Research Article

Genetic polymorphisms in DNA repair genes (hOGG1 & APE1) and their association with oral cancer susceptibility in rural Indian population: a hospital based case-control study

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Abstract

Background: Smoking and alcohol related head and neck cancer is a major concern of health risk in urban and rural areas of India.

Objective: This study was aimed to determine the frequency of polymorphisms in DNA repair genes, hOGG1 at codon (cd) 326 and APE1 at cd 148 in patients of oral cancer from Maharashtra and to evaluate their association with oral cancer development.

Materials and Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to analyze gene polymorphisms in 200 patients with oral cancer and 200 age and sex matched disease-free controls were used.

Result: The results obtained from the present study indicated that there was no significant difference in the genotype distribution between oral cancer patients and controls for APE1 ($p > 0.05$). The result showed that allele frequencies of hOGG1Cys326 (OR = 8.73; 95% CI = (2.51-30.39); $p = <0.0001$) genotype significantly increased the risk of head and neck cancer.

Conclusion: This study indicates that polymorphisms in cd326 of hOGG1 gene could play a role in modifying genetic susceptibility of individual to head and neck cancer in Maharashtrian patients.

KEYWORDS: hOGG1, *APE1*, PCR-RFLP, genetic polymorphism

Introduction

Oral cancer is the most common cancer in the world increased significantly in last few years where cancer of the oral cavity, oropharynx, and larynx constitute a major public health problem. Oral cancer is the 5th common type of cancer worldwide and 2nd in the developing world.[1] In the Indian subcontinent, head and neck cancer (HNC) is the common malignancy, accounting up to 40–50% of all malignant cancers.[2] In India, the major risk factors of HNC are tobacco and alcohol,[3] and account for more than 75% of HNC but specific carcinogenic mechanisms are unclear. The reactive oxygen species (ROS) derived from metabolites of tobacco and alcohol are known to cause oxidative damage to cellular DNA in the form of serious lesions like single strand breaks (SSBs), double-strand breaks (DSBs), the most serious DNA damage, which if not repaired or misrepaired, may result in genomic instability and cancer development.[4] Such DNA damage can be corrected by some DNA repair mechanisms such as base excision repair (BER), nucleotide excision repair (NER),

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double strand break repair (DSBR) and, DNA mismatch repair (DMR) which play a crucial role in maintenance of genomic integrity.[5] But it is not yet clear which DNA repair pathways are the most important for protection against HNC.

Among these various types of oxidative DNA damages, the formation of 8-hydroxy-2-deoxyguanine (8-OHdG) is one of the most abundant oxidative products of cellular DNA, believed to play an important role in carcinogenesis,[6] because it is highly mutagenic agent causing GC to TA transversions during DNA replication. The human 8-oxoguanine DNA glycosylase 1 (hOGG1) and apurinic/apyrimidinic endonuclease 1 (APE1) genes play an important role in the DNA repair pathway[7] where hOGG1 has primary activity to remove the 8-OHdG from DNA through the BER mechanism whereas APE1 repairs basic AP sites in DNA.

It is believed that along with the environmental factors, host factors including individual's genetic susceptibility are also likely to play a role in the development of HNC. Genetic susceptibility is related to genetic polymorphisms of various genes, including those involved in DNA repair pathway. Few molecular epidemiologic studies have evaluated the association of HNC with functional genetic variants in the BER genes,[8] but in some of the studies the results are contradictory rather than convincing. Also, the earlier observations were inconsistent in terms of their roles in oral cancer susceptibility and therefore the influence of these genes polymorphisms is still unclear. It is also doubtful which DNA repair pathway genes may be more important for protection against oral cancer. In earlier studies, it has been shown that polymorphisms in BER pathway genes especially XRCC1, XRCC4, XRCC5, XRCC7 and NER pathway gene XPD codon 199 plays an important role in susceptibility of oral cancer in rural population of Maharashtra.[9-10] In continuation with this, it was also hypothesized that the inherited polymorphisms in some gene products i.e., hOGG1 and APE1 may contribute to genetic susceptibility to oral cancer. To test this hypothesis, the present study proposed to investigate the associations of hOGG1, APE1 gene polymorphisms with the development of oral cancer in Maharashtrian population.

Materials and methods

Study subjects

This study was a hospital based, case-control study conducted in rural areas of Maharashtra from India. The study participants included 200 newly diagnosed HNC patients, and 200 healthy with age and sex matched individuals. Trained interviewers used a structured questionnaire to collect personal interview data from the participants.

Genomic DNA isolation from whole blood

Five milliliter (mL) of whole blood from patients and normal controls was collected in vacutainer after receiving formal consent. Genomic DNA extraction was carried out from the peripheral blood sample using Purelink genomic DNA extraction and purification Kit (Invitrogen, Life technologies) following the manufacturer's instructions.

Genotyping assays

Genotyping of hOGG1 and APE1 genes were performed by PCR-RFLP methods with appropriate primer sets (Table 1). The primers were designed to amplify the regions of DNA that contain polymorphic sites of interest: (A) hOGG1 Ser326Cys codon326 in the exon-7 and (B) APE1 Asp 148Gln codon 148 in the exon-5. The PCR amplification was carried out separately under different conditions in 20 micro liter (μL) reaction mixtures containing PCR buffer (10 mili molar (mM) Tris-HCl (pH 9.0), 50 mM KCl 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 10 picomole (pmol) of each primer listed in Table 1, 1U *Taq DNA* polymerase (GeNei, Merck Bioscience) and 100 nanogram (ng) of purified genomic DNA template. The reaction mixtures were subjected to PCR amplification with a master cycler gradient PCR (Eppendorf). After performing PCR program, the PCR products were analyzed by agarose gel electrophoresis. The agarose gels were stained with ethidium bromide (10 mg/mL) and visualized under UV Transilluminator. After confirmation of DNA amplification, each PCR product was digested with appropriate restriction enzyme as shown in Table 1 for genotyping. 10 μL of the PCR products were digested at 37°C

Table 1: Details of PCR and RFLP procedures and expected products for hOGG1 and APE1 genes

Gene	Primers Forward / Reverse	PCR conditions	PCR Product	Restriction enzyme	Restriction products
hOGG1 (C1245G) Ser326Cys cd326 Exon7	5′-ctg ttc agt gcc gac ctg cgc cga-3′ 5′-atc ttg ttg tgc aaa ctg ac-3′	95°C- 5 m, 35 cycles of 95°C- 30 s, 64°C- 30 s, 72°C- 30 s, 72°C- 5 m	247 bp	2U of MboII	Ser/Ser: 224bp, 23bp Ser/Cys: 247bp, 224bp, 23bp Cys/Cys: 247bp
APE1 (T2197G) Asp148Glu cd 148 Exon5	5′-ctg ttt cat ttc tat agg cta-3′ 5′-agg aac ttg cga aag gct tc-3′	95°C- 5 m, 35 cycles of 95°C- 20 s, 55°C- 20 s, 72°C- 20 s, 72°C- 5 m	164 bp	1U of BfaI	Asp/Asp: 144 bp. 20 bp Asp/Glu: 164bp, 144bp 20bp Glu/Glu: 164bp

overnight with specific restriction enzymes in 20 µL reaction mixtures containing buffer supplied with each restriction enzyme. After the overnight incubation, digestion products were then separated on a 6% PAGE gel at 100 volts (V) for 30 m stained with silver staining and photographed with gel documentation system (BioRad Laboratories).

Statistical analysis

The associations between the hOGG1 and APE1 genotypes and risk of HNC with or without smoking and drinking history were studied using odds ratio (OR). Both the univariate and multivariate logistic regression analyses were employed to calculate the adjusted ORs and 95% confidence intervals (CIs) to determine the cancer risk associated with genotypes.

Ethical approval

This study was approved by the Institutional Ethics Committee of Krishna Institute of Medical Sciences.

Result

Characteristics of the study subjects

The characteristics of age and sex matched cases and controls are represented in Table 2. The (Mean ± SD) age in years was 53.70 ± 13.34 for the cases and 52.60 ± 12.44 ($P < 0.05$) for the controls, however, there was no significant difference in mean age between cases and controls.

Association hOGG1 and APE1 genotype variants with oral cancer risk

The present study examined the polymorphism Ser326Cys of hOGG1 and Asp148Gln of APE1 genes in a total of 200 subjects and equal number of controls.

(A) hOGG1 (Ser326Cys) codon326 in exon-7

Table 3 displays the distribution of genotypes and frequency of alleles of the G23592A polymorphisms in patients

Table 2: Distribution comparisons of selected demographic characteristics of HNC cases and healthy controls from rural areas of Maharashtra

Variable	**Cases *N* = 200**		**Controls *N* = 200**		***P*-Value based on χ^2**
Age (Mean ± SD) years	**53.70 ± 13.34**		**52.60 ± 12.74**		
	No.	**(%)**	**No.**	**(%)**	
≤ 50	83	41.50	90	45.00	
51–60	51	25.50	55	27.50	0.67
61–70	53	26.50	43	21.50	
>70	13	06.50	12	06.00	
Sex					
Male	137	68.50	137	68.50	1.00
Female	63	31.50	63	31.50	
Tobacco smoking Status					
Smokers Current	152	76.40	47	23.50	<0.001
Non smokers	47	23.60	153	76.50	
Alcohol status					
Drinkers	134	68.75	26	12.50	<0.001
Non-drinkers	66	31.25	174	87.50	
Diet					
Vegetarian	39	19.50	39	19.50	
Non-vegetarian	07	3.50	07	3.50	1.00
Mixed	154	77.00	154	77.00	
Education					
High School	131	65.50	131	65.00	
High School graduate (12 y)	27	13.50	28	14.00	0.96
College /Graduate	42	21.00	41	20.50	
Economic status					
Middle	62	31.00	60	30.00	0.92
Poor	138	69.00	140	70.00	
Family history of Cancer					
Yes	12	6.00	0	0.00	<0.001
No	188	94.00	200	100	

Table 3: The genotype frequencies of hOGG1*and* APE1 gene variants in untreated HNC patients and controls.

GENE	Genotype	CASES (*n* = 200) (%)	CONTROL (*n* = 200) (%)	Odds' Ratio (95% CI)	*P* value	Adjusted Odds Ratio (95% CI)	*P* value
hOGG1 (C1245G) Ser326Cys cd326 Exon7	Ser/Ser	65 (32.50)	71 (25.50)	1		1	
	Ser/Cys	111 (55.50)	126 (73.00)	0.93 (0.63-1.46)	0.85	1.062 (0.58-13.91)	0.84
	Cys/Cys	24 (12.00)	3 (1.50)	8.73 (2.51-30.39)	<0.0001*	16.30 (3.84-76.33)	<0.001
	Ser/Cys+Cys/Cys	135 (67.50)	129 (64.50)	1.14 (0.75-1.72)	0.52	1.26 (0.72-2.28)	0.38
APE1 (T2197G) Asp148Glu cd 148 Exon5	Asp/Asp	27 (13.50)	25 (12.50)	1		1	
	Asp/Glu	170 (85.00)	175 (87.50)	0.89 (0.50-1.61)	0.72	1.58 (0.86-3.12	0.99
	Glu/Glu	3 (1.50)	0 (00.00)	0.92 (1.17-5.01)	0.22	0.84 (0.46-1.52)	0.57
	Asp/Glu+ Glu/Glu	173 (86.50)	175 (85.50)	0.91 (0.51-1.64)	0.76	0.83 (0.34-1.88)	0.61

* indicates significant odds ratio ($p < 0.05$)
p value determined based on χ^2

Table 4: Stratification analysis of the demographic factors including age, tobacco smoking and alcohol drinking and distribution of genotypes of the hOGG1 and APE1 genes in the patients with HNC and the control group from population of Maharashtra.

Gene	Genotype	Demographic Factors							
		Age (Cases/Control)		Sex (Cases/Control)		Smoking status (Cases/Control)		Drinking status (Cases/Control)	
		≤ 50 *N* = 122/150	> 50 *N* = 198/250	Male *N* = 200//260	Female *N* = 120/140	Smokers *N* = 253/80	Nonsmokers *N* = 67/320	Drinkers *N* = 220/50	Non-drinkers *N* = 100/350
hOGG1 (C1245G) Ser326Cys cd326 Exon7	Ser/Ser	29/34	36/36	45/47	18/24	51/17	14/54	18/12	9/59
	Ser/Cys + Cys/Cys	55/57	80/73	95/90	42/39	102/30	33/99	116/14	57/115
	OR (95% CI)	1.13 (0.60-2.10	0.91 (0.52-1.59)	0.90 (0.55-1.49)	0.69 (0.32-1.47)	0.88 (0.44-1.74)	0.77 (0.38-1.57)	0.18 (0.07-0.45)	0.30 (0.14-0.66)
	P value	0.69	0.74	0.70	0.34	0.71	0.48	0.0003	0.002
APE1 (T2197G) Asp148Glu cd 148 Exon5	Asp/Asp	8/12	19/13	18/16	9/9	21/8	6/17	18/3	9/22
	Asp/Glu + Glu/Glu	75/77	98/98	119/122	54/53	134/40	40/135	117/23	56/152
	OR (95% CI)	0.68 (0.26-1.76)	1.46 (0.68-3.12)	1.15 (0.56-2.36)	0.98 (0.36-2.66)	0.78 (0.32-1.90)	1.19 (0.44-3.22)	1.17 (0.32-4.33)	1.11 (0.48-2.55)
	P value	0.43	0.32	0.69	0.97	0.59	0.73	0.80	0.80

with HNC and controls. The frequency of *hOGG1Ser/Ser* wild type homozygotes was 32.50% in cases and 25.50% in controls whereas *hOGG1Cys/Cys* variant homozygotes was 12.0% in cases and 1.50% in controls. The frequency of *hOGG1Ser/Cys* heterozygotes was 55.50% in cases and 73.0% in controls (Table 3). Compared to *hOGG1Ser/Ser* genotype, the variant genotype *hOGG1Cys/Cys* was associated with oral cancer risk (OR=8.73; 95% CI = (2.51-30.39, $P < 0.0001$) of HNC. The *hOGG1Cys/Cys* variants are extremely high and contribute significantly to the risk of HNC in the rural population of south western Maharashtra.

(B) APE1 (Asp148 Glu) Codon 148 in exon-5

The amplification of APE1 codon 148 resulted in 147 bp. The PCR amplified products upon treatment with BfaI yielded wild-type (2197T) alleles of 144 and 20 bp fragments, and the polymorphic (G) allele produces 164 bp (Figure 1B). The frequency of *APE12197TT* homozygotes was 13.50% in cases and 12.50% in controls whereas the frequency of *APE12197GG* allele was lower but not significant in the cases (1.50%) than in the controls (0.0%). The frequency of *APE1T2197G* heterozygotes was 85.0% in cases and 87.50% in controls (Table 3). Thus, the T→G polymorphisms in exon 5 at nucleotide did not result in an amino acid change at codon 148 in cases as compared to controls.

Discussion

In this hospital based case-control study, the relationship between newly reported genotype polymorphisms of DNA repair genes especially involved in BER pathway and the elevated risk for oral cancer particularly in tobacco and alcohol users from the rural areas of Maharashtra has been investigated. To evaluate the association of hOGG1 and APE1

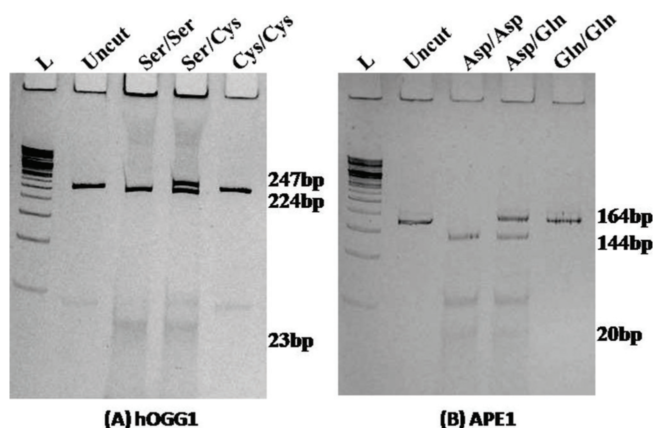

Figure 1: Agarose gel image showing nucleotide polymorphism by PCR-RFLP of (A) hOGG1 codon326 in the exon-7 (Lane 1: 100bp DNA ladder, lane 2: Uncut PCR product, lane 3: Ser/Ser genotype lane 4: Ser/Cys genotype, lane 5: Cys/Cys genotype and) and (B) APE1 codon 148 in the exon-5. (Lane 1: 100 bp DNA ladder, lane 2: Control cut, lane 3: Asp/Asp genotype, lane 4: Asp/Gln genotype, lane 5: Gln/Gln genotype.

variants, the risk of HNC crude and adjusted ORs and their 95% CIs were calculated using both homozygous genotypes or combined with their respective heterozygous genotypes. Comparable wild type genotype frequencies of APE1 codon 148 showed wide distribution in the Maharashtrian population in controls as well as HNC cases. Also, when investigated the relationship between the polymorphisms of hOGG1 and the risk of oral cancer in a Maharashtrian population, association between the hOGG1 codon 326 at Ser 326 Cys polymorphism and smoking and drinking related oral cancer has been found.

The polymorphism in DNA repair genes has been extensively investigated for its associations with cancer risk and the results were conflicting in different types of cancer or different populations. Several epidemiological studies have investigated the association between genetic polymorphisms in hOGG1, APE1 and susceptibility to several kinds of cancers including lung,[11] stomach,[12-13] prostate,[14] breast cancers[15] among different ethnic including Brazilians, Japanese and Chinese populations. Very few studies from India have reported the genetic polymorphisms in the DNA repair genes, hOGG1 and, APE1 with respect to a variety of cancer risks including gastric,[16] gallbladder,[17] prostate cancers.[18] These previous observations suggest that hOGG1Ser326Cys, APE1Asp148Gln polymorphisms may or may not influence different cancer susceptibility in different populations with varied incidence of cancer, whereas few other studies failed to find positive evidence for hOGG1 polymorphisms in orapharyngeal carcinoma risk.[19] However, very limited information is available on the association of genetic polymorphisms of DNA repair genes including hOGG1, APE1 genes and their susceptibility to oral cancer from rural population of Maharashtra where the rate of tobacco and alcohol consumption is very high. Therefore in this study, the relationship between the development of oral cancer and genetic polymorphisms in hOGG1 and APE1 genes from a pool of unexplored Maharashtrian population has been investigated.

Conclusion

In conclusion to this knowledge, this study is the first one to show that hOGG1 Ser326Cys polymorphism was associated with susceptibility to oral cancer in Maharashtrian population.

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