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Original Research Article

Association of CYP2E1 genetic polymorphism with risk of Oral pre cancer and cancer in North Indian population

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Abstract

Purpose: To evaluate the association of CYP2E1 promoter (-1091) C/T polymorphisms with the susceptibility of Oral pre cancer and cancer patients in North Indian population.
Methods: A total 250 patients with oral pre cancer and cancer, 250 controls were genotypes for the CYP2E1 (C1091T) gene polymorphism. Genotypes were identified by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP). Genotype frequencies were evaluated by Chi-square test and Odds ratio (OR) relative risk.
Results: The average CYP2E1(C1091T) genotype frequencies of CC, CT and TT were calculated 80.80%, 5.20%, 14.00% in cases and 54.80%, 13.20% and 32.00% in healthy controls; respectively.
Conclusion: We conclude that the CYP2E1 (C1091T) polymorphism was significantly associated in Oral pre cancer and cancer patients.
Keywords: Oral pre cancer and cancer, CYP2E1, PCR-RFLP, Gene polymorphism

1. Introduction

Oral cancer is one of the eight most common cancers in the world and occurs more often in males in developing countries. The use of tobacco is an established etiological factor in the development of cancer of the oral cavity [1]. Tobacco is consumed in both smoking and smokeless forms. Aromatic amines such as 4-aminobiphenyl and heterocyclic amines such as ph1p4 are present in cigarette smoke. Panmasala consumption is associated with the consumption of smokeless tobacco with the concomitant use of several additives that can alter cancer risk. Oral squamous cell carcinoma is the most common malignancy of the oral cavity [2]. Many environmental factors like smoking and alcohol consumption and genetic factors like oncogenes and tumor suppressor genes are implicated in the development of oral cancer [2]. Several factors related to angiogenesis, inflammation and thrombosis have also been associated with oral oncogenesis [1-3].

Both genetic and environmental factors are involved in the development of cancer. The environment gene interaction on carcinogenesis has been well demonstrated by Phase I and II enzymes that are involved in the metabolism of carcinogens. Some of these enzymes are polymorphic in genotypes with correspondence variation in their activities. The Phase I enzymes, CYP2E1 activate many environmental pro carcinogens by adding or exposing their functional groups [4]. These enzymes act on different substrates, and some of them are inducible by environmental factors. CYP2E1 inducible by ethanol and plays an important role in the metabolic activation of N-Nitrosoamines. The genetic polymorphism of CYP2E1 has been associated with the risk of lung cancer [5] and hepatocellular carcinoma [6]. An interaction between CYP2E1 polymorphism and cigarette smoking was also Observe in the development of hepatocellular carcinoma.

Investigation of the association between risk of cancer development and *C*YP2E1 gene polymorphism is of significant interest, because this enzyme is involved in metabolism of aniline, vinyl chloride, and urethane and participates in the activation of N-nitrosodimethylamine, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone, and nicotine-dependent *N*-nitrosonornicotine.[7-12] All these compounds initiate malignancies of various sites, including stomach,

oesophagus, liver, and lung.[13] CYP2E1 catalytic activity was found to display individual differences, which can lead to increased risks of tumour development. Changes in enzymatic activity may be a consequence of genetic polymorphism or gene induction by xenobiotics. Indeed, a correlation was found between mRNA level, CYP2E1catalytic activity, and transcriptional activity of polymorphic gene variants. The *C*YP2E1 gene is present in the population in various polymorphic forms. Polymorphisms detectable by DraI and TaqI digestion are not thought to affect transcription or function of the enzyme coded for by the gene. In contrast, the variant detectable by RsaI digestion (called the c2 variant) contains polymorphic base substitution sites in a region of the gene that is not transcribed but that appears to be involved in the transcriptional regulation7of CYP2E1 expression. It is also demonstrated that CYP2E1 synthesis and catalytic activity levels are changed under exposure to environmental factors. This can result in the development of acquired susceptibility to diseases. This study discusses the association of CYP2E1 polymorphism with individual human susceptibility to leukoplakia than transformation to malignancies.

Human CYP2E1 is constitutively expressed in the liver and to lesser extent in other organs and tissues, including human urothelial cells. It is a key enzyme in the metabolic activation of many low-molecular-weight carcinogens, such as vinyl chloride, benzene, and tobacco-specific nitrosamines [14,15]. CYP2E1(7632) polymorphisms, have been frequently studied. CYP2E1 is a DraI polymorphism caused by a T to A at 7632 in intron 6. CYP2E1 does not affect gene transcription but may have an effect on the catalytic activity of the enzyme.CYP2E1 is a PstI polymorphism caused by a G to C at 1293 in the non-coding region. G and C are named c1 and c2 alleles, respectively. Studies showed that CYP2E1 modulates transcription of the gene in vitro [16]. Frequency of the c2 allele and reported associations between this polymorphism and risk for many types of cancer, including bladder, have been inconsistent and varied among ethnicgroups[17].

2. Materials and Method

The study was conducted on 250 patients with previously treated and histologically confirmed oral pre cancer and cancer who were registered at department of Oral Pathology & Microbiology, King George's Medical University and 250 healthy controls. Informed written consent was obtained from all subjects. Ethical clearance was obtained from institutional ethical committee.

2.1 Determination of CYP2E1 (C1091T) genes

Venous blood samples were collected in EDTA tubes and stored at −80 °C, till DNA extraction. Genomic DNA extraction from blood samples was carried out by salting out method.(18) The CYP2E1(C1091T) polymorphism was analysed by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Genomic DNA was amplified using the following PCR conditions: 94˚C for 2 min, 30 cycles at 94˚ C for 20 sec, 55˚C for 30 sec, 72˚C for 30 sec, and finally 72˚C for 10 min. The primers used for amplification of the CYP2E1 (C1091T) gene polymorphisms were as follows: forward primer 5'-**CCA GTC GAG TCT ACA TTG TCA** -3'and reverse primer 5'-**TTC ATT CTG TCT TCT AAC TGG** -3'. Amplification success of samples was monitored on 2% agarose gel by Gel electrophoresis. There after the PCR products (413-bp) were divided and separately subjected to PstI restriction enzyme digestions. The mixture was incubated overnight at 37˚C for digestion. The digested product was electrophoresed on 3 % agarose gel at 80 V for 1 h. The presence of restriction site on chromosomes yielded to fragments of 118- and 295-bp for the PstI digested (Fig: 1).

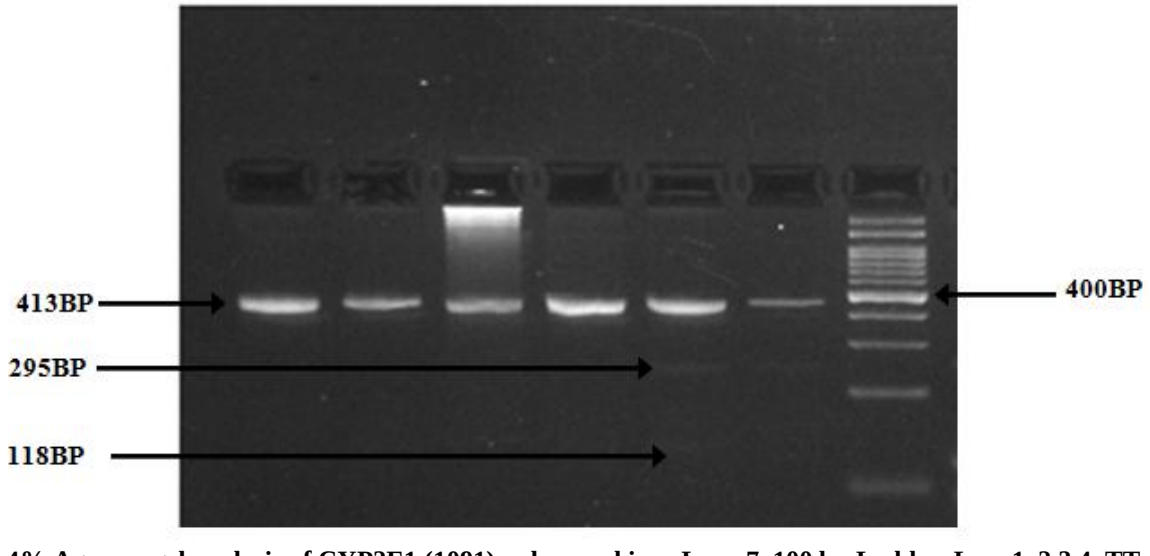

Figure 1: 4% Agarose gel analysis of CYP2E1 (1091) polymorphism. Lane 7 100 bp Ladder, Lane1, 2,3,4 TT genotype 413 bp, Line 5,6 CT genotype 413, 118, 295 bp.

2.2 Statistical analysis

The significance of this study was evaluated by Chi-square test. Odds ratio (OR) was calculated as an estimate of relative risk of having disease according to the relative frequency of different genotypes among the cases as well as the controls. ORs are given with 95% confidence interval (CI).

3. Results

The demographic profile included age, gender, relative environmental risk factors and tumor staging which may contribute the progression of Oral pre cancer and cancer. In this study, age [(<20-40 64.00%, 52.40%), (<40-70 36.00%, 47.60%)], sex [(60% male, 70% male),(40% female, 30% female)] in Oral pre cancer & cancer patients and controls respectively. In this study we also observed masala tobacco chewing (28.00%, 18.00%), smoking (24.00%, 12.00%) and alcohol consumption (16.00%, 4.00%), no habit (32.00%, 66.00%) in Oral pre cancer & cancer patients and controls respectively. In this study we observed that masala tobacco chewing, smoking and alcohol consumption, no habit are significantly (p b 0.05) associated with oral pre cancer and cancer. The occurrence of tumor stages [I (30.40%) II (27.20), III (22.40) and (20.00) IV], tumor T status (≤T2 60.40%, >T2 39.60%) was presented in this study (Table2). The average CYP2E1(C1091T) genotype frequencies of CC, CT and TT were calculated 80.80%, 5.20%, 14.00% in cases and 54.80%, 13.20% and 32.00% in healthy controls; respectively. The observed CYP2E1 (C1091T) high expression mutant T allele frequency was 16.60% in cases and 38.60% in healthy controls (Table 2). We correlated the CYP2E1 genotypes of Oral pre cancer and cancer patients under different groups i.e. age, gender, smoking, tobacco chewing, alcohol consumption, clinical tumor stage (I+II and III+IV), tumor T status which are shown in (Table 1).

Table 1: Demographic Profile

Demographic Character	Cases (n=250)	Control (n=250)	P- value
Male	150 (60%)	175 (70%)	0.3131
Female	100 (40%)	75 (30%)	0.1236
Age			
<20 – 40	160 (64.00%)	131 (52.40%)	0.2010
<40- 70	90 (36.00%)	119 (47.60%)	0.1088
Habit			
No habit	80 (32%)	165 (66%)	0.0011*
Alcohol consumption	40 (16%)	10 (4%)	0.0011*
Smoking	60 (24%)	30 (12%)	0.0051*
Masala, tobacco chewing	70 (28%)	45 (18%)	0.0455*
TNM staging			-
Tumor Stage I II III IV	 76 (30.40%) 68 (27.20%) 56 22.40%) 50 (20.00%)	-	-
Tumor T Status ≤T2 >T2	 151 (60.40%) 99 (39.60%)	-	-

*=significant value

Table 2: The genotype and allele frequencies of CYP2E1 promoters polymorphisms in oral pre cancer and cancer patients and controls

SNP	Cases (n=250)	Controls (n=250)	p- value	Odds Ratio	95% CI
CYP2E1 (C1091T) Genotypes					
CC	202 (80.80%)	137 (54.80%)	-	-	-
CT	13 (5.20%)	33 (13.20%)	<0.0001*	3.743	1.901-7.370
TT	35 (14.00%)	80 (32.00%)	<0.0001*	3.370	2.143-5.300
C	417 (83.40%)	307 (61.40%)	-	-	-
T	83 (16.60%)	193 (38.60%)	<0.0001*	3.158	2.348-4.249

*=significant value

4. Discussion

We did not observe the association between genotype at the PstI-RsaI sites of CYP2E1and the risk of oral cancer on this population. But polymorphism at these sites modulated the risk of cancer in Chinese, [19] Caucasian, and African-American population. [20] This difference in observation between this part of Indian population and other populations may be because variant c1 allele at PstI and RsaI sites is very less frequent in this population and alcohol drinkers are few in this population compared with Chinese and Caucasian populations. So this may be one of the reasons for which we could not detect the effect of this variant allele in this part of Indian population. At the DraI site, the combined *CC* + *CD* genotypes (i.e., expected risk-genotypes) did not modulate the risk of the diseases when all patients and controls were compared. This finding is consistent with reports on Japanese and Caucasians but is in contrast with a report on upper aerodigestive tract in Caucasians. An observation also noticed with lung and oral cancer patients [21]. Such a high effect of low-dose tobacco habits had been explained by individual's susceptibility and formation of higher DNA carcinogens adduct due to polymorphism in some metabolic genes (e.g., GST, *CYP2E1*) [22,23]. So attributable risk should be accessed in a larger case-control study, exposed to low tobacco dose [24]. The apparent absence of risk of oral cancer in heavy tobacco users may be due to the overwhelming effect of heavy tobacco dose on these genotypes or due to the effect of small sample size being reduced due to the stratification of the samples. The lack of association at high tobacco dose also suggests that study of polymorphism in other metabolic and/or DNA repair genes may provide important finding to explain this phenomenon.

In this study, we examined association between polymorphisms in CYP2E1 gene and risk of oral cancer among Indian tobacco users. Incidences of oral cancer are more in men than in woman because of sex differences in tobacco habits (e.g. smoking, chewing etc). In the present study, we found that CYP2E1 (C1091T) CT, TT is extremely significantly associated with Oral pre cancer and cancer.

In conclusion, It can be concluded that the present study provides evidence of the correlation between CYP2E1 gene polymorphism with risk of Oral pre cancer and cancer. The CT, TT genotype and T allele are significantly associated with the risk of Oral pre cancer and cancer. This finding suggests that CYP2E1 may be used as a diagnostic marker for effective management of Oral pre cancer and cancer in future, though further studies with larger sample sizes will be necessary to confirm this.

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