RESEARCH ARTICLE

Phospholipase C Epsilon 1 (PLCE1 rs2274223A>G, rs3765524C>T and rs7922612C>T) Polymorphisms and Esophageal Cancer Risk in the Kashmir Valley

Manzoor Ahmad Malik[1], Meenakshi Umar[1], Usha Gupta[1], Showkat Ali Zargar[2], Balraj Mittal[1]*

Abstract

Background: Phospholipase C epsilon 1 (PLCE1) encodes a member of the phospholipase family of proteins that play crucial roles in carcinogenesis and progression of several cancers including esophageal cancer (EC). In two large scale genome-wide association studies (GWAS) single nucleotide polymorphisms (SNP, rs2274223A>G, rs3765524C>T) in PLCE1 were identified as novel susceptibility loci of esophageal cancer (EC) in China. The aim of the present study was to investigate this finding in Kashmir Valley, a high risk area. Materials and Methods: We determined genotypes of three potentially functional SNPs (rs2274223A>G, rs3765524C>T and rs7922612C>T) of PLCE1 in 135 EC patients, and 195 age and gender matched controls in Kashmiri valley by PCR RFLP method. Risk for developing EC was estimated by binary logistic regression using SPSS. Results: The selected PLCE1 polymorphisms did not show independent association with EC. However, the $G_{2274223}T_{3765524}T_{7922612}$ haplotype was significantly associated with increased risk of EC (OR=2.92; 95% CI=1.30-6.54; p=0.009). Smoking and salted tea proved to be independent risk factors for EC. Conclusions: Genetic variations in PLCE1 modulate risk of EC in the high risk Kashmiri population.

Keywords: PLCE1 polymorphisms - PCR/RFLP - haplotype - esophageal cancer - Kashmir Valley

Asian Pac J Cancer Prev, **15 (10)**, 4319-4323

Introduction

Carcinomas of the esophagus have diverse incidence patterns and risk factors (Brown et al., 2002). The etiology of these cancers is incompletely understood, like many other malignancies. Overall, esophageal cancer incidence and mortality have fallen dramatically over the past 70 years (Parkin, 2008). Despite its recent decline, esophageal cancer (EC) is the eighth most commonly occurring cancers in the world (Parkin et al., 1993). Within the Indian subcontinent, the Valley of Kashmir presents a strikingly different picture where incidence of esophageal cancer have been reported to exceed 40% of all cancers and incidence is 3-6 times higher than various metropolis cancer registries in India (Khuroo et al., 1992; Siddiqi et al., 1992). Some of the genetic and environment factors have been reported to be associated with an increased risk of esophageal cancer in Kashmir valley (Siddiqi et al., 1988; Malik et al., 2009; 2012; Chikan et al., 2012).

A number of epidemiological studies have been used as a tool to find out common susceptibility markers of EC, however the results of only few studies were concordant when replicated in other independent populations. The development of genome-wide association study (GWAS) has a major impact on the discovery of susceptibility genes for complex disease and exploring the relationship between a large number of single nucleotide polymorphisms (SNPs) and disease predisposition. A GWAS for PLCE1 was published in 2010 and identified significant associations with rs2274223 (Arg1927His) and rs3765524 (Ile1777Thr) on chromosome 10q23 in Chinese population. These two SNPs result in missense mutations in the coding region, rs2274223 (Arg1927His) and rs3765524 (Ile1777Thr) (Abnet et al., 2010; Wang et al., 2010).

The PLCE1 belongs to the phospholipase family that catalyzes the hydrolysis of polyphosphoinositides to generate secondary messengers, such as inositol-1,4,5 trisphosphate and diacylglycerol. Therefore, PLCE1 is involved in cell growth and differentiation (Wing et al., 2003). Epidemiological study have shown that PLCE1 functions as an effector of Ras and is a major factor in progression of various cancers, intestine (Li et al., 2009), skin (Bai et al., 2004), bladder (Ou et al., 2010), colorectal (Wang et al., 2008) and head and neck (Bourguignon et al., 2006). These findings suggest that single nucleotide

*[1]Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, [2]Department of Gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Kashmir, India *For correspondence: balraj@sgpgi.ac.in, balrajmittal@gmail.com*

polymorphisms (SNPs) in PLCE1 that affect the gene expression or protein functions, may affect the risk of some cancers. However, this finding lacks independent replication in many populations (Bye et al., 2012; Palmer et al., 2012). Therefore, we hypothesized that GWAS discovered SNPs in the PLCE1 gene are may be associated with esophageal cancer risk in patients from Kashmir valley. To test this hypothesis, we replicate association of three polymorphisms (rs2274223A>G, rs3765524C>T and rs7922612C>T) PLCE1 polymorphisms with susceptibility to EC in Kashmir valley a high risk area.

Materials and Methods

This case control study comprised histopathologically confirmed cases with esophageal (EC) cancer (135) and healthy controls (195) from the population of Kashmir valley. The sample size of the present study was adequate to provide 80% power. All subjects were unrelated ethnic Kashmiri residents, referred from the Departments of Gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar from May 2006 to July 2008. Patients were excluded if they had non-malignant conditions like corrosive esophageal injury, Achalasia injury, Barrett's esophagus, gastro-esophageal reflux disease (GERD) and non-ulcer dyspepsia. The controls were also recruited from Sher-i-Kashmir Institute of Medical Sciences, Srinagar, who come for their routine checkup and were diagnosed as non-severe ailment and no malignancy. All the individuals were personally interviewed for their age, occupation, demographic features, dietary habits (Haak=brassica oleraceae; Wur=red chilies), usage of hot noon chai (salted tea) and smoking habits. Tobacco usage included smoking of cigarette, or hukka (water pipe). Written informed consent was obtained from all participants in the study. The research protocol was approved by the ethical committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow (28th Ethics Committee Meeting on May 14, 2004). Sample collection, storage and transport were complied with guidelines of the committee. Blood samples were collected in EDTA and the genomic DNA was extracted from peripheral blood leukocytes pellet using the standard salting-out method (Miller et al., 1998). The quality and quantity of DNA was checked by gel electrophoresis and spectrophotometry using Nanodrop Analyser (ND-1000) spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA). The ratio of absorbance at 260 and 280 nm of DNA was around 1.7-1.9. The isolated DNA was stored at -70°C.

Genotyping

The genotyping of all three PLCE1 gene polymorphisms were carried through PCR RFLP method and detailed methodology is shown in Table 1. The genotypes of PLCE1 genetic variants were assigned on the basis of band sizes. In case of PLCE1 rs2274223 A>G polymorphism, A allele showed a band of 242 bp and G allele yielded two fragments of 155 bp and 87 bp. PLCE1 rs3765524 C>T C allele resulted in 326 bp fragment and the variant allele, designated T allele resulted in 200 bp and 126 bp fragments. The wild type C allele of PLCE1 rs7922612 C>T polymorphism on restriction digestion showed two fragments of 197bp and 143 bp, however the variant T allele was resistant to digestion and yielded a single fragment of 340 bp. More than 15% of the samples were randomly selected for confirmation, and the results were 100% concordant.

Statistical analysis

Demographic characteristics of patients and controls were described as frequencies and percentages, whereas descriptive statistics of patients and controls were presented as mean and standard deviations for continuous measures. Statistical significance of frequency differences between patients and control groups was evaluated using the χ^2 test. Deviation from the Hardy-Weinberg equilibrium in controls was assessed using the χ^2 test; p value was considered significant at <0.05 level. Effective sample sizes were calculated by Quanto software version 1.2. The same controls were used for analyzing two sets of cancer cases. Association was expressed as odds ratios (OR) for risk estimation with 95% confidence intervals (95% CI). All analyses were performed using the SPSS statistical analysis software, version 15.0 (SPSS, Chicago, IL, USA).

Results

Population characteristics

The mean age of healthy subjects (controls) and patients with EC was 57.98yrs±12.67 and 60.38yrs±8.41 respectively (t-test p value=ns). EC was highly prevalent in males (68.1%) than in females. In patients with EC, squamous cell carcinoma (SCC) histopathology was common (76.3%). Smoking habit (Hukka) showed significantly higher risk in EC (OR=21.45; 95%CI=11.63-39.55; p=0.0001) patients. Individuals consumed salted-tea in a range of 2-8 cups per day; and median consumption of tea was 4 cups per day. So, we grouped individuals in to ≤4 cups or >4 cups per day and individuals consumed salted tea >4 cups per day were regarded as high salted tea consumers. Higher consumption of salted tea was also found to be associated with increased risk of EC (OR=14.86; 95%CI=8.42-26.25; p value=0.0001) (Table

Table 1. Detailed Methodology Used in Study

Gene	SNP ID	Chromosome 10 position	Nucleotide change	Primer sequences	Amplicon size	Restriction enzymes	Method	Genotyping
PLCE1 A>G	rs2274223	96066341(+)	A to G	F-GTCTCTGGTCAGAATGTGTG R-CAGTGGAATGATTCTCTGAG	242bp	BstU1	PCR/RFLP	AA: 242bp GG:155 bp +87 bp
PLCE1 C>T	rs3765524	96058298(+)	C to T	F-CTACACTAATCTGCGGCTAA R-TCAGTCTGGTAGTTGAGTGC	326bp	DpnII	PCR/RFLP	CC: 326 bp TT : 200 bp+126 bp
PLCE1 C>T	rs7922612	95811439(+)	C to T	F-CATGCCCATCCAAAGGATAC R-ACAAATGAAGGCAGCCAAGT	340bp	HhaI	PCR/RFLP	CC: 197 bp 143 bp TT:340bp

Table 2. Demographic Characteristics of Esophageal Cancer Patients and Healthy Controls of Kashmir Valley

Variables		Healthy controls n=195	Esophageal cancer patients n=135	OR* (95%CI)	p value
Mean Age±SD		57.98yrs±12.67	60.38yrs±8.41	-	
Sex	Male	139 (71.3%)	92 (68.1%)	-	
	Female	56 (28.7%)	43 (31.9%)		
Histology	Aden carcinoma		32 (23.7%)	-	
	Squamous cell carcinoma		103 (76.3%)		
Smoking**	Smokers (Hukka)	38 (20.5%)	106 (84.1%)	21.45 (11.63-39.55)	0.0001
Salted tea intake**	≤4 cups daily	159 (85.9%)	36 (28.6%)		
	>4 cups daily	26 (14.1%)	90 (71.4%)	14.86 (8.42-26.25)	0.0001
Alcohol consumption		Nil	Nil	Nil	

*Age and gender adjusted odds ratio; **Data missing

Table 3. Frequency Distribution of PLCE1 rs2274223A>G, rs3765524C>T and rs7922612C>T and Risk Assessment in Esophageal Cancer Cases and Control's

Genotypes	Controls (195) N (%)	Esophageal cancer (135) N (%)	OR* (95%CI)	p value
PLCE1 rs2274223A>G				
AA	100 (51.28)	65 (48.15)	1 (Reference)	
AG	78 (40.0)	58 (42.96)	1.10 (0.69-1.76)	0.66
GG	17 (8.72)	12 (8.89)	1.12 (0.50-2.51)	0.77
A	279 (71.50)	189 (70.00)	1 (Reference)	
G	111 (28.50)	81 (30.00)	1.04 (0.73-1.50)	0.79
PLCE1 rs7922612C>T				
CC	90 (46.15)	57 (42.2%)	1 (Reference)	
CT	85 (43.59)	61 (45.2)	1.11 (0.69-1.78)	0 .65
TT	20 (10.26)	17 (12.6)	1.33 (0.64-2.77)	0.43
C	266 (68.20)	176 (65.20)	1 (Reference)	
T	124 (31.80)	94 (34.80)	1.07 (0.77-1.50)	0.67
PLCE1 rs3765524C>T				
CC	109 (55.9)	77 (57.0)	1 (Reference)	
CT	74 (37.9)	49 (36.3)	0.90 (0.56-1.45)	0.68
TT	12 (6.2)	9 (6.7)	1.03 (0.41-2.59)	0.94
C	293 (75.10)	204 (75.60)	1 (Reference)	
T	97 (24.90)	66 (24.40)	0.95 (0.66-1.37)	0.79

*Age and gender adjusted odds ratio

Table 4. PLCE1 rs2274223A>G, rs3765524C>T and rs7922612C>T Haplotype Frequencies Among Study Subjects and Risk Assessment of EC

Haplotype	Controls (390) N (%)	EC Patients (270) N (%)	OR* (95% CI)	p value
$A_{2274223}C_{3765524}C_{7922612}$	188 (48.2)	116 (43.0)	Reference	---
$G_{2274223}C_{3765524}C_{7922612}$	73 (18.7)	55 (20.4)	1.22 (0.80-1.86)	0.351
$A_{2274223}T_{3765524}T_{7922612}$	69 (17.7)	56 (20.7)	1.31 (0.86-2.00)	0.203
$G_{2274223}T_{3765524}T_{7922612}$	25 (6.4)	6 (2.2)	0.39 (0.16-0.98)	0.104
$A_{2274223}C_{3765524}T_{7922612}$	21 (5.4)	15 (5.6)	1.16 (0.58-2.33)	0.683
$G_{2274223}C_{3765524}T_{7922612}$	10 (2.6)	18 (6.7)	**2.92 (1.30-6.54)**	**0.009**[a]
$G_{2274223}T_{3765524}C_{7922612}$	4 (1.0)	3 (1.1)	1.22 (0.27-5.53)	0.801
$A_{2274223}T_{3765524}C_{7922612}$	0 (0)	1 (0.4)	NC	NC

Age and gender-adjusted odds ratio. NC=Not Calculated. Bonferroni's corrected p value=0.027[a]; Significant values shown in bold

2). None of patients or controls reported consumption of alcohol, so interaction of alcohol intake with genetic variations could not be analyzed. No association was observed between intakes of Brassica oleraceae or red chillies with EC (data not shown).

Association of genetic variants of PLCE1 (rs2274223A>G, rs3765524C>T and rs7922612C>T) polymorphism with susceptibility to EC

The genotype and allele distributions of three SNPs (rs2274223A>G, rs3765524C>T and rs7922612C>T) in cases and controls are shown in Table 3. The observed genotype frequencies for all these three polymorphisms were in Hardy-Weinberg equilibrium in the controls. The single locus analyses revealed that genotype distributions of these three polymorphisms were not significantly different between overall cases and controls (p=0.77 for rs2274223, p=0.43 for rs7922612 and p=0.94 for rs3765524, respectively). Also at allele level we did not find any significant association (Table 3).

PLCE1 (rs2274223A>G, rs3765524C>T and rs7922612C>T) haplotype analysis and risk of EC

A total of eight haplotypes were observed in the study subjects. The frequency of haplotype $G_{2274223}T_{3765524}T_{7922612}$ was higher in patients as compared to controls and presented statistically significant increased risk of EC (OR=2.92; 95% CI=1.30-6.54; P=0.009) even after Bonferroni's correction (0.027) (Table 4).

Association of PLCE1 rs2274223A>G, rs3765524C>T and rs7922612C>T gene polymorphisms with tumor histopathology and environmental factors like smoking and salted tea

While analyzing the genotypes with histology (squamous cell carcinoma and adenocarcinoma) none of

Table 5. Interaction of PLCE1 rs2274223A>G, rs3765524C>T and rs7922612C>T Genotypes and Smoking in Modulation of EC Risk

Genotype		Controls* Smoker	Controls* Non-smoker	Esophageal cancer* Smoker	Esophageal cancer* Non-smoker	OR**(95%CI)	p value
PLCE1 rs2274223 A>G	AA	27 (56.3)	76 (48.4)	57 (49.6)	15 (51.7)	1 (Reference)	
	AG	15 (31.3)	68 (43.3)	48 (41.7)	10 (34.5)	1.38 (0.64-2.96)	0.39
	GG	6 (12.5)	13 (8.3)	10 (8.7)	4 (13.8)	0.65 (0.20-2.05)	0.46
PLCE1 rs7922612 C>T	CC	18 (37.5)	75 (47.8)	51 (44.3)	11 (37.9)	1 (Reference)	
	CT	22 (45.8)	67 (42.7)	50 (43.5)	15 (51.7)	0.80 (0.37-1.71)	0.57
	TT	8 (16.7)	15 (9.6)	14 (12.2)	3 (10.3)	0.60 (0.21-1.72)	0.34
PLCE1 rs3765524C>T	CC	21 (20.4)	82 (79.6)	57 (80.3)	14 (19.7)	1 (Reference)	
	CT	15 (21.1)	56 (78.9)	41 (89.1)	5 (10.9)	0.99 (0.44-2.23)	0.99
	TT	2 (18.2)	9 (81.8)	8 (88.9)	1 (11.1)	1.34 (0.25-7.10)	0.72

*Data missing in some subjects; **Age and gender adjusted odds ratio; OR was calculated for smokers only

Table 6. Interaction of PLCE1 rs2274223A>G, rs3765524C>T and rs7922612C>T Genotypes and Salted Tea in Modulation of EC Risk

Genotype	Controls* Tea(Cups)/day		Esophageal cancer* Tea(Cups)/day		OR**(95%CI)		p value
	≤4	>4	≤4	>4			
PLCE1 rs2274223A>G							
AA	86 (50.9)	19 (52.8)	22 (48.9)	47 (47.5)	1	(Reference)	
AG	69 (40.8)	13 (36.1)	20 (44.4)	43 (43.4)	1.31	(0.57-3.01)	0.51
GG	14 (8.3)	4 (11.1)	3 (6.7)	9 (9.1)	0.94	(0.25-3.46)	
PLCE1 rs7922612C>T							
CC	79 (46.7)	15 (41.7)	18 (40.0)	41 (41.4)	1	(Reference)	
CT	72 (42.6)	17 (47.2)	20 (44.4)	47 (47.5)	1.31	(0.57-3.01)	0.51
TT	18 (10.7)	4 (11.1)	7 (15.6)	11 (11.1)	0.94	(0.25-3.46)	0.92
PLCE1 rs3765524C>T							
CC	90 (86.5)	14 (13.5)	17 (23.6)	55 (76.4)	1	(Reference)	
CT	10 (14.3)	60 (85.7)	29 (64.4)	16 (35.6)	0.54	(0.09-3.23)	0.50
TT	2 (18.2)	9 (81.8)	6 (66.7)	33 (33.3)	1.04	(0.99-1.09)	0.11

*Data missing in some subjects; **Age and gender adjusted odds ratio; OR was calculated for >4 cup tea consumption in cases and controls

the PLCE1 genetic variants were associated with risk in EC (data not shown). We examined the possible interactions of PLCE1 (rs2274223, rs7922612 and rs3765524) genotypes with smoking and salted tea consumption and risk of EC in study subjects. Although both smoking and salted tea were independent risk factors for EC in the Kashmir Valley, we did not find any enhanced risk of EC with PLCE1 genotypes in subjects with smoking and salted tea intake habits (Table 5 and Table 6).

Discussion

In present study, we investigated the associations between three novel, potentially functional SNPs of PLCE1 (rs2274223, rs3203713 and rs11599672) with risk of EC in Kashmir valley. In the present study, we observed that although PLCE1 polymorphism did not independently modulate risk of EC, however, one PLCE1 haplotypes was found to be associated with significant increased risk of EC in Kashmiri population.

Recently in Han Chinese populations, polymorphism in PLCE1 was identified as a risk factor for GC and EC by two contemporary independent GWAS (Abnet et al., 2010; Wang et al., 2010). Also two replicated independent studies have also validated the association of PLCE1 rs2274223A>G polymorphism with head and neck cancer and gastric cancer in Chinese population (Luo et al., 2011; Ma et al., 2011). Recently, a metaanalysis of PLCE1 rs2274223 A>G polymorphism also suggested its involvement in conferring increased risk of esophageal cancer risk (Umar et al., 2013).

PLCE1 rs2274223 A>G was found to increase the risk of esophageal and gastric cancers in genome wide association study (GWAS). This nonsynonymous SNP present in exon 26 results in histidine to arginine amino acid change in the $Ca2^{+}$-dependent lipid binding domain of the protein. Although the exact functional relevance of the polymorphism is not known, Hu et al. (2000) have shown significant reduced staining of PLCE1 protein in the ESCC tissues of the rs2274223 GG genotype compared to that in the AG genotype. Also, one study have shown a downregulation of the PLCE1 mRNA expression in tumor tissues of colorectal cancer, gastric adenocarcinoma, and esophageal squamous cell carcinoma compared to respective normal tissues (Wang et al., 2008).

In addition to possible exposure to well known risk factors (like smoking and salted tea) for esophageal and gastric cancers, the people of valley have many unique social, cultural and dietary features which are different from rest of world. Salted tea used by people is prepared by using baking soda (sodium bicarbonate) and common salt (sodium chloride) and boiled for few hours before consuming. It has been suspected that the salts might cause thermal injury to esophageal and gastric epithelium (Khuroo et al., 1992). Several previous studies have attributed high incidence of esophageal and gastric cancers in Kashmir to considerable amount of nitroso compounds in raw foodstuffs and use of hot salted tea (Khuroo et al., 1992; Siddiqi et al., 1992). Salted tea used in Kashmir valley has considerable amounts of N-nitrosoproline (NPRO) (360 micrograms/kg) and N nitrosopipecolic acid (NPIC) (5870 micrograms/kg) which may impart risk for EC in this area (Siddiqi et al., 1988). In the present study salted tea was significantly associated with increased high risk for EC (OR=18.179; p=0.001). Also our results show significant independent association of smoking (Hukka) with EC (OR=21.465; p=0.0001). But in gene environment interactions no such association was observed. Brassica oleraceae or red chillies also did not show any association with EC. It appears that genetic variants of PLCE1 modulate susceptibility to EC independent of environmental interactions.

In conclusion, the association of the PLCE1 polymorphisms seem important in carcinogenesis of EC in Kashmiri Valley. It is worthwhile conducting additional population-based studies in large sample size before its clinical application.

Acknowledgements

The study was supported as fellowship grant from Indian Council of Medical Research (ICMR), New Delhi India.

References

Abnet CC, Freedman ND, Hu N, et al (2010). A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet*, **42**, 764-7.

Bai Y, Edamatsu H, Maeda S, et al (2004). Crucial role of phospholipase Cepsilon in chemical carcinogen-induced skin tumor development. *Cancer Res*, **64**, 8808-10.

Bourguignon LY, Gilad E, Brightman A, Diedrich F, Singleton P (2006). Hyaluronan-CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co-activation, phospholipase C epsilon-Ca2+ signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells. *J Biol Chem*, **281**, 14026-40.

Brown LM, Devesa SS (2002). Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am*, **11**, 235-56.

Bye H, Prescott NJ, Lewis CM, et al (2012). Distinct genetic association at the PLCE1 locus with oesophageal squamous cell carcinoma in the South African population. *Carcinogenesis*, **33**, 2155-61.

Chikan NA, Shabir N, Shaff S, Mir MR, Patel TN (2012). N-nitrosodimethylamine in the Kashmiri diet and possible roles in the high incidence of gastrointestinal cancers. *Asian Pac J Cancer Prev*, **13**, 1077-9.

Hu H, Yang J, Sun Y, et al (2012). Putatively functional PLCE1 variants and susceptibility to esophageal squamous cell carcinoma (ESCC): a case–control study in eastern Chinese populations. *Ann Surg Oncol*, **19**, 2403–10.

Khuroo MS, Zargar SA, Mahajan R, Banday MA (1992). High incidence of oesophageal and gastric cancer in Kashmir in a population with special personal and dietary habits. *Gut*, **33**, 11-5.

Li M, Edamatsu H, Kitazawa R, Kitazawa S, Kataoka T (2009). Phospholipase Cepsilon promotes intestinal tumorigenesis of Apc(Min/+) mice through augmentation of inflammation and angiogenesis. *Carcinogenesis*, **30**, 1424-32.

Luo D, Gao Y, Wang S, et al (2011). Genetic variation in PLCE1 is associated with gastric cancer survival in a Chinese population. *J Gastroenterol*, **46**, 1260-6.

Ma H, Wang LE, Liu Z, Sturgis EM, Wei Q (2011). Association between novel PLCE1 variants identified in published esophagealcancer genome-ide association studies and risk of squamous cell carcinoma of the head and neck. *BMC Cancer*, **20**, 258.

Malik MA, Upadhyay R, Mittal RD, et al (2009). Role of xenobiotic-metabolizing enzyme gene polymorphisms and interactions with environmental factors in susceptibility to gastric cancer in Kashmir Valley. *J Gastrointest Cancer*, **40**, 26-32.

Malik MA, Zargar SA, Mittal B (2012). Role of NQO1 609C>T and NQO2 -3423G>A gene polymorphisms in esophageal cancer risk in Kashmir valley and meta analysis. *Mol Biol Rep*, **39**, 9095-104.

Miller SA, Dykes DD, Polesky HF (1998). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.

Ou L, Guo Y, Luo C, et al (2010). RNA interference suppressing PLCE1 gene expression decreases invasive power of human bladdercancer T24 cell line. *Cancer Genet Cytogenet*, **200**, 110-9.

Palmer AJ, Lochhead P, Hold GL, et al (2012). Genetic variation in C20orf54, PLCE1 and MUC1 and the risk of uppergastrointestinal cancers in Caucasian populations. *Eur J Cancer Prev*, **21**, 541-4.

Parkin DM (2008) The role of cancer registries in cancer control. *Int J Clin Oncol*, **13**, 102-11.

Parkin DM, Pisani P, Ferlay J (1993) Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer*, **4**, 594-606.

Siddiqi M, Kumar R, Fazili Z, Spiegelhalder B, Preussmann R (1992). Increased exposure to dietary amines and nitrate in a population at high risk of oesophageal and gastric cancer in Kashmir (India). *Carcinogenesis*. **13**, 1331-5.

Siddiqi M, Tricker AR, Preussmann R (1988). The occurrence of preformed N-nitroso compounds in food samples from a high risk area of esophageal cancer in Kashmir, India. *Cancer Lett*, **39**, 37-43.

Umar M, Upadhyay R, Mittal B (2013) PLCE1 rs2274223 A>G polymorphism and cancer risk: a meta-analysis. *Tumour Biol*, [Epub ahead of print].

Wang LD, Zhou FY, Li XM, et al (2010). Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at PLCE1 and C20orf54. *Nat Genet*, **42**, 759-63.

Wang X, Zbou C, Qiu G, et al (2008). Screening of new tumor suppressor genes in sporadic colorectal cancer patients. *Hepatogastroenterology*, **55**, 2039-44.

Wing MR, Bourdon DM, Harden TK (2003). PLC-epsilon: a shared effector protein in Ras-, Rho-, and G alpha beta gamma-mediated signaling. *Mol Interv*, **3**, 273-80.