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MICROSATELLITE INSTABILITY IN ORAL SQUAMOUS CELL CARCINOMA WITH REFERENCE TO TUMOUR SUPPRESSOR GENE BRCA 1

Oral Pathology

Dr. Sivakumar R*	Assistant Professor, Dept. of Oral pathology, Govt. Dental College Thiruvananthapuram. *Corresponding Author
Dr. VT Beena	Principal, Govt. Dental College Kottayam.
Dr. Nalina Kumari K R	Formerly Associate Prof. & Head, Division of Dental Care, Regional Care Centre, Thiruvananthapuram.
Dr. R. Heera	Professor & Head of the Department, Dept. of Oral pathology Govt. Dental College Thiruvananthapuram.
Dr. Swagatika Panda	Professor, Dept. of Oral pathology, Institute of dental sciences Siksha o Anusandhan University, Odisha.
Dr. K. Navin Nair	Formerly Senior Resident, Dept. of Oral pathology, Govt. Dental College, Alappuzha.
Dr. Moinac Banerjee	Human Molecular Genetics Laboratory, RGCB Thiruvananthapuram.

ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy representing up to 80 to 90% of all malignant neoplasms of the oral cavity. Microsatellite alterations characterized by microsatellite instability (MI) and loss of heterozygosity (LOH) have been implicated as a possible means of early detection of head and neck malignancy. The objective of this study was to investigate the incidence of microsatellite alteration in relation to BRCA 1 tumor suppressor gene in OSCC in south Kerala population. A total of 37 patients histopathologically diagnosed with OSCC with no other malignant and premalignant conditions were considered for the study. Blood and tumor samples were obtained and DNA from these were isolated and microsatellite alteration in relation to BRCA 1 tumor suppressor gene was analyzed in these samples using D17S579 microsatellite marker flanking the region and MI and LOH were assessed. It was concluded that molecular alterations in BRCA 1 gene is an early event in oral multi step carcinogenesis.

KEYWORDS

Oral squamous cell carcinoma, microsatellite instability, BRCA 1, LOH

INTRODUCTION

Oral squamous cell carcinoma is the common oral carcinoma with varied clinical presentations. It accounts for more than 90% of malignant lesions of the oral cavity. [1] The Asian continent has the highest incidence and mortality rates of oral cavity and oropharyngeal cancers among all other countries. [2] Inactivation of tumor suppressor genes and activation of oncogenes have been considered to play an important role in the multistep process of human tumorigenesis [3]. Clonal evolution due to progressive accumulation of genetic alteration in proto oncogenes and tumor suppressor genes are the principal hypothesis for neoplastic development. [4] Microsatellite alterations (MSA) characterized by MSI and LOH have been proposed as a possible way of early detection of head and neck cancer. Microsatellites(also known as short tandem repeats or simple sequence repeats) are sequences of DNA comprising of multiple copies of repeat unit of 1 to 6 base pairs. They are common, polymorphic and distributed widely throughout the genome. Tumours exhibiting microsatellite instability are said to have replication error (RER phenotype) [5]. in affected carcinomas the number of repeat units in the microsatellite increases or decreases, although the composition of the unit itself is unaffected. ([6] BRCA1 is a tumor suppressor gene linked to DNA recombination and repair process being of importance for its role in regulation of RAD51 and H2AX [7].Though extensive studies have been conducted in breast carcinoma, ovarian cancers and gastric carcinoma regarding the role of BRCA 1 gene, studies regarding its role in OSCC is very limited. In this study we aim at detecting microsatellite alterations including MSI and LOH in BRCA 1 gene in chromosome 17 using a highly polymorphic dinucleotide microsatellite marker D17S579 flanking the gene in south Kerala population.

MATERIALS AND METHODS:

A prospective study was carried out for a period of 1 year and based on strict selection criteria 37 subjects native to the south Indian state of Kerala diagnosed with OSCC without any other superimposed premalignant or malignant conditions were recruited for the study. A proforma was prepared to record patient demographic details like age, gender, site of the lesion, deleterious oral habits and histopathological grading of tumor. The study was approved by the institutional ethical committee Govt. Dental College, Trivandrum. 3 ml of peripheral blood sample was collected and stored at -70 degrees for DNA studies. After biopsy half of the tumor tissue was sent for histopathological analysis and remaining half was stored at -70 degrees for DNA studies. The tissue samples were included in the study after histopathological confirmation of OSCC. DNA was isolated from all tissue and blood samples using conventional phenol chloroform method. DNA concentration was assessed by spectrometer absorbance between 260 nm and 280 nm.A260/A280 between 1.7 and 1.8 were considered as good quality DNA. Primer for microsatellite marker D17S579 flanking the BRCA 1 gene was designed and synthesized with fluorescent label. PCR was set with primer Taq Polymerase and true allele PCR premix. To increase the sensitivity of the microsatellite assay Monoplex PCR was done with initial denaturation at 95°C for 12 minutes followed by 94°C for 30 minutes and final extension at 72°C. The amplified DNA fragments were then subjected to capillary electrophoresis and fluorescence data was analyzed by Gene Mapper software which uses adjustable parameters like range, peak detection settings, size range tabulation of peaks and size calling. The automatic allele calls were confirmed visually by examining the Gene scan electropherograms. Graphic peak patterns of electropherograms in each patient's blood DNA were considered normal and microsatellite alteration in tissue were studied.

Microsatellite instability 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 or absences of stutter peaks were also carefully considered. Tumor samples were labeled Microsatellite stable (MSS)if no alteration was found(Fig 1.5). Statistical analysis was done by Chi-square or Fischer test as required. 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 computed using equation R=(A1)(N2)/(A2)(N1) where A1 and A2 are peak height of alleles from tumor tissue and N1 andN2 are peak heights of alleles from DNA of blood. LOH was scored when ratio was <0.67 or >1.5(Fig 1.6). Homozygous samples were not

considered for scoring.

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%) , retromolar area and alveolus in 2 cases each(5% each),1 each in soft palate and hard palate and 3 in gingiva (Fig 1.1). 14 tumor samples were well differentiated (38%),18 were moderately differentiated (49%)and 5 tumors were poorly differentiated(14%) (Fig 1.2).

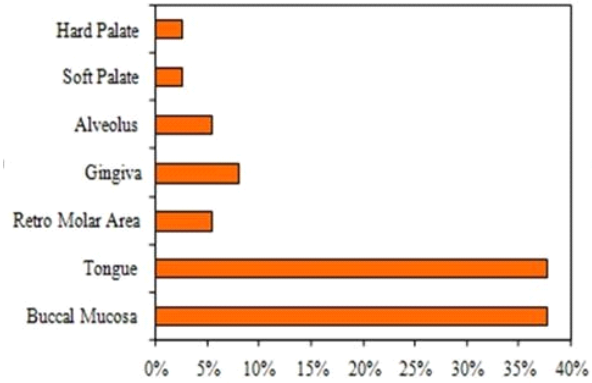

Fig 1.1.: Distribution Based On Region

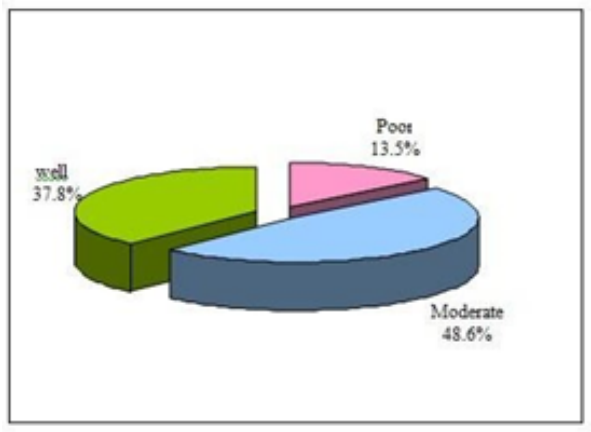

Fig 1.2: Distribution Based On Differentiation

Frequency of Microsatellite instability with reference to microsatellite marker D17S579 was 60%. (Out of 30 informative cases 18 showed MI positivity (Fig 1.3). Frequency distribution of LOH in relation to marker D17S579 was 16 %.(Of the 30 informative cases only 5 cases showed LOH)(Fig 1.4). The study by microsatellite marker D17S579 also showed mono allelic lengthening in 12 cases, biallelic lengthening in 3 cases, mono allelic shortening in 2 cases and biallelic shortening in 3 cases. The clinicopathological parameters like age, gender, habits, site of lesion, habits, histopathological differentiation of tumor showed no significant association with microsatellite alteration.

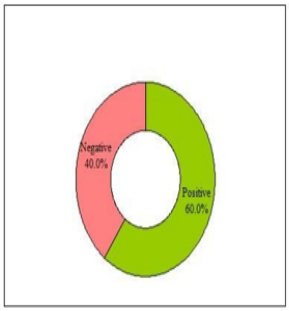

Fig 1.3: MSI in relation to D 17S 579

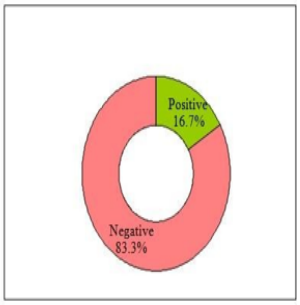

Fig 1.4: LOH in relation to D 17S 579

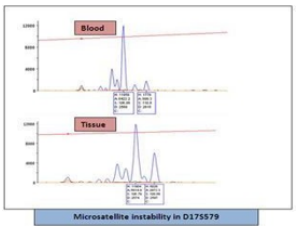

Fig 1.5: MSI in relation to D17S579

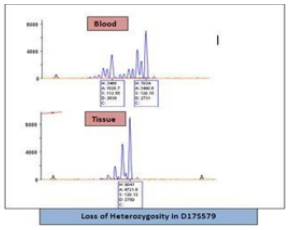

Fig 1.6: LOH in relation to D17S579

DISCUSSION:

Head and neck squamous cell carcinoma affects 700000 individuals per year making it the sixth leading cause of cancer related morbidity worldwide [8],[9] . Genetic changes that lead to loss or changes in tumor suppressor genes are known to contribute to oral carcinogenesis [10].Those alleles when present in normal tissues and undergoing persistent deletion I tumors could be used as markers for screening and early identification of population at risk of developing head and neck lesions [11]. Allelic losses at homozygous deletions and polymorphic loci are key events of tumor suppressor genes [3] .

In the present study we have analyzed the microsatellite alteration in relation to tumor suppressor gene BRCA 1 in chromosome 17 with highly polymorphic microsatellite marker D17S579 flanking the region in 37 OSCC patients belonging to Malayalam speaking Kerala population. In the results we got the frequency of Microsatellite instability with reference to marker D17S579 as 60%. The frequency of LOH in relation to D17S579 was 16.7 %. This data clearly shows a strong association of microsatellite alteration in BRCA 1 tumor suppressor gene in oral squamous cell carcinoma. The result agrees with the other studies relating the BRCA 1 gene and oral squamous cell carcinoma [7] [12]. Microsatellite instability and loss of heterozygosity in relation to BRCA 1 gene indicates the early genomic instability and changes in the gene in oral squamous cell carcinoma. The genomic instability in relation to BRCA 1 reflects the replication errors which result in erroneous recombinations and ultimately manifesting as notable variations in the length of microsatellites. Since these changes are associated with early carcinogenesis the identification of them at such an early stage can be very significant in early diagnosis and treatment. These variations can be used as an effective marker of OSCC in relation to this gene and can be used for genetic instability assessment which in turn reflects the risk of the population in relation to head and neck carcinoma. Early genomic instability assessment will help in early diagnosis and early intervention and will definitely improve prognosis of the disease.

Current evidence suggests that mutations in BRCA 1 do not directly result in tumor formation, but instead causes genetic instability, exposing cells to high risks of malignant transformation. FA /BRCA (DSB) pathway has been reported to be involved in transformation of oral premalignant lesion (Oral Leukoplakia) to OSCC [13]. It has been reported that the product of BRCA 1 putative tumor suppressor gene interacts directly with p53 and transcriptionally activates p21.Together with p53 gene alteration, alteration in RCA 1 gene could contribute to genomic instability by selectively activating p21 in ulcerative colitis associated carcinogenesis. It has been reported that BRCA1 selectively coactivates the p53 transcription factor towards genes that direct DNA repair and cell cycle arrest but not towards those that direct apoptosis. Studies also indicate that p53 is involved in inhibiting recombination by both BRCA1 dependent and independent mechanisms and there is functional link between p53 suppression and BRCA1 promotion in regulation of HR activity at transcription level and possible post transcription level.[14, 15] The cumulative effect of these two genes in oral carcinogenesis has to be investigated further.In our study the clinicopathological parameters like age, gender, habits, site of lesion, histopathological differentiation of tumors were assessed for microsatellite alteration to find out any significant relation is there between these parameters and microsatellite instability in relation to BRCA 1 gene. The clinical parameter tobacco habit was again subdivided into tobacco smoking and tobacco chewing. The differentiation of tumours considered were well, moderate and poorly differentiated. In our study it was clearly found that there was no significant correlation between these clinical pathological parameters and MI. This result is in agreement with the previous studies done with tumor suppressor genes [16] . In the present study monoplex PCR was 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 the error is negligible. Hence it can be concluded that the microsatellite alterations in BRCA tumor suppressor gene is an early event in oral multistep carcinogenesis and the detection of the same in early stages of the disease can have diagnostic and prognostic significance.

CONCLUSION:

The present study showing a significant association of microsatellite alteration in relation to BRCA 1 tumor suppressor gene in oral squamous cell carcinoma clearly indicates that molecular alterations in in BRCA 1 gene is an early event in oral multi step carcinogenesis. The study also reveals that the clinical parameters and histopathological grading has no significant association with microsatellite alterations in relation to this gene.

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