes. Science. 1991 Nov 22;254(5035):1138-46.
[3]. Mahale A, Saranath D. Microsatellite alterations on chromosome 9 in chewing tobacco-induced oral squamous cell carcinomas from India. Oral oncology. 2000 Mar 1;36(2):199-206.
[4]. Mironov NM, Aguelon MA, Potapova GI, Omori Y, Gorbunov OV, Klimenkov AA, Yamasaki
[5]. H. Alterations of (CA) n DNA repeats and tumor suppressor genes in human gastric cancer. Cancer research. 1994 Jan 1;54(1):41-4.
[6]. Dillon EK, De Boer WB, Papadimitriou JM, Turbett GR. Microsatellite instability and loss of heterozygosity in mammary carcinoma and its probable precursors. British journal of cancer. 1997 Jul;76(2):156-62.
[7]. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science. 1991 Jul 5;253(5015):49-53.
[8]. Greenblatt MS. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res.. 1994;54:4855-78.
[9]. Tsuzuki T, Tsunoda S, Sakaki T, Konishi N, Hiasa Y, Nakamura M. Alterations of retinoblastoma, p53, p16 (CDKN2), and p15 genes in human astrocytomas. Cancer: Interdisciplinary International Journal of the American Cancer Society. 1996 Jul 15;78(2):287-93.
[10]. (9) Porter PL. Molecular markers of tumor initiation and progression. Current opinion in genetics & development. 2001 Feb 1;11(1):60-3.
[11]. ARAI K, SHIBAHARA T, YAMAMOTO N, YAKUSHIJI T, TANAKA C, NOMA H.
[12]. Frequent allelic loss/imbalance on the short arm of chromosome 3 in tongue cancer. The Bulletin of Tokyo Dental College. 2001;42(3):151-7.
[13]. Nunn J, Scholes AG, Liloglou T, Nagini S, Jones AS, Vaughan ED, Gosney JR, Rogers S, Fear S, Field JK. Fractional allele loss indicates distinct genetic populations in the development of squamous cell carcinoma of the head and neck (SCCHN). Carcinogenesis. 1999 Dec 1;20(12):2219-28.
[14]. Field JK, Kiaris H, Risk JM, Tsiriyotis C, Adamson R, Zoumpourlis V, Rowley H, Taylor K, Whittaker J, Howard P. Allelotype of squamous cell carcinoma of the head and neck: fractional allele loss correlates with survival. British journal of cancer. 1995 Nov;72(5):1180.
[15]. Adamson R, Jones AS, Field JK. Loss of heterozygosity studies on chromosome 17 in head and neck cancer using microsatellite markers. Oncogene. 1994 Jul;9(7):2077-82.

[16]. Beder LB, Gunduz M, Ouchida M, Fukushima K, Gunduz E, Ito S, Sakai A, Nagai N, Nishizaki K, Shimizu K. Genome-wide analyses on loss of heterozygosity in head and neck squamous cell carcinomas. Laboratory investigation. 2003 Jan;83(1):99-105.
[17]. Bockmühl U, Schwendel A, Dietel M, Petersen I. Distinct patterns of chromosomal alterations in high-and low-grade head and neck squamous cell carcinomas. Cancer research. 1996 Dec 1;56(23):5325-9.
[18]. Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R, Harris CC. Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. Nucleic acids research. 1996 Jan 1;24(1):141-6.
[19]. Panda S, Beena VT, Sivakumar R, Choudhary K, Nalinakumari KR, Sathyan S, Banerjee M. Microsatellite instability in D2S123 flanking the hMSH2 gene in oral squamous cell carcinoma in South India. Clinical Cancer Investigation Journal. 2015 Jan 1;4(1):22.
[20]. Glavač D, Volavšek M, Potočnik U, Ravnik-Glavač M, Gale N. Low microsatellite instability and high loss of heterozygosity rates indicate dominant role of the suppressor pathway in squamous cell carcinoma of head and neck and loss of heterozygosity of 11q14. 3 correlates with tumor grade. Cancer genetics and cytogenetics. 2003 Oct 1;146(1):27-32.
[21]. Choi TH, Chung WB, Hong SH, Kim JA, Na SO, Jang HJ, Sohn YK, Kim CS, Kim JW. Microsatellite instability and p53 gene mutation in oral squamous cell carcinoma. Journal of the Korean Association of Oral and Maxillofacial Surgeons. 2000;26(4):337-44.
[22]. El-Naggar AK, Hurr K, Huff V, Clayman GL, Luna MA, Batsakis JG. Microsatellite instability in preinvasive and invasive head and neck squamous carcinoma. The American journal of pathology. 1996 Jun;148(6):2067.
[23]. Field JK, Kiaris H, Howard P, Vaughan ED, Spandidos DA, Jones AS. Microsatellite instability in squamous cell carcinoma of the head and neck. British journal of cancer. 1995 May;71(5):1065-9.
[24]. Dasgupta S, Mukherjee N, Roy S, Roy A, Sengupta A, Roychowdhury S, Panda CK. Mapping of the candidate tumor suppressor genes' loci on human chromosome 3 in head and neck squamous cell carcinoma of an Indian patient population. Oral oncology. 2002 Jan 1;38(1):6-15.

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