RESEARCH ARTICLE

Polymorphisms in Heat Shock Proteins A1B and A1L (HOM) as Risk Factors for Oesophageal Carcinoma in Northeast India

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Abstract

Background: To investigate polymorphisms in heat shock proteins A1B and A1L (HOM) and associated risk of oesophageal carcinoma in Northeast India. Materials and Methods: The study includes oesophageal cancer (ECA) patients attending general outpatient department (OPD) and endoscopic unit of Gauhati Medical College. Patients were diagnosed based on endoscopic and histopathological findings. Genomic DNA was typed for HSPA1B1267 and HSPA1L2437 SNPs using the polymerase chain reaction with restriction fragment length polymorphisms. Results: A total of 78 cases and 100 age-sex matched healthy controls were included in the study with a male: female ratio of 5:3 and a mean age of 61.4±8.5 years. Clinico-pathological evaluation showed 84% had squamous cell carcinoma and 16% were adenocarcinoma. Dysphagia grades 4 (43.5%) and 5 (37.1%) were observed by endoscopic and hispathological evaluation. The frequency of genomic variation of A1B from wild type A/A to heterozygous A/G and mutant G/G showed a positive association [chi sq=19.9, p= <0.05] and the allelic frequency also showed a significant correlation [chi sq=10.3, with cases vs. controls, OR=0.32, p≤0.05]. The genomic variation of A1L from wild T/T to heterozygous T/C and mutant C/C were found positively associated [chi sq= 7.02, p<0.05] with development of ECA. While analyzing the allelic frequency, there was no significant association [chi sq= 3.19, OR=0.49, p=0.07]. Among all the risk factors, betel quid [OR =9.79, Chi square= 35.0, p<0.05], tobacco [OR = 2.95, chi square=10.6, p<0.05], smoking [OR=3.23, chi square=10.1, p<0.05] demonstrated significant differences between consumers vs. non consumers regarding EC development. Alcohol did not show any significant association [OR= 1.34, chi square=0.69, p=0.4] independently. Conclusions: It can be concluded that the present study provides marked evidence that polymorphisms of HSP70 A1B and HSP70 A1L genes are associated with the development of ECA in a population in Northeast India, A1B having a stronger influence. Betel quid consumption was found to be a highly significant risk factor, followed by smoking and tobacco chewing. Although alcohol was not a potent risk factor independently, alcohol consumption along with tobacco, smoking and betel nut was found to contribute to development of ECA.

Keywords: Oesophageal cancer - heat shock proteins - HSPA1B1267 and HSPA1L2437 - risk factors

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Introduction

Oesophageal cancer (ECA) is the eighth most common type of cancer worldwide (Zhang et al., 2009). Squamous cell carcinoma of the oesophagus is the third leading cancer in men and fourth leading cancer in women in India. In India the highest incidence of ECA patients are recorded in Assam and rest of the North eastern region of India (Chitra et al., 2004).

Only about 10% of the ECA patients show a 5-year survival rate, this is mainly due to the symptomless progression in the early stages of cancer and poor prognosis (Yang et al., 2010). Oesophageal cancer occurs in two main histopathological types; squamous cell carcinoma (SCC) and adenocarcinoma (ADC). Squamous cell carcinoma, in most cases; occur in the middle or upper one third of the oesophagus and adenocarcinoma in the lower one third or Gastro-Oesophageal Junction (Ke et al., 2002). Both external and internal factors contribute to the pathogenesis of ECA. The external factors are mainly related to different life style factors and habits and internal factors are the genetic variations. Epidemiological studies indicate that betel nut, alcohol and tobacco consumption along with smoking are major risk factors for ECA (Chen et al., 2011).

HLA polymorphism is implicated in conferring genetic

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susceptibility to a large number of immune-mediated diseases, including some cancers in several populations including Asian and African populations (Kummee et al., 2007). HLA-linked, heat shock protein 70 (HSP70) genes are of particular interest because their involvement in tumor immunity and cancer pathogenesis makes them cancer susceptibility candidate genes (Rohde et al., 2005).

Heat shock proteins are highly conserved proteins expressed in normal physiological condition at low levels but their expression increase in response to a variety of stress stimuli, including free radical, toxic metal ion exposure etc. Moreover, pathological stimuli such as hormonal overstimulation or viral/bacterial infection can induce a substantial increase of intracellular synthesis and secretion of Hsp proteins. Hsp proteins are divided in to six families based on their molecular weight. Hsp70 is encoded by a single exon of 1926 bp and its protein includes 641 amino acids (Meimaridou et al., 2009). Hsp70 protein is expressed across mammalian species both constitutively and in response to cellular stress or injury (Boiocchi et al., 2014). The functions of Hsp70 within the cell are primarily homeostatic in nature and include assistance with protein folding and prevention of protein aggregation and as inductors of anti apoptotic mechanisms (Lovett et al., 2014). But when released to extracellular space, Hsp70 acts as a potent damage-associated molecular pattern (DAMP) to stimulate the innate and adaptive immune response via interactions with Toll like receptor (TLR) 2 and TLR4, resulting in the release of proinflammatory cytokines such as IL-1beta, TNF-alpha, and IFN-beta (Vabulas et al., 2002). In addition Hsp70 promotes ubiquitination and degradation of misfolded proteins (Asea et al., 2000).

Some of the genes encoding the Hsp proteins which have been well studied and characterized are Hsp70-1, Hsp70-2 (HspA1B) and Hsp70-Hom (A1L). These genes are located in class III region of the human Major Histocompatibility Complex (MHC) on chromosome 6 (Daugaard et al., 2007). HspA1A and HspA1B genes encode an identical protein, the Hsp70 protein, whereas the HspA1L gene encodes a non heat inducible protein that shares 90% sequence identity with Hsp70 protein (Milner et al., 1992). It has been proposed that the Hsp70 plays an important role in tumor development, treatment, and prognosis and has distinct immunologic mechanisms affecting tumor cells and promoting cell growth (Wang et al., 2005). In cancer cells, the expression of Hsp70 is abnormally high and the protein may participate in oncogenesis and in resistance to chemotherapy. Several single nucleotide polymorphisms (SNPs) have been described in these genes which are still under intense scrutiny for possible association as risk factors to different cancers (Favatier et al., 1997). Predisposition to cancer may be conferred by genetic polymorphisms that arise from SNPs in certain pertinent genes (Hanahan et al., 2011). These genetic polymorphisms might partly explain the individual differences in susceptibility towards ECA. Since the population in the North eastern states of India is ethically distinct, the genetic makeup may render this population to be more susceptible towards development of ECA in association with certain high risk habits.

Materials and Methods

Patient population

The study includes 78 cases and 100 age and sex matched healthy controls attending the outdoor and endoscopic unit at Department of Gastroenterology, Gauhati Medical College Hospital, Guwahati, for the period of April 2012 to April 2014 with prior consent. Patients observed to have abnormal ulceroproliferative growth in any part of the oesophagus were selected. Endoscopic biopsy samples were taken and histopathological evaluation was performed to confirm diagnosis of cancer. Disease history was recorded for all subjects, along with life style factors and habits like history of alcohol consumption, betel nut chewing, tobacco chewing, smoked food and smoking. Patients were categorized on the basis of histopathological report or dysphagia grade based on modified O'Rourke dysphagia grading system (Waters et al., 2002).

Sample preparation

4ml of whole blood was collected in EDTA vials by simple venipuncture method from ECA patients and stored at 4°C. Genomic DNA was isolated from whole blood using standard technique (Sambrook et al., 1990).

PCR and RFLP analysis for A1B and A1L(HOM) genes

A1B:DNA samples were amplified with product size 1117bp for A1B using primers 5'-CATCGACTTC TACACGTCCA-3' (forward) and 5'-CAAAGTCCTTGAGTCCCAAC-3' (reverse) and subjected to restriction digestion with PstI (Thermo scientific) as previously described (Chouchane et al., 1997) The DNA-lacking polymorphic PstI site 1267 (rs1061581) within the HSPA1B gene generates a product of 1117 bp after restriction in case of the A allele, whereas the PstI site produced two fragments of 936 and 181 bp for the G allele.

A1L: PCR amplification for A1L was done using primers 5'-GGACAAGTCTGAGAAGGTAC AG-3' (forward) and 5'-GTAACTTAGATTCAGGTCTGG-3' (reverse) with product size of 878bp and subjected to restriction digestion with NcoI (Thermo Scientific) as previously described (Chouchane et al., 1997). At allele position 2437 T/C (rs2227956) the presence of two fragments of 551bp and 327bp represents the T allele whereas the undigested 878bp product represents the C allele.

Statistical analysis

Genotypic and allelic frequencies were compared by using the Mantel-Haenzel, chi-square test or the Fisher's exact test. Statistical significance was defined as $p<0.05$. Strength of association rate was assessed by odds ratio (OR) statistics. The statistical tool EPIINFO (version 7; July 2014) was used.

Results

Demographic features

A total of 100 age and sex matched healthy controls

with no previous indication of malignancy along with 78 ECA cases were included in this study. Sex ratio was 5:3 (M:F), with mean age of 61.4±8.52 years. The mean duration of dysphagia for patients was found to be 84±64 days. The distribution of cases and controls are shown in Table:1. Highest number of cases were in the age group 65-74 years. Male-preponderance was observed. Majority of the cases were from rural area with higher illiteracy.

Clinico-pathological evaluation

Clinico-pathological evaluation showed 84% patients developed tumor at upper end of the oesophagus with pathologically distinguished squamous cell carcinoma and 16% were evaluated as adenocarcinoma with tumor growth at lower end of the oesophagus or GE junction. Endoscopic evaluation showed majority of the patients with dysphagia grade 4 (43.5%) and 5 (37.1%) (Table 2).

Table 1. Demographic Characteristics of Oesophageal Cancer and Healthy Controls Included in the Study

Characterisics	Cases N=78(%)	Controls N= 100 (%)
Age (yrs)		
<25	1(1.3)	6(6.0)
25-34	1(1.3)	4(4.0)
35-44	7(8.9)	8(8.0)
45-54	16(20.5)	20(20.0)
55-64	23(29.4)	30(30.0)
65-74	26(33.3)	28(28.0)
75+	4(5.1)	4(4.0)
Sex		
Male	46(58.9)	70(70.0)
Female	32(41.0)	30(30.0)
Residence		
Urban	26(33.3)	58(58.0)
Rural	52(66.6)	42(42.0)
Education		
Literate	32(41.1)	84(84.0)
Illiterate	46(58.9)	16(16.0)

Table 2. The modified O'Rourke dysphagia staging system (Stages of Swallowing status)

Stage	Magnitude of Symptoms	PercentageN=78 (%) of Patients
1	Asymptomatic	4(5.12)
2	Solids with some dysphagia	5(6.41)
3	Soft or pureed food only	6(7.6)
4	Liquids only	34(43.5.)
5	No swallowing at all	29(37.1)

Correlation between different life style factors and habits in cases and controls

Among all the life style factors and habits, betel quid [OR=9.79, chi square=35.0, p <0.05], tobacco [OR=2.95, chi square=10.6, p<0.05], smoking [OR=3.23, Chi square=10.1, p <0.05] has significant correlation when compared with healthy controls between consumers and nonconsumers. (Table:4)

Association of Hsp A1B gene and oesophageal cancer

While analyzing the frequency of genomic variation of A1B from wild type A/A to heterozygous A/G and mutant G/G with cases vs. controls, a positive association was found [chi sq=19.9, p< 0.05] and the allelic frequency also showed a significant correlation [chi sq=10.3, OR=0.32; p< 0.05]. These results may indicate that the variation in A1B genotype can be relative risk to develop ECA [Table 3]

While taking the history of patient regarding their food habits and life style we observed the combined consumption of different food habits of smoking, tobacco, alcohol, betel nut. This combination has been shown in Table5. Statistical analysis showed that different combination of these food habits gives a significant association to develop the genetic variation in A1B gene in ECA. Significant association was seen in case of combination of

smoking & alcohol [chi sq=51.8, OR=10.5, p<0.05]; smoking & betel nut [chi sq=29.7, OR=0.19, p<0.05]; smoking & tobacco [chi sq=8.05, OR=0.15, p <0.05]; alcohol & betel nut [chi sq=5.91, OR=4.05, p<0.05]; betel nut & tobacco [chi sq=20.5, OR=0.26, p<0.05]; smoking ,

Table 4. Correlation between Different Life Style Factors and Habits in Patients with Oesophageal Cancer Cases and Healthy Controls

Factors	Case N =78 (%)	Control N=100(%)	OR	Chi-square	P
Betel Quid					
Chewers	70(89.7)	24(30.7)	9.79	35	<0.05
Non chewers	8(10.2)	26(33.3)			
Tobacco					
Chewers	46(58.9)	16(20.5)	2.95	10.6	<0.05
Non chewers	32(41)	34(43.5)			
Smoking					
Smokers	37(47.4)	11(14.1)	3.23	10.8	<0.05
Non Smokers	41(52.5)	39(50)			
Alcohol					
Drinker	26(33.3)	18(23)	1.34	0.69	0.4
Non drinker	52(44.4)	32(41)			

Table 3. Genomic and Allelic Frequency of A1B gene in Esophageal Cancer Patients with Respect to Control Sample

	Genomic Frequency					Allelic Frequency				
A1B	A/A n(%)	A/G n(%)	G/G n(%)	Chi-square	P value	A n(%)	G n(%)	Chi-square	P value	OR
Case (n=78)	29(37.1)	12(15.3)	37(47.4)	19.9	<0.05	70(45)	86(55)	10.3	<0.05	0.32
Control (n=100)	62(62)	20(20)	18(18)			144(72)	56(28)			
A1L	TT(%)	TC(%)	CC(%)	Chi-square	P value	T(%)	C(%)	Chi-square	P value	OR
Case (n=78)	48(61.5)	28(35.8)	2(2.5)	7.02	<0.05	124(79)	32(20.5)	3.19	0.07	0.49
Control (n=100)	78(78)	22(22)	0			178(89)	44(11)			

Table 5. Genomic Distribution of A1B gene in Different Combinations of Life Style Factors and Habits in Cases and Control

A1B	A/A n (%)	A/G +G/G n (%)	Chi square	OR	P value
Smoking & Alcohol					
Cases (N=17)	11 (64.7)	7 (35.3)	51.8	10.5	<0.05
Control (N=28)	4 (14.3)	24 (85.7)			
Smoking & Betel nut					
Cases (N=33)	13 (39.4)	20 (60.6)	29.7	0.19	<0.05
Control (N=18)	14 (77.8)	4 (22.2)			
Smoking &Tobacco					
Cases (N=18)	8 (44.5)	10 (55.5)	8.05	0.15	<0.05
Control (N=24)	22 (91.6)	4 (8.4)			
Alcohol & Betel nut					
Cases (N=22)	14 (64)	8 (36)	5.91	4.05	<0.05
Control (N=26)	8 (31)	18 (69)			
Alcohol&Tobacco					
Cases (N=18)	11 (61.1)	7 (38.9)	1.4	0.7	0.23
Control (N=26)	18 (69.2)	8 (30.8)			
Betelnut &Tobacco					
Cases (N=42)	16 (38)	26 (62)	20.5	0.26	<0.05
Control (N=20)	14 (70)	6 (30)			
Smoking &Alcohol&Betel nut					
Cases (N=15)	9 (60)	6 (40)	1.05	0.74	0.76
Control (N=12)	8 (67)	4 (33)			
Smoking &Alcohol&Tobacco					
Cases (N=11)	6 (54.5)	5 (45.5)	57.7	0	<0.05
Control (N=4)	4 (100)	0			
Smoking &Alcohol&Tobacco&Betelnut					
Cases (N=7)	3 (43)	4 (57)	7.18	2.26	<0.05
Control (N=8)	2 (25)	6 (75)			

Table 6. Genomic Distribution of A1L gene in Different Combinations of Life Style Factors and Habits in Cases and Control

A1L	T/T n (%)	T/C +C/C n (%)	Chi square	OR	P value
Smoking & Alcohol					
Cases (N=18)	13 (72.2)	5 (27.8)	9.16	0.32	>0.05
Control (N=18)	16 (89)	2 (11)			
Smoking & Betel nut					
Cases (N=33)	25 (76)	8 (24)	5.82	0.39	>0.05
Control (N=12)	10 (83.3)	2 (16.7)			
Smoking &Tobacco					
Cases (N=17)	13 (76.4)	4 (23.6)	9.71	2.67	<0.05
Control (N=22)	12 (54.5)	10 (45.5)			
Alcohol & Betel nut					
Cases (N=22)	15 (68.1)	7 (31.9)	1.38	1.42	0.23
Control (N=30)	18 (60)	12 (40)			
Alcohol&Tobacco					
Cases (N=18)	12 (66.6)	6 (33.4)	9.99	0.33	<0.05
Control (N=14)	12 (85.7)	2 (14.3)			
Betelnut &Tobacco					
Cases (N=41)	27 (66)	14 (34)	17.2	0.2	<0.05
Control (N=22)	20 (91)	2 (9)			
Smoking &Alcohol&Betel nut					
Cases (N=15)	11 (73.3)	4 (26.7)	31.9	5.49	<0.05
Control (N=12)	4 (33.3)	8 (66.7)			
Smoking &Alcohol&Tobacco					
Cases (N=11)	8 (73)	3 (27)	45.87	8.11	<0.05
Control (N=8)	2 (25)	6 (75)			
Smoking &Alcohol&Tobacco&Betelnut					
Cases (N=12)	9 (75)	3 (25)	1.55	1.48	0.21
Control (N=6)	4 (67)	2 (33)			

alcohol & tobacco [chi sq=57.7, OR=0; p<0.05]; smoking , alcohol, tobacco & betel nut [chi sq=7.18, OR=2.26, p<0.05].

Association of Hsp A1L gene and oesophageal cancer

The genomic variation of A1L from wild T/T to heterozygous T/C and mutant C/C were found positively associated [chi sq=7.02; p =<0.05] to develop ECA when analyzed in cases vs. control. While analyzing the allelic frequency there was not any significant association [chi sq=3.19, OR=0.49, p=0.07]. [Table 3]

The patient history of their food habits and life style were analyzed in different combination. The statistical analysis of combined consumption of different food habits of smoking, tobacco, alcohol, betel nut was done. This combination has been shown in Table 6. Significant correlation was found in genomic distribution of A1L, between cases and controls in combined consumption of smoking & alcohol [chi sq=9.16, OR=0.32; p<0.05]; smoking & betel nut [chi sq=5.82, OR=0.39; p<0.05]; smoking & tobacco [chi sq=9.71, OR=2.67, p<0.05]; alcohol & tobacco [chi sq=9.99, OR=0.33, p<0.05]; betel nut & tobacco [chi sq=17.2, OR=0.2, p<0.05]; smoking , alcohol & betel nut [chi sq=31.9, OR=5.49, p<0.05]; smoking, alcohol & tobacco [chi sq=45.87, OR=8.81, p<0.05]. [Table 6]

Discussion

Oesophageal cancer is a common and polygenic malignant cancer caused by complex interactions between genetic and environmental factors. The demographic distribution showed male predominance in these patients in the age group of 65-74years. Majority of the cases were from rural having poor literacy rate. Ganesh et al., 2009 also reported the male predominance in another population with higher age group in Mumbai, Western India. This study reveals high incidence of ECA in males with higher consumption of betel quid, tobacco, alcohol and smoking habits. In a trend similar to previously reported studies in South Indian population (Chitra et al., 2004) Squamous cell carcinoma was found to be the more dominant over adenocarcinoma in Northeast Indian population.

Among studied risk factors betel quid, tobacco, smoking and alcohol, we found betel quid to be strongly associated and potent risk factor in development of ECA in comparison with controls from Northeast India. Similar association has also been reported for betel quid in Indian population as a whole (Akhtar et al., 2012) and from North east India (Kurkalang et al., 2014) previously. Smoking and ECA have been associated by other studies from different regions of the world (Dar et al., 2012; Wang et al., 2015). Our study also corroborates these findings. Tobacco consumption has been consistently reported to be a high risk factor for ECA in various studies (Yang et al., 2005; Ganesh et al., 2009). In a previous study in the same population, the risk associated with chewing of betel nut was found to be higher than those for tobacco, smoking and alcohol consumption at all levels of consumption (Phukan et al., 2001).

Alcohol consumption was found to be a non significant risk factor for oesophageal carcinoma in our study. Prabhu et al., 2013 reported that there was no role of alcohol in development of ECA in Asian population. In another study conducted in Western Maharashtra, alcohol consumption percentage was the least among other risk factors such as tobacco chewing and smoking (Giri et al., 2014) among ECA patients. Alcohol had very little effect on development of ECA whereas smoking was found to be a fivefold more potent risk factor (Holmes et al., 2007). Contrasting data have also been reported where positive correlation of alcohol on ECA was shown (Pandeya et al., 2009). In some of the earliest studies in the Indian population alcohol drinking was shown to be a significant risk factor (Gopala et al., 2013). Combined effect of Alcohol and tobacco or betel quid consumption in the development of ECA has been previously documented (Lee et al., 2005). Our study has also revealed similar scenario of synergistic effect of alcohol consumption along with smoking, betel quid and tobacco.

Genetic variants of HSP70 (A1B1267 and A1L2437) was evaluated previously as a risk to develop chronic hepatitis and hepatocellular carcinoma in Indian population where A1B1267 was more significant to develop the disease than A1L2437 (Medhi et al., 2013). Another study supported the similar conclusion in Croatian population to develop chronic obstructive pulmonary disease where there was no evidence for the association of A1L2437 (Matokanovic et al., 2012).

In our study, we evaluated the influence of HSPA1B and HSPA1Lgene variants in gene on ECA risk by association analysis in 78 ECA patients and 100 cancer-free control samples from Northeast India. No previous data has been reported on A1B and A1L from this region of India. The A→G substitution in A1B1267 is a silent mutation which does not result any variation in the protein (Vargas-Alarcon et al., 2002) but the heterozygous A/G variant can alter the expression of A1B by interfering with the secondary structure and mRNA stability (Wu et al., 2004), thus may affect its antiapoptotic as well as immune modulatory functions, resulting in predisposition and prognosis of different types of cancers. Previously it is a reported marker in different diseases like nasopharyngeal carcinoma, Parkinson's disease, renal diseases (Wu et al., 2004). Whereas T→C substitution in A1L2437 results in amino acid substitution of Met→Thr at 493 position of amino acid sequence in the protein (Fekete et al., 2006). However, in another study has shown no association in the allelic and genotypic distribution between patients and controls for these two polymorphisms in Chinese population (Liu et al., 2007).

Our data suggests that the homozygous variant (G/G) genotype of A1B is associated with increased risk of ECA when compared with control samples from Northeast India. Whereas in case of A1L gene the ancestral genotype (T/T) was found more in cases and controls than the homozygous variant (C/C). Statistically when compared all the genotypes of each gene with control, a significant association was found with P<0.05 to develop ECA in North eastern part of India.

A contradictory conclusion was reported in Costa Rican population where the heterozygous (A/G) genotype of A1B1267 was associated with increased risk of gastric

cancer and duodenal ulcer than the homozygous (G/G) genotype. Where as in case of A1L2437 homozygous variant genotype (C/C) were significantly less susceptible to gastric cancer than homozygous wild (T/T) genotype, that is concordance to our study. (Ferrer-Ferrer et al., 2013). The allelic frequency of A1L2437 is not associated to develop the oesophageal carcinoma in our study. Another contradictory result was reported in Hungarian population (Szondy et al., 2012) where G/G genotype of A1B1267 is a negative prognostic factor for survival in SCLC patients. A meta analysis also has recently reported (Kuang et al., 2014) where the heterozygous polymorphism of A1B1267 may contribute to susceptibility of cancer, in a Caucasian population.

Similar findings with our study was reported in Chinese, Han and Tunisian populations where homozygous was associated with an increased risk of developing cancer but not in heterozygous genotype (Mestiri et al., 2001). A significant association of the allele G of A1B gene with spondyloarthropathies in a Mexican population has been reported (Vargas-Alarcon et al., 2002). In our study the and G/G genotype of A1B1267 and T/T genotype of A1L2437 was found significantly associated with the patients of ECA, when compared with control samples from this population. The similar conclusion was reported on hepatocellular carcinoma and chronic hepatitis cases in Indian population, it was observed that the homozygous variant allele was implicated as a possible risk factor. (Medhi et al., 2013).

It can be concluded that the present study provides marked evidence that Polymorphisms of A1B1267 and A1L2437 genes are associated with the development of ECA in a population in Northeastern region of India. The A1L2437 is not associated to develop the disease with respect to the allelic distribution compared with controls. However, one of the limitations of this study is the limited sample size. Larger and well designed studies are warranted to confirm our observations. Particularly the gene-environment interactions and mechanisms of A1B and A1L in regulating the proliferation of ECA cells are important facets of research. Moreover, data from this study showed betel quid consumption to be a highly significant risk factor, followed by smoking and tobacco chewing. Alcohol consumption alone was found as a non significant risk factor but joint effect with smoking, betel nut and tobacco significantly increases the risk of developing oesophageal carcinoma in North Eastern population of India.

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