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Lack of the BRAF^{V600E} Mutation in Oral Squamous Cell Carcinoma

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Abstract

The aim of this study was to estimate the frequency of BRAF (V600E) mutation in oral squamous cell carcinoma (OSCC) in the south Indian population. In the present study we analyzed the V600E BRAF gene mutation in 100 cases with oral squamous cell carcinoma (OSCC), using a PCR-DHPLC method. Our analyses illustrated that no V600E BRAF gene mutation was detected among the 100 selected patients. Our study is the first one in which the frequency of BRAF (V600E) mutation in oral squamous cell carcinoma (OSCC) was not reported in the south Indian population. This suggests that BRAF V600E mutations may not be playing a role in these OSCC patients.

Introduction

Oral cancer is one of the most commonly occurred cancers worldwide. It is the sixth most common cancer occurring globally and accounts for 30% of all cancers in India (Elango, J. K et al, 2006). Oral cancer arises from different regions of the oral cavity. About 90% of oral malignant neoplasms are oral squamous cell carcinomas (OSCC). In India Oral squmaous cell carcinoma (OSCC) is the most common cancer in males and it is the third most common cancer in Indian females (Rai R, et al 2004). Consequently, there has been an increasing focus on identifying key genetic players that may contribute to OSCC pathogenesis, with the overall goal of preventing onset and progression of disease.

BRAF is a B-type Raf kinase, located in chromosome 7, and is the most potent activator of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MEK-ERK) pathway. This pathway plays a role in mediating cellular response to cell growth. BRAF mutations have been described in about 15% of all human cancers. Activating mutations of BRAF are found in colorectal cancers (CRCs), melanomas, ovarian tumors, lung and thyroid cancers (Davies et al., 2002). The most common hotspot mutation in the BRAF gene is a thiamine transversion to adenine at nucleotide position 1799 (T1799A) in exon 15. This causes a conversion of valine to glutamate of amino acid 600 in the BRAF protein. This mutation disrupts the kinase function, which leads to constitutive activation and downstream signaling along the MAPK signaling pathway. This mutation predisposes to apoptosis inhibition, increases invasiveness, and occurs during carcinogenesis (Mercer, et al 2003).

BRAF mutational status is a strong predictor for overall survival not only in the metastatic setting but also in earlier-stage diagnoses. BRAF gene is previously implicated in HNSCCs, there is little data regarding their involvement in OSCCs. In the present study, we analyzed the status of the BRAF gene V600E mutation to elucidate a possible role of these genes in the oral squamous cell carcinoma.

Materials & Methods

Study Population

Oral squamous cell carcinoma (OSCC) patients were assessed on the basis of clinical and pathological examinations. This Study is a Hospital-based study conducted in South Indian population. All incidents of OSCC cases were newly diagnosed during the study period Ethics Committee approved the study for the benefit of humans in general. The procedures followed were in accordance with the ethical standards of responsible committee of the Institutes / Hospitals, to participate in a face-to-face interview using a structured questionnaire.

Selection Criteria

Senior pathologists confirmed all diagnoses. We interviewed and collected the data about the patient's demographic factors. We collected the information on habitual risk factors and previous cancer diagnoses. The clinical information for these cases were obtained from medical records like tumor size and stage of cancer.

Sample Collection

Based on the above criteria, a total of 100 OSCC patients were enrolled for genotyping study. About 5ml Blood samples were collected in from pathology lab after diagnosis. All the samples diagnosed mainly as OSCC. Sampling was done from MNJ Cancer Hospital, one of the major Cancer Hospitals in Hyderabad, Andhra Pradesh between the period June 2013 to January 2016.

Genotyping of the BRAF^{V600E} Polymorphism

Genomic DNA was extracted from whole blood according to standard procedures used by our group. PCR amplification was carried out using the given primers, 5'-TCATAATGCTTGCT-CTGATAGGA-3' (Forward primer) and 5'-GGCCAAAAATTTAATCAGTGGA-3' (Reverse primer) synthesized at Bio serve Biotechnologies (Hyderabad, India). A 3 step PCR amplification was performed as described earlier (Mohan, et al 2013), briefly a 25 µl reaction was set up containing 0.2mM of each dNTP, 1X buffer, 1.5 mM $MgCl_2$ and 2U of Taq DNA polymerase (Bioserve, India). 30 cycles were performed with denaturation at 94 ^{o}C for 30 seconds, annealing at 55 ^{o}C for 30 seconds and elongation at 72 ^{o}C for 30 seconds. The amplified PCR products were subjected to denaturing high-performance liquid chromatography (dHPLC).

Results

Biological Characteristics of the study population

In the present study 100 blood samples from Oral squamous cell carcinoma (OSCC) patients were used. Distribution of the selected demographic characteristics and risk factors in control subjects and OSCC patients is shown in Table 1. The demographic profile included sex, age, and various habitual risk factors involved in the progression of OSCC. Out of 100 OSCC cases, 45 (45%) patients were male and 55 (55%) patients were female. In the control group 55(55%) were male and 45(45%) were female. Ages ranged from 9-87 years for cases and 21-76 years for controls. The mean age of OSCC patients and healthy controls at the time of diagnosis was 50.53 and 55.27 years, respectively. OSCC patients were divided into four groups according to age at diagnosis; these were < 25, 26-45, 46-65, and >66 years. Incidence of OSCC cases and a control group was higher in the age groups 46-65 (60% and 70%) years when compared to other age groups. In this study we observed that tobacco chewing (32%, 30%), alcohol + tobacco chewing (17% & 13%) alcohol smoking (12%, 6%) and

alcohol + smoking + tobacco chewing (10%, 6%) in OSCC patients and controls respectively. According to histological differentiation of tumor grades, 27%, 39% and 34%, patients were classified in three grades, poor, moderate, or well grade, respectively. In our study we found that well and moderate groups were high than poor.

Analysis of Genotype frequency of BRAF V600E

BRAF genotyping analysis

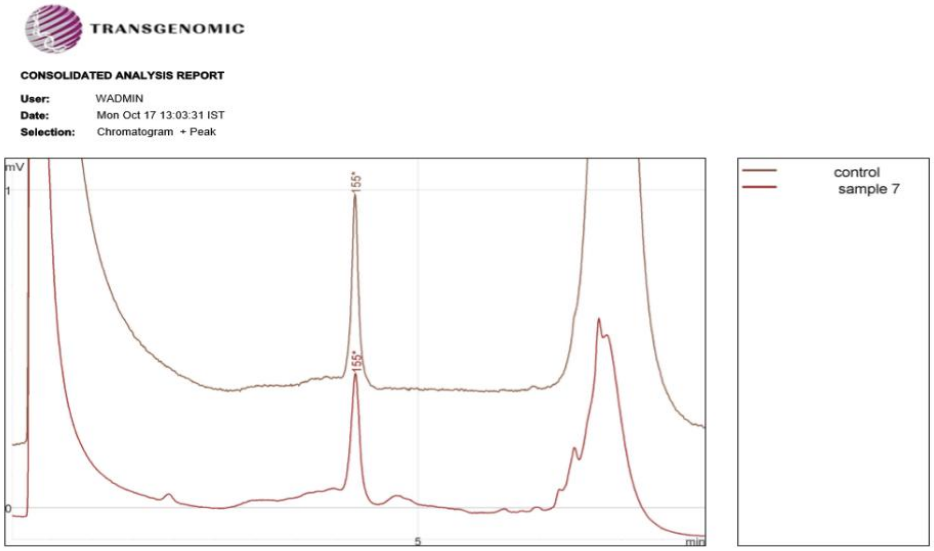
BRAF polymorphism in DNA obtained from biopsy samples of 100 OSCC patients was done and compared to 100 healthy age matched control volunteers. BRAF polymorphism was analyzed by PCR-DHPLC. All the heteroduplexes were aligned. There are 3 peaks in the chromatorgram, the first peak is the injection peak, middle peak is the DNA sample and the last peak is the primer peak. The heteroduplexes formed a sharp peak without any abnormality and no mutation was observed in all these cases. Sequencing was done and no mutation was observed. In the present study, V600E polymorphism was not found in both OSCC cases and controls. We show that there was no significant difference between the patients and controls, and there was no relation in risk of OSCC between cases and controls for BRAF V600E polymorphism.

Table 1: Clinical Characteristics of the OSCC Patient & Healthy controls

Clinical Characteristics	n = 100 (Cases)	n = 100 (Controls)
Gender		
Males	45(45%)	55(55%)
Females	55 (55%)	45 (55%)
Mean age & Range Males	50.53/9-87	
Mean age & Range Females	55.27/30-75	
Age Distribution		
26-45	28 (28%)	22(22%)
46-65	60 (60%)	70(70%)
66 and above	12 (12%)	8 (8%)
Habitual Risk		
Alcoholics	16 (16%)	3 (3%)
Smokers	32 (32%)	13 (13%)
Tobacco chewing	52 (52%)	30 (30%)
Alcohol + Smoking	12 (12%)	6 (6%)
Alcohol + Tobacco chewing	17 (17%)	13(13%)
Smoking + Tobacco chewing	1 (1%)	4 (4%)
Alcohol + Smoking + Tobacco chewing	10(10%)	6 (6%)
No Habits	10(10%)	25 (25%)
Site of Diagnosis		
Tongue	23(23%)	
Buccal mucosa (BM)	35 (35%)	
Mandible	12 (12%)	
Oral Cavity	10 (9%)	
Retromolartrigone	8(8%)	
Floor of mouth	4(4%)	
Lip	3(3%)	
Base of tongue	2 (2%)	
Maxilla	2 (2%)	
Palate	1 (1%)	
Staging		
Stage 1	6 (105)	
Stage 2	21 (19%)	
Stage 3	39 (50%)	
Stage 4	34(21%)	

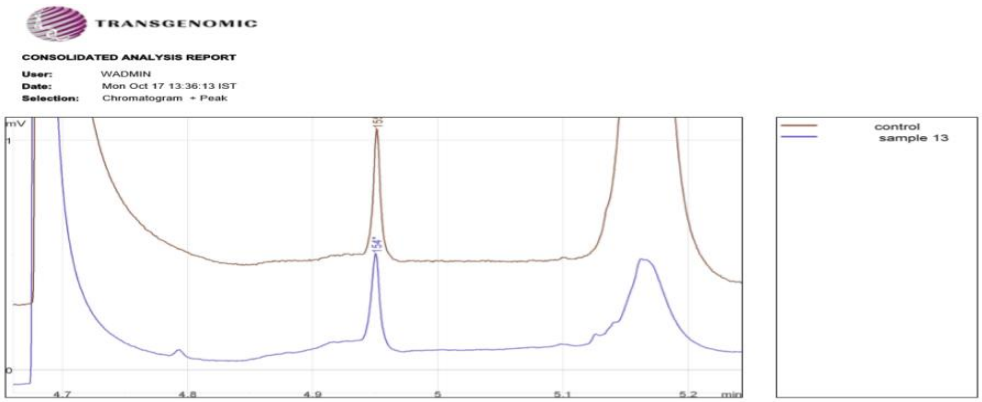
BRAF chromatogram figures:

BRAF case # 7



TCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCCTTTACTTACTACACCTCAGATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGTGAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTTGTGGATGGTAAGAATTGAGGCTATTTTTCCACTGATTAAATTTTTGGCC

BRAF case # 13



TCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCCTTTACTTACTACACCTCAGATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGTGAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTTGTGGATGGTAAGAATTGAGGCTATTTTTCCACTGATTAAATTTTTGGCC

Discussion

The V600E mutation is in the part of BRAF that passes along the cell growth signal. When the BRAF mutation occurs, the pathway becomes constitutively activated, abolishing Ras activation and ATP requirements. Such deregulation is essential for melanoma cells' proliferation and survival, since the mutant protein is ten times more active than the wild type (Sekulic et al., 2008; Vidwans et al., 2011). BRAF mutations occur in 40-70% of cutaneous melanomas, with V600E mutations accounting for >90% of mutations. BRAF mutations seem to predict clinical response to either BRAF or MEK inhibitors in melanoma and other tumors. BRAF V600E mutations do not respond to anti-EGFR monoclonal antibody therapy. BRAF V600E mutations have been found to have significantly

worse overall survival, progression-free survival and response rates to conventional chemotherapy.

In this study, we assessed the BRAFV600E mutation frequency in OSCC patients. Our analyses illustrated that no V600E BRAF gene mutation was detected among the 100 selected patients. This suggests that BRAF V600E mutations may not be playing a role in these OSCC patients. Our results were remarkably similar to the mutation rates reported by Shelly et al. (2005). BRAF gene is previously implicated in HNSCCs, there is little data regarding their involvement in OSCCs Davies et al. (2002) examined 19 HNSCC and failed to detect specific mutations. Weber, et al. (2003) reported that 3% mutation frequency of BRAF, in their pharynx and hypopharynx specimens but none in oral specimens. BRAF mutations occurring in exon region 15 in canine oral cancer specimens and found no mutations in their cohort of samples (Shelly, et al 2005). Findings of the present and previous studies failed to associate this mutation with oral squamous cell carcinoma. Although somatic mutations of BRAF are not frequent events in OSCC, as suggested by our study, the detection of these mutations is important to support the notion that the *RAS-RAF-MEK-ERK-MAP* kinase and *PIK3CA-PTEN-AKT* pathways are involved in OSCC tumorigenesis.

Conclusion

We conclude that the BRAF V600E mutation in oral squamous cell carcinoma (OSCC) was not reported in the south Indian population. This suggests that BRAF V600E mutations may not be playing a role in these OSCC patients. Sequencing was done and no mutation was observed. This may be due to small cohort of samples and other studies are still needed to determine the role of genetic and epigenetic alterations in the activation of this oncogene in HNSCC.

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