Original Article

An Oncogenic Role of Astrocyte Elevated Gene-1 in Oral Squamous cell Carcinoma

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Abstract

Background: *The role of oncogenes and tumor suppressor genes in Oral squamous cell carcinoma (OSCC) have already been found in numerous studies. Astrocyte Elevated Gene-1(AEG-1) is an oncogene that is overexpressed in many cancer, including OSCC.*

Objective: *With an aim of understanding the genetic basis of squamous cell carcinoma among the Bangladeshi population, this study has been carried out to find out the genetic expression in AEG-1 gene with OSCC patients.*

Materials and Methods: *Malignant tissue was collected from 24 patients with OSCC and 14 normal patients. RNA was extracted using RNeasy Mini Kit(Qiagen). Then, cDNA synthesis and RT-qPCR were performed to find out the expression of AEG-1 gene.*

Results: *Among twenty four patients, fourteen patients(58.3%) with OSCC had over-expression of AEG-1 gene in compared to control, which was 3 fold increased in OSCC patients.*

Conclusion: *In sum, according to the study, it has been indicated that the over-expression of the AEG gene has an oncogenic role in development of OSCC in Bangladeshi patients. However, further studies on the expression of the AEG-1 gene have to be done with more samples for a better understanding of their role in the formation of OSCC.*

Key Words: *Oral squamous cell carcinoma, AEG-1 gene, Oncogene.*

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Introduction

Oral cancer is the sixth most common cancer in the world and oral squamous cell carcinoma (OSCC) is more than 90% of all oral cancers, accounting approximately 630,000 new patients diagnosed annually resulting in more than 350,000 deaths every year.[1] The incidence rate of this cancer is not distributed uniformly as three-quarters of affected people reside in the developing countries and it is much more common in Southeast Asia,[2] while in developed countries it is less common. Differences in incidence rates across the world are mainly due to the types of oral cancer-associated lifestyles, practiced by different groups of people.[3] The major oral cancer risk factors are: tobacco smoking, alcohol drinking and betel quid chewing etc.[4] Despite the progressing in diagnostic techniques, SCC has a high incidence in different parts of the world.

Many histopathologic and clinical criteria have been used to evaluate the prognosis of oral squamous cell carcinoma, yet, the most important prognostic factor is TNM staging. Now a days, there is much interest in novel molecular markers for predicting of tumor prognosis and estimating of overall survival rate of patients.[5] To improve the outcomes of advanced OSCC, we need to find specific early biomarkers for the diagnosis and prognosis and develop new targeted therapies based on an understanding of the molecular mechanisms of the disease.

Astrocyte elevated gene 1 (AEG-1) expression is up-regulated in various human cancers and plays an important role in tumorigenesis and progression and also related with poor survival. This protein also known as metadherin is a transmembrane protein that has a role in cancer development and progression, was originally identified by rapid subtraction hybridization as a human immunodeficiency virus 1– or tumor necrosis factor α–inducible transcript in primary human fetal astrocytes.[6,7]

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The human AEG-1 gene is located on chromosome 8q22, consists of 12 exons and 11 introns, and is amplified in several cancers such as HCC and breast cancer.[8] Overexpression of AEG-1 showed that it could promote proliferation of many cancer cells, including breast and prostate tumors, while knocking down AEG-1-induced apoptosis and inhibiting growth.[9] AEG-1 gene is an oncogene that is frequently overexpressed in oral cancer including OSCC and plays a significant role in tumor growth, invasion, metastasis, angiogenesis and chemoresistance.[10]

AEG-1 expression positively correlates with tumor progression, especially in the metastatic stage, and in vivo studies using nude mice and metastatic models with various cancer cell lines and transgenic and knockout mouse models point out that AEG-1 overexpression induces an aggressive, angiogenic and metastatic phenotype, and AEG-1 knockdown or knockout markedly hampers tumor initiation, growth and metastasis.[11,12]

AIMS AND OBJECTIVES:

GENERAL: To find out the role of AEG-1 gene in oral squamous cell carcinoma.

SPECIFIC:

1. To find out the oncogenic or tumor suppressive role of AEG-1 gene in oral squamous cell carcinoma.
2. To find out the clinicopathological correlation with AEG-1 expression with OSCC.

Materials and Methods

Sample collection: 24 cases of oral squamous cell carcinoma (OSCC) and 14 control patients were included in this study. Firstly cancer tissues of oral cavity and fresh frozen tissues of gingiva flaps were taken from cancer patients of OSCC and healthy normal non-cancer individuals as control respectively. The collected tissues were preserved in RNAlater solution. All samples and related clinical data were collected from Bangabandhu Sheikh Mujib Medical University (BSMMU).

RNA isolation: The collected tissues were sectioned using a microtome and homogenized using WiseTis HG-15A homogenizer. RNA were extracted from 700 mm of tissue sections using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. The quality and concentration of the RNA was measured using the Implen NanoPhotometer P300 Primer design: Primers for the project was designed using Primer Blast (www.ncbi.nlm.nih.gov/tools/primer-blast/) and later was verified by NCBI-BLAST cDNA synthesis: Total RNA (3 µg) was reverse-transcribed to synthesize complementary DNA (cDNA) by QuantiTect Reverse Transcription Kit (Qiagen) according to manufacturer's instruction. The kit provides a fast and convenient procedure that requires only 20 minutes to synthesize first-strand cDNA and eliminates genomic DNA contamination.

Real time RT-PCR: Real time RT-PCR was performed to quantify the level of eIF3e mRNA in test and control subjects. Real time PCR amplification of cDNAs was carried out in a 15 µl reaction mixture containing SYBR Green PCR Master Mix (Qiagen) and primers according to manufacturer's instruction. The expression of eIF3e mRNA was normalized to the expression of the 'housekeeping gene' 18S ribosomal RNA (RRN18S). The comparative CT method was used for relative quantification of the amount of mRNA for each sample normalized to RRN18S.

Statistical analysis: Statistical analysis was conducted using an Excel spreadsheet and IBM SPSS version 22. Significant integration was identified using Chi-squared test. Expression correlated with sociodemographic and clinicopathological parameters were calculated using Chi-square or Fisher's exact test.

Results

A total of 24 patients with male-female ratio was 1.18:1, where the mean age was 52.5(+-13.6). The majority (41.7%) of the patients were 41 to 50 age groups [Table I]. Maximum 50% of patients had cancer at buccal mucosa then 16.66% patients had cancer at tongue. 8.33% of patients had cancer at retromolar trigone, vestibule, maxilla both buccal mucosa and vestibule of the mouth. [Table II].

Out of 24 patients, 58.3%(14) patients had overexpression of the AEG-1 gene [Fig 1] and it was more than 3 fold increased [Table III], where 41,7% (8) patients had less or normal expression in comparison to normal subjects. In this study, we also examined several variables to find out the correlation with AEG-1 gene expression but no significant relation was found between other clinicopathology and AEG-1 gene expression.

Table I: Age Distribution of Patients

Age group	Frequency	Percent	Cumulative Percent
less than 30 years	1	4.2	4.2
31 -40 years	2	8.3	12.5
41 -50 years	10	41.7	54.2
51 -60 years	6	25.0	79.2
over 60 years	5	20.8	100.0
Total	24	100.0	

Table II: Site of Oral Cancer

Site of Lesion	Frequency	Percent
Buccal mucosa	12	50.0
vestibule	2	8.33
retromolar area	2	8.33
tongue	4	16.66
maxilla	2	8.33
buccal mucosa and vestibule	2	8.33
Total	24	100.0

Table III: 3.03 fold increased of Gene expression

Sample	No. of Cases	Mean Ct (18S RNA)	Mean Ct (int6 gene)	Normalized Mean Ct	Mean 2^ - △ △ Ct	P value	Mean fold incresed
Control	14	9.37	21.51	12.14			
OSCC	24	9.50	20.06	10.56	.33	<0.05	3.03

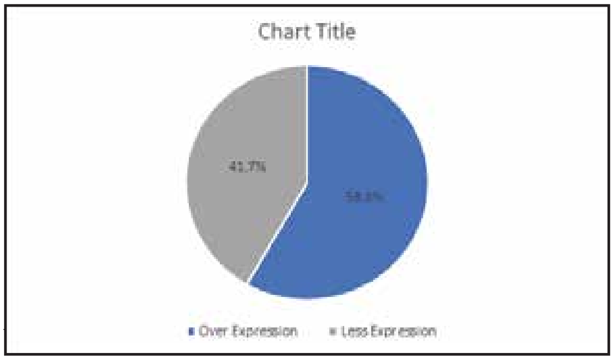

Fig 1: Frequency distribution of Gene expression

Discussion

In this study, we evaluated AEG-1 gene expression in 24 cases of OSCC patients and tried to find out the correlation with some clinicopathological features. There was 58.3% patients had overexpression of the AEG- gene. The higher expression of AEG-1 in oral squamous cell carcinoma compared to normal oral mucosa is a confirmation for its role in carcinogenesis and progression of OSCC. Other studies have shown that tumor cells in tongue carcinoma, metastatic cancers and oral squamous cell carcinoma exhibit increased expression of AEG-1, [12.14.15] which is consistent with the results of present study.

Though in this present study we found AEG-1 expression in OSCCs was greater than in normal oral mucosa but no association was found with any of the clinicopathologic factors, age and gender of patients, tumor location, smoking history, tumor staging and grading, metastasis to lymph nodes and distant metastasis.[14,15] It might be due to small sample size of this study which is the limitations of this study further studies with more samples have to be done to find out the clinicopathological correlation.

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Conclusion

According to this study, it has been indicated that the increase of expression of the AEG-1 gene among the OSCC patients could be treated as an oncogenic role of the Aeg-1gene. The increased expression of the AEG gene has an association with the development of OSCC in Bangladeshi patients.

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