

Full Length Research Paper

A novel *CYP1A1* gene polymorphism and the risk of head and neck cancer in Pakistani population

Nosheen Masood*, Fraz Arshad Malik, Ishrat MahJabeen, Ruqia Mahmood Baig and Mahmood Akhtar Kayani

Cancer Genetics Laboratory, Department of Biosciences, CIIT, Islamabad. Pakistan.

Accepted 4 March, 2011

Several polymorphisms in the *CYP1A1* locus have been identified and their genotypes appear to exhibit population frequencies that depend on ethnicity. In this study, we assessed the role of *CYP1A1* genotype in 388 head and neck cancer patients in Pakistani population via a case-control study. Polymerase chain reaction (PCR) and single stranded conformational polymorphism assays were used. Most of the patients (51%) enrolled for the study, were from the age group of 40 to 60 years (± 16.59). Mean age of the cancer patients involved in the study was 48 ± 16.59 years. Statistical analysis has shown that, tobacco users have more chances of head and neck cancer ($P < 0.05$) than non tobacco users, whereas male to female ratio is 1:1 ($P > 0.05$). Jobless persons are more prone to head and neck cancer ($P < 0.01$) compared with employers and housewives. After the genetic analysis, it was found that no already reported variants of *CYP1A1* gene were found in Pakistani population. A novel mutation in *CYP1A1* gene at exon 2 in 21 patients ($P < 0.001$, Odd Ratio (OR) = 9.4 and 95% confidence interval (CI) 1.3 to 70.8) has been found with a serine formation instead of tyrosine at amino acid 110. The patients showing this mutation have the mean age of 51.75 (± 15.7). Therefore, mutation in *CYP1A1* gene may be one of several factors that increase the chance of developing head and neck cancer.

Key words: Cytochrome P450 1A1 gene (*CYP1A1*), head and neck cancer (HNC), mutation, novel polymorphism, Pakistani population.

INTRODUCTION

Head and neck cancer (HNC) includes the cancers of oral cavity, larynx and pharynx and is the sixth most frequent cancer worldwide and is particularly, high in south East Asian countries (Johnson 1991; Parkin et al., 2002). In Pakistan, incidence of head and neck cancer is 40.1% of all the cancers (Hanif et al., 2009) and the second most frequent type of prevalent cancers (Faheem, 2007). In France, cancer of pharynx is more common in men, while cancer of oral cavity is more common in women in India. In Europe and Japan,

(Makimoto et al., 2000) marked rise in incidence of HNC has been reported in last few decades (Sankaranarayanan et al., 1998; Morelato and Lopez, 2006).

Multitudes of factors are responsible for occurrence of HNC like lifestyle, dietary habits and mental stress (Jensen et al., 2010). Workers of mines and nickel or wood industry are more prone to HNC (Tevfik et al., 2007). Environmental factors like chewing tobacco and alcohol are given special attention in relation to head and neck cancer (Sabitha et al., 2010). Exposure to synthetic or natural chemical compounds present in the environment is usually associated with HNC. Xenobiotics metabolizing enzymes are responsible for metabolism of many exogenous chemicals that are toxic, mutagenic or carcinogenic. Carcinogen detoxifying enzymes include the phase I enzymes involved in the detoxification of carcinogens and either neutralize them or change them into electrophilic compounds that are detoxified by the phase II enzymes (Rajani et al., 2003).

*Corresponding author. E-mail: nosheenmasood@hotmail.com.
Tel: 0092-334-5890589.

Abbreviations: ***CYP1A1***, Cytochrome P450 1A1 gene; **HNC**, head and neck cancer; PCR, polymerase chain reaction; GSTM1, glutathione-S-transferase isozyme M1; GSTP1, glutathione-S-transferase isozyme P1; GSTT1, glutathione-S-transferase isozyme T1; OR, odd ratio; CI, confidence interval.

The principal enzymes responsible for phase I reaction belong to cytochrome P-450 multigene family. The cytochrome P450 1A1 enzyme functions by the addition of oxygen atom into the toxic chemical and initiate detoxification and elimination by increasing hydrophilicity (Guengerich and Shimada 1991). Cytochrome P450 1A1 gene (*CYP1A1*) is located on chromosome 15q22-24 and encodes aromatic hydrocarbon, hydroxylase that converts polycyclic aromatic hydrocarbons (PAHs) (Shimada et al., 1989) to carcinogen and is predominantly expressed in extrahepatic tissues including lungs (Anttila et al., 1992).

The cytochrome P-450 that are known to exhibit polymorphism include *CYP1A1*, *CYP1B1* (Arrind et al., 2008), *CYP2A6*, *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP2E1* (Rajani et al., 2003). Polymorphism of *CYP1A1* gene has been studied with relation to different cancers including head and neck cancer (Sabitha et al., 2010). Four different sequence polymorphisms have been reported in *CYP1A1* gene, first known as *CYP1A1*2* involves a T₆₂₃₅ to C transition in the 3' noncoding region (Kawajiri et al., 1990; Jun et al., 2010), second known as *CYP1A1*3* involve a A₄₈₈₉ to G transition in exon 7 (Jun et al., 2010; Hayashi et al., 1991), third known as *CYP1A1*4* involves a T₅₆₃₉ to C transition in intron 7 (Crofts et al., 1993) and fourth known as *CYP1A1*5* involves a C₄₈₈₇ to A transition in exon 7 (Jun et al., 2010; Cascorbi et al., 1996). The present study is aimed at evaluating the role of environmental factors in head and neck cancer risk along with *CYP1A1* gene polymorphisms in Pakistani population.

MATERIALS AND METHODS

Identification of patients and normal controls

The present case-control study consisted of 388 cases with pathologically confirmed head and neck cancer along with age and sex matched 150 cancer free normal individuals as controls. They were recruited from National Oncology and Radiotherapy Institute (NORI) and Pakistan Institute of Medical Sciences (PIMS), Islamabad from March 2008 to September 2009 with a prior approval from Ethical Committees of both university and hospitals.

All study subjects participated on a volunteer basis. All subjects were personally interviewed according to a structured questionnaire. They were asked about area of cancer, age, tobacco addictions and occupational exposures. Blood was collected from subjects with their informed consent. Information concerning alcohol intake was found fairly unreliable and was disregarded due to Muslim community. However, our experiences with patients and control blood donors have shown that, majority are tobacco addicted in the form of betel quids and moist snuff. Subjects' blood was sampled before starting the therapy.

Sample collection and DNA isolation

Blood samples were collected in EDTA-containing tubes and stored at -20°C until further use. DNA was isolated, using organic protocol with phenol-chloroform extraction as previously described (Baumgartner-Parzer et al., 2001; Vierhapper et al., 2004).

Electrophoresis was performed on isolated DNA on 1% ethidium-bromide stained agarose gel and photographed (BioDocAnalyze Biometra). 5 ng dilutions were made of each DNA isolated and stored at 4°C until use.

Primer designing and polymerase chain reaction (PCR)

Primers for 7 exons of *CYP1A1* were synthesized by using primer 3 input software version 0.4.0 (Table 1) and BLAST using NCBI PRIMER BLAST. 2 µl DNA (10 ng/µl) was added to a 20 µl PCR mixture composed of 2 µl PCR buffer, 2 µl of each primer (10 mM), 0.24 µl deoxynucleotide triphosphate (25 mM) and 0.2 µl Taq polymerase (5 u/µl). The reaction mixture was placed in 9700 thermal cycler of ABI systems for 5 min at 94°C and subjected to 30 cycles at 94°C for 25 s, annealing temperature for 1 min and 72°C for 1 min, followed by a final step at 72°C for 10 min and held at 4°C.

Amplification products were resolved on a 2% ethidium bromide-stained agarose gel along with 100 bp DNA ladder. All the patients and control DNA was amplified for all the 7 exons of *CYP1A1* gene with exon specific primers. All of the photographs of gel electrophoresis were read by two technicians blind to each other's assessments.

Single strand conformational polymorphism

PCR product was analyzed by single stranded conformational polymorphism (SSCP) using the procedure described by Patricia et al. (2009) and Amalio et al. (1993) with some minor modifications. SSCP results were analyzed with gel documentation system (BioDocAnalyze Biometra) after ethidium bromide staining and photographed. The samples showing mobility shifts from the controls were then sequenced.

Sequencing

21 samples were screened out from SSCP and were sequenced from MacroGen (Korea). Reverse primer was used for sequencing. The sequenced results were made forward complementary before analysis using BioEdit v 7.0.5 software and analyzed. Statistical analysis was performed by using SPSS statistics 17.0 software and GraphPad Prism 5 Demo for calculating odd ratios, 95% confidence interval and standard error.

RESULTS

Highly significant difference in number of patient with cancer of oral cavity ($P < 0.01$) was found compared with pharyngeal and laryngeal cancers. In cancer of oral cavity, pharynx and larynx, difference in number of male patients compared with females was statistically non significant ($P > 0.05$).

Statistically significant increase in incidence of head and neck cancer was observed in age group of 40 to 60 years ($P < 0.05$). The mean age of cancer patients was 48 years (± 16.59), whereas the mean age of controls was 46 (± 17.69) years. A higher number of males had cancers of oral cavity and laryngeal cancer, whereas females had a higher frequency of pharyngeal cancer cases as shown in Figure 1. Surprisingly no statistical

Table 1. Primer sequences synthesized for different exons of *CYP1A1*.

| Primer name | Primer sequence |
|------------------------------|-----------------------|
| <i>CYP1A1</i> Exon1 Forward | TACAGGCACCGAGATGTGTC |
| <i>CYP1A1</i> Exon1 Reverse | AGTCCTGGAGGCACCAAAAT |
| <i>CYP1A1</i> Exon2a Forward | GTTTCCCCTTTCCCTGACAC |
| <i>CYP1A1</i> Exon2a Reverse | CAGGTAGCAGGAGGTTGAGG |
| <i>CYP1A1</i> Exon2b Forward | CCGACCTCTACACCTTCACC |
| <i>CYP1A1</i> Exon2b Reverse | CCCATGCAGTTCCTCTTACC |
| <i>CYP1A1</i> Exon3 Forward | GACCAGACCTGGATGGAGAG |
| <i>CYP1A1</i> Exon3 Reverse | TGACTGTGTCAAACCCTGGA |
| <i>CYP1A1</i> Exon4 Forward | TGTGTCCTTCCTGTGCTCAA |
| <i>CYP1A1</i> Exon4 Reverse | AACACAGGGACAAGATGGATG |
| <i>CYP1A1</i> Exon5 Forward | AGGTAGTGGCTCCCTTCAA |
| <i>CYP1A1</i> Exon5 Reverse | TGTCCCTCCCCTAACCTA |
| <i>CYP1A1</i> Exon6 Forward | GACACGGCATGGGAGACA |
| <i>CYP1A1</i> Exon6 Reverse | ATGGACAGGAGGATCAATGC |
| <i>CYP1A1</i> Exon7a Forward | GCATTGATCCTCCTGTCCAT |
| <i>CYP1A1</i> Exon7a Reverse | CAGAGGCAAGTCCAGGGTAG |
| <i>CYP1A1</i> Exon7b Forward | TGTCTACCTGGTCTGGTTGG |
| <i>CYP1A1</i> Exon7b Reverse | CCTCCAGGACAGCAATAAGG |
| <i>CYP1A1</i> Exon7c Forward | CTGCCAAGAGTGAAGGGAAG |
| <i>CYP1A1</i> Exon7c Reverse | AACACAGAATGGGGTTCAGG |

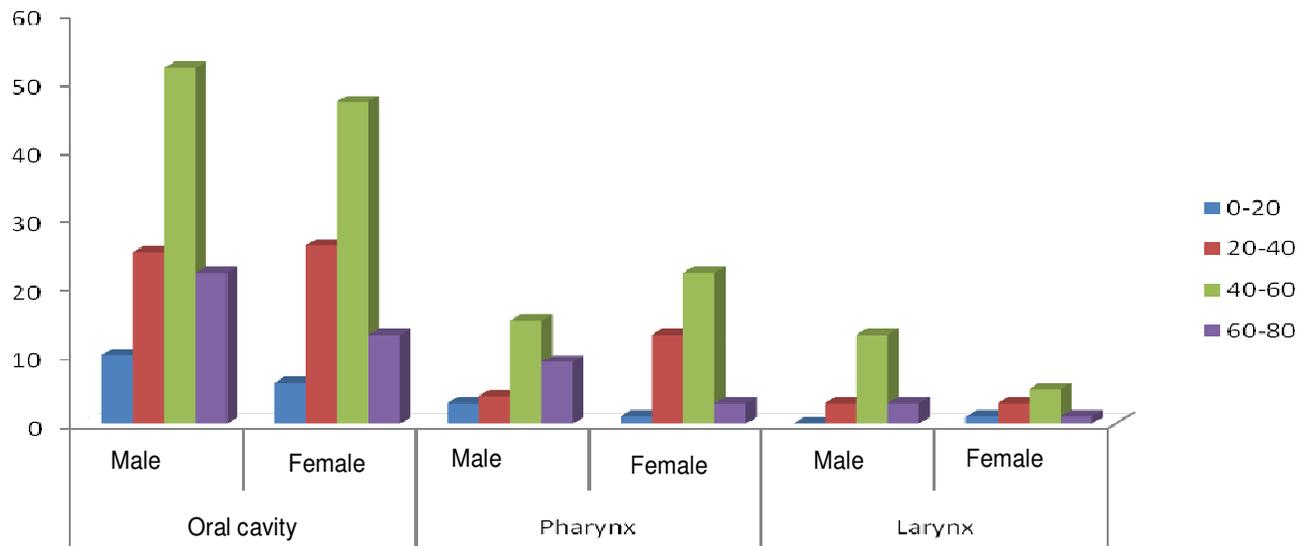


Figure 1. Frequency of head and neck cancer cases with reference to age bands, area of cancer and sex.

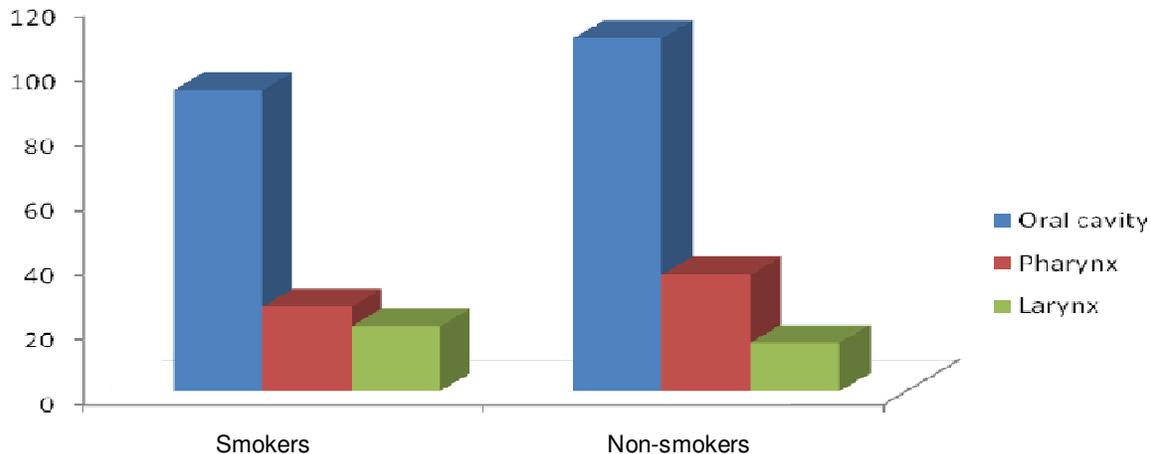


Figure 2. Comparison of different areas of cancer with respect to smoking status in head and neck cancer patients.

variations, with respect to gender were observed ($P > 0.05$) and male to female ratio was found to be 1:1 (Figure 1).

This study shows that persons using any form of tobacco (cigarettes, cigars, bidhi, moist snuff) and betel nuts are more affected by HNC ($P < 0.05$) compared with nontobacco users (Figure 2). In tobacco addicted patients, highest number of oral cancer cases were present (66%) compared with pharyngeal (24%) and laryngeal (10%) cancers.

In jobless persons, a statistically significant increase ($P < 0.01$) in incidence of head and neck cancer was found compared with housewives, businessman and labors. A lower incidence of head and neck cancer was observed in businessman and office employers (14%) when compared with labors (17%) and housewives (20%) shown in Figure 3.

Figure 4 shows that, no polymorphism in either of the reported variants *CYP1A1*2*, *CYP1A1*3*, *CYP1A1*4* and *CYP1A1*5* has been observed in this study consisting of 388 head and neck cancer cases and 150 controls. A novel substitution mutation of A_{2842} with C (Figure 5 and Table 2) was observed in 21 patients ($P < 0.001$, odd ratio (OR) = 9.4 and 95% confidence interval (CI) 1.3 to 70.8), whereas no control showed this mutation in exon 2. This A_{2842} to C mutation causes a change in DNA sequence from TAC to TCC and resulting UCC which codes serine, whereas wild type UAC codes for tyrosine. This tyrosine to serine mutation is in the conserved P450 domain and not in the transmembrane domain. In 21 patients with *CYP1A1* mutation in 2nd exon, 62% are female (OR 1.6, 95% CI 0.09 to 29.8) and 38% are males (OR 0.6, 95% CI 0.03 to 11.3), while 76% have cancer of oral cavity, 14% of pharynx and 10% of laryngeal cancer (OR 3.2, 0.17 and 0.11, respectively). The mean age of patients, showing A to C substitution mutation, was 51 ± 15.7 years. This mutation was present in more patients with a job (OR 0.75, 95% CI 0.04 to 13.7) when

compared with jobless (OR 0.62, 95% CI 0.03 to 11.3) and house wives (OR 0.2, 95% CI 0.01 to 4.62).

DISCUSSION

The main aim of this case control study was to evaluate the key risk factors for head and neck cancer (HNC). The current study clearly demonstrates that, environmental factors along with genetic polymorphism of *CYP1A1* gene are the main cause of head and neck cancer. Oral cavity cancer is the most prevalent area of head and neck cancer followed by pharynx and then larynx in our data. These findings are in accordance with the previous findings by Llewellyn et al. (2004), Shiboski et al. (2000) and Ping et al. (2007) differing only in increased incidence of HNC. In Americans and Caucasians, incidence of oral cancer has increased in the past few years; however, pharyngeal cancer is not common in most of the world populations (Parkin et al., 2002).

The mean age of our study patients was 48 years and highest HNC cases were observed in age group of 40 to 60 years. The age is an uncontrollable variable with the increase in age particularly between 40 to 60 years the risk of head and neck cancer is increased (Shanmugaratnam et al., 1982). This study reveals that, age more than 45 years should be considered as a risk factor for HNC development as found in earlier studies (Llewellyn et al., 2004) and once HNC is developed, the survival, particularly >80 years of age, is reduced when compared with patients <65 years age (Clayman et al., 1998). Mean age of pharyngeal cancer patients in South East Asia is lower compared with Western countries (Yu and Yuan, 2002).

In the present study, 50% were males and equal females. This shows that the males and females are equally affected by head and neck cancer. This finding is in contrast with the findings from earlier studies which

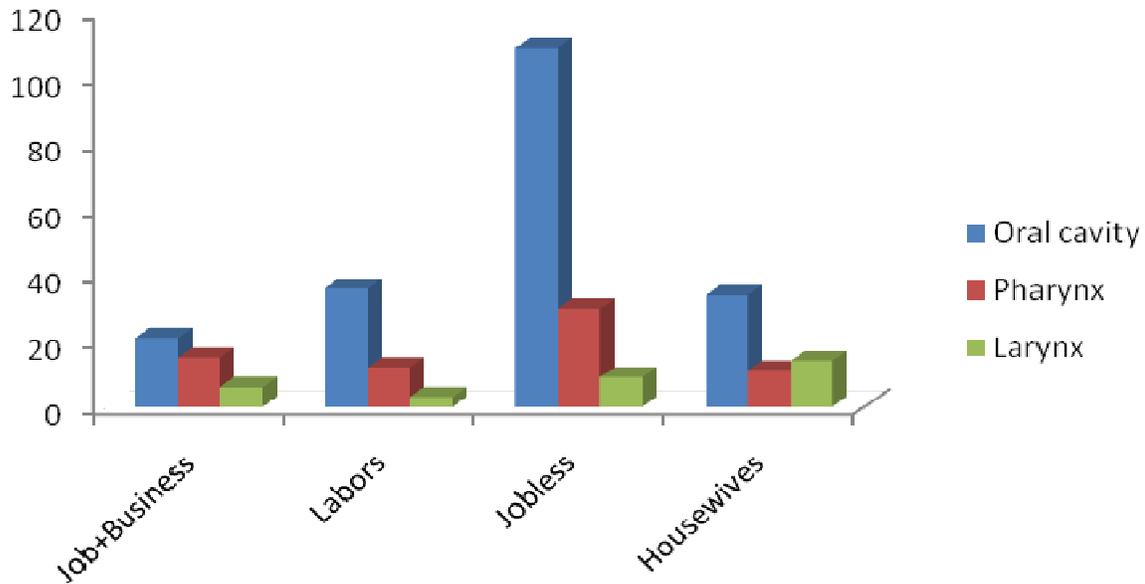


Figure 3. Comparison of number of patients with respect to area of cancer and occupation.

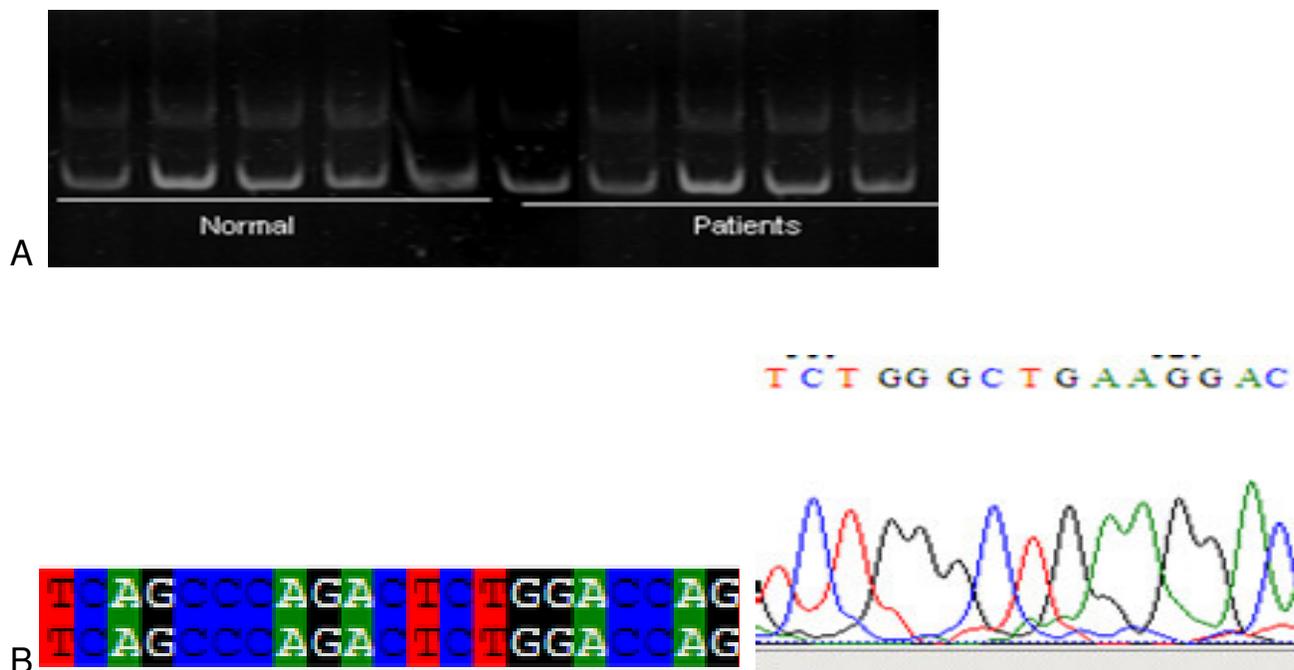


Figure 4. (A) No mobility shift in exon 7 *CYP1A1* gene of patients and normal controls in ethidium bromide stained PAGE; (B) no difference in sequencing results (upper sequence) compared with original sequence of *CYP1A1* gene exon 7 using

reported a 2:1 male to female ratio (Toefil et al., 2007) and that of Abdulmir et al. (2008) who found no association of age with sex ratio and areas of head and neck cancer in Asian patients.

The smoking status is always expected to be a key risk factor in development of cancer of any origin particularly HNC. In this study, 63.79% were smokers and 36.21% were non-smokers. The P value with Fishers exact test is

($P < 0.05$) which show that incidence of HNC in smokers is approximately two fold compared with non-smokers. Such results have frequently been reported in other parts of the world (Jun et al., 2010; Hecht et al., 1993). A study in the USA have shown that, heavy smokers die due to carcinoma of larynx 32 folds earlier than age-adjusted non-smokers and of oral cavity cancer, more than 24 folds earlier compared with non-smokers (Berrino and

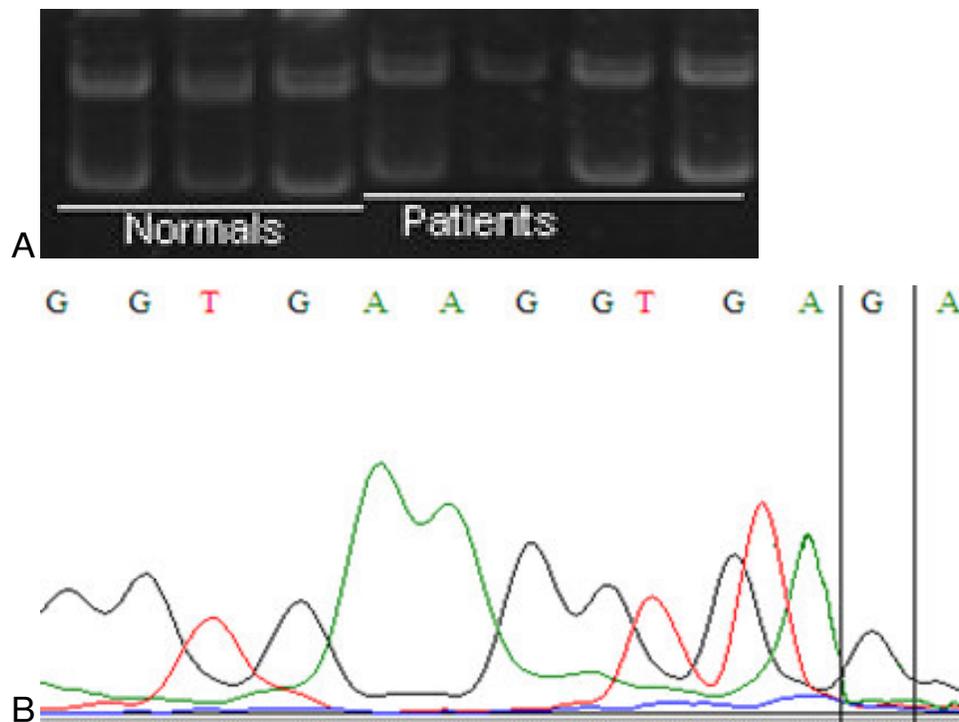


Figure 5. (A) Difference in mobility of exon 2, *CYP1A1* gene, between patients and normal controls in ethidium bromide stained PAGE; (B) G instead of T in sequencing compared with original sequence of *CYP1A1* gene exon 2 using BioEdit software v 0.7.0.

Table 2. Statistical data of patients having *CYP1A1* exon 2 mutation at aminoacid 110.

| Variable | A to C mutation | OR (CI 95%) | P value |
|----------------------|-----------------|--------------------------|--------------|
| Total patient | 21 | 9.4 (1.3 to 70.8) | 0.007 |
| Gender | | | |
| Female | 62% | 1.6 (0.09- 29.8) | 0.4 |
| Male | 38% | 0.6 (0.03-11.3) | 0.4 |
| Age | | | |
| Mean | 51.75 | (±15.7) | |
| <48 | 52% | 1.1 (0.06- 20.0) | 1 |
| >48 | 48% | 0.9 (0.05- 16.5) | 1 |
| Smoking | | | |
| Yes | 62% | 1.6 (0.09- 29.8) | 0.4 |
| No | 38% | 0.6 (0.03-11.3) | 0.4 |
| Occupation | | | |
| Job | 9 | 0.75 (0.04- 13.7) | 1 |
| Jobless | 8 | 0.62 (0.03- 11.3) | 1 |
| house wives | 4 | 0.2 (0.01- 4.62) | 1 |
| Area of cancer | | | |
| Oral Cavity | 76% | 3.2 (0.17- 61.02) | 0.03 |
| Pharynx | 14% | 0.17 (0.01- 3.4) | 1 |
| Larynx | 10% | 0.11 (0.005- 2.4) | 1 |

Gatta, 1998). These findings are also in agreement with earlier studies (Eaden et al., 2000; Lee et al., 2004; Llewellyn et al., 2004) in different populations where it has been established that, smoking plays a significant role in pathogenesis of HNC.

Interestingly, HNC is more common in jobless individuals due to increased tobacco addictions and mental stress when compared with on job individuals. This might be the reason that, house wives have least percentage of head and neck cancer (Abdulmir et al., 2008). Increased incidence of HNC in jobless individuals may also be attributed to increased mental stress (Jensen et al., 2010) as in Pakistan it has increased significantly (Pope et al., 1983; Bhugra, 2004). The results suggested that, the occurrence of head and neck cancer (HNC) is related to age, smoking and the occupation status.

CYP1A1 is involved in the activation of many polycyclic aromatic hydrocarbon and aromatic amines by enzyme aryl hydrocarbon hydrolase (Bartsch et al., 2000). In the present case-control study, none of the already reported mutations were observed. However, a novel mutation in exon 2 of *CYP1A1* gene was observed which causes tyrosine to change in serine at amino acid number 110 of *CYP1A1* gene. Tyrosine to serine substitution mutation causes a change in conserved domain of cytochrome P450. This mutation cause, a change in the protein structure as an aromatic amino acid is changed into a non aromatic amino acid and subsequently, gene function is also altered. Aside from being a proteogenic amino acid, tyrosine has a special role by virtue of the phenol functionality. It occurs in proteins that are part of signal transduction processes. Tyrosine is a precursor to neurotransmitters and increases plasma neurotransmitter levels (particularly dopamine and norepinephrine). Therefore, this mutation might lead to imbalanced functional activity in detoxification process as *CYP1A1* is a key enzyme that converts PAHs into active carcinogens (Hecht et al., 1993; Bartsch et al., 2000). PAHs present in tobacco smoke activate transcription of the *CYP1A1* gene and increase pulmonary *CYP1A1* activity several fold (Omiecinski et al., 1990). Mutated *CYP1A1* gene can not convert carcinogen into a hydrophilized form required for phase II enzyme activation (glutathione-S-transferase isozymes GSTM1, GSTP1 and GSTT1) for the process of detoxification.

The current mutation of *CYP1A1* gene along with environmental factors may be one of the several factors causing head and neck cancer. Polymorphisms in *CYP1A1* gene and alterations in their expression and function, may increase or decrease carcinogen activation/detoxification followed by a variation of cancer risk (Shen et al., 2002; Jourenkova et al., 1998; Olshan et al., 2000; Lea et al., 2007; Matullo et al., 2001; Park et al., 2002). However, the ultimate phenotypic effect reflects a complicated network of interactions between genes and environmental factors. This study focuses on only one gene *CYP1A1* with environmental factors in HNC; how-

ever, determining the role of genetic polymorphisms as cancer risk factor would require studies aiming at the integrated analysis of many genes involved in cancer development.

ACKNOWLEDGEMENTS

All authors would like to acknowledge the patients and normal individual who contributed in this research work and also the hospital and staff for their cooperation. We would like to thank COMSATS and HEC for financial assistance.

REFERENCES

- Abdulmir AS, Hafidh RR, Abdulmuhamen N, Abubkar F, Abbas KA (2008). The distinctive profile of risk factors of nasopharyngeal carcinoma in comparison with other head and neck cancer types. *BMC Public Health*. 8: p. 400.
- Amalio T, Paul I, Francine M, Tobias S, Thomas B (1993). Direct, automated detection of rifampin-resistant mycobacterium tuberculosis by polymerase chain reaction and single-strand conformation polymorphism analysis. *Antimicrob. Agents chemother.* 37(10): 2054-2058.
- Anttila S, Vainio H, Hietanen E, Camus AM, Malaveille C, Brun G (1992). Immunohistochemical detection of pulmonary cytochrome P-4501A and metabolic activities associated with P4501A1 and P4501A2 isozymes in lung cancer patients. *Environ. Health Perspect.* 98: 179-182.
- Arrind P, Singh A, Parag P, Shah A, Neeraj M, Jeroen TM (2008). Genetic polymorphisms in Cytochrome P4501B1 and susceptibility to Head and Neck Cancer. *Mutat. Res.* 639(1-2): 11-19.
- Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K (2000). Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol. Bio. Prev.* 9: 3-28.
- Baumgartner-Parzer S, Schulze E, Waldhäusl W, Pauschenwein S, Rondot S, Nowotny P (2001). Mutational spectrum of the steroid 21-hydroxylase gene in Austria: identification of a novel missense mutation. *J. Clin. Endocrinol. Metab.* 86: 4771-75.
- Berrino F, Gatta G (1998). Variation in survival of patients with head and neck cancer in Europe by the site of origin of the tumours. *Eur. J. Cancer*, 34: 2154-2161.
- Bhugra D (2004). Migration and mental health. *Acta. Psychiatr. Scand.* 109: 243-258.
- Cascorbi I, Brockmoller J, Roots I (1996). A C4887A polymorphisms in exon 7 of human CYP1A1: Population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res.* 56: 4965-4969.
- Clayman GL, Eicher SA, Sicard MW, Razmpa E, Goepfert H (1998). Surgical outcomes in head and neck cancer patients 80 years of age and older. *Head Neck.* 20(3): 216-223.
- Crofts F, Cosmo GN, Taioli E, Currie D, Toniolo P, Garte SJ (1993). A novel CYP1A1 gene polymorphism in African-Americans. *Carcinogenesis*, 14: 1729-1731.
- Eaden J, Abrams K, Ekbohm A, Jackson E, Mayberry J (2000). Colorectal cancer prevention in ulcerative colitis: a case-control study. *Alimentary Pharmacol. Therap.* 14(2): 145-153.
- Faheem A (2007). Cancer registration in Pakistan: contemporary state of affairs. *Asia. Pac. J. Cancer Prev.* 8: 452-456.
- Guengerich FP, Shimada T (1991). Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.* 4: 391-407.
- Hanif M, Zaidi P, Kamal S, Hameed A (2009). Institution-based cancer incidence in a local population in Pakistan: nine year data analysis. *Asia. Pac. J. Cancer Prev.* 10(2): 227-230.
- Hayashi SI, Watanabe J, Nakachi K, Kawajiri K (1991). Genetic linkage

- of lung cancer associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P-450 gene. *J. Biochem.* 110: 407-411.
- Hecht SS, Carmella SG, Murphy SE, Foiles PG, Chung FL (1993). Carcinogen biomarkers related to smoking and upper aerodigestive tract cancer. *J. Cell. Biochem.* 17: 27-35.
- Jensen CA, Hu JJ, Case LD, Browne JD, Gilbert J, Metzner-Sidurski J, Franzmann E, Frizzell B, Schneider C, Shaw EG (2010). Stress and depression in head and neck cancer patients by primary surgery versus primary radiotherapy treatment modality. *J. Clin. Oncol.* 28: p. 15.
- Johnson NW (1991). A global view of the epidemiology of oral cancer, in risk markers for oral diseases. Cambridge: University Press, pp. 3-27.
- Jourenkova N, Reinikainen M, Bouchardy C, Dayer P, Benhamou S, Hirvonen A (1998). Larynx cancer risk in relation to glutathione S-transferase M1 and T1 genotypes and tobacco smoking. *Cancer. Epidemiol. Bio. Prev.* 7: 19-23.
- Jun T, Ming Y, Xin N, Dianke Y, Jugao F, Wen T (2010). Genetic polymorphisms in cytochrome P450 genes are associated with an increased risk of squamous cell carcinoma of the larynx and hypopharynx in a Chinese population. *Cancer genet. cytogenet.* 196: 76- 82.
- Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinoda N, Watanabe J (1990). Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P-450 1A1 gene. *FEBS. Lett.* 263: 131-133.
- Lea IA, Jackson MA, Li X, Bailey S, Peddada SD, Dunnick JK (2007). Genetic pathways and mutation profiles of human cancers: site and exposure-specific patterns. *Carcinogenesis*, 28(9): 1851-1858.
- Lee SO, Kim NJ, Choi SH, Kim TH, Chung JW, Woo JH (2004). Risk factors for acquisition of imipenem-resistant *Acinetobacter Baumannii*: a case-control study. *Antimicrob. Agents. Chemother.* 48(1): 224-28.
- Llewellyn CD, Jhonson LW, Warnakulasuriya KA (2004). Risk factors for oral cancer in newly diagnosed patients aged 45 years and younger: a case-control study in Southern England. *J. Oral. Pathol. Med.* 33(9): 525-532.
- Makimoto K, Oda H, Higuchi S (2000). Is heavy alcohol consumption an attributable risk factor for cancer-related deaths among Japanese men? *Alcohol. Clin. Exp. Res.* 24: 382-385.
- Matullo G, Palli D, Peluso M, Guarrera S, Carturan S, Celentano E (2001). XRCC1, XRCC3, XPD gene polymorphisms, smoking and 32P-DNA adducts in a sample of healthy subjects. *Carcinogenesis*, 22: 1437-1445.
- Morelato RA, Lopez SA (2006). Oral cancer mortality in the province of Cordoba, Argentine republic in the period 1975- 2000. A comparative study with other populations. *Med. Oral. Patol. Oral. Cir. bucal.* 11: 230-235.
- Olishan A, Weissler M, Watson MA, Bell D (2000). GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use and risk of head and neck cancers. *Cancer. Epidemiol. Bio. Prev.* 9: 185-191.
- Omiecinski CJ, Redlich CA, Costa P (1990). Induction and developmental expression of cytochrome P4501A1 messenger RNA in rat and human tissues: detection by the polymerase chain reaction. *Cancer Res.* 50: 4315-4321.
- Park J, Lee S, Jeon H, Bae N, Chae S, Joo S (2002). Polymorphism of the DNA repair gene XRCC1 and risk of primary lung cancer. *Cancer Epidemiol. Bio. Prev.* 11: 23-27.
- Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB (2002). Cancer incidence in five continents, vol. VIII. IARC scientific publications No. 155 Lyon: IARC.
- Patricia S, Melissa M, Robert HG, Lizeth P, Luz C, Mirko JZ (2009). Sputum PCR-single-strand conformational polymorphism test for same-day detection of pyrazinamide resistance in tuberculosis patients. *Clin. Microbiol.* 47(9): 2937-2943.
- Ping HC, Tien YS, Pei SH, Chi CT, Yi HY, Ying CL (2007). Prognostic factors associated with the survival of oral and pharyngeal carcinoma in Taiwan. *BMC Cancer*, 7: p. 101.
- Pope HG, Ionescu-Pioggia M, Yurgelun-Todd D (1983). Migration and manic-depressive illness. *Compr. Psychiatry.* 24: 158-65.
- Rajani B, Aparna K, Shama B (2003). Polymorphism at CYP and GST gene loci and susceptibility to tobacco related cancers. *Proc. Indian. Natn. Sci. Acad.* 69(1): 35-48.
- Sabitha K, Vishnuvardhan MR, Kaiser J (2010). Smoking related risk involved in individuals carrying genetic variants of CYP1A1 gene in head and neck cancer. *Cancer Epidemiol.* 34(5): 587-592.
- Sankaranarayanan R, Masuyer E, Swaminathan R (1998). Head and neck cancer: a global perspective on epidemiology and prognosis. *Anticancer Res.* 18: 4779-4786.
- Shanmugaratnam K (1982). Nasopharynx. In: Schottenfeld D, Fraumeni Jr. JF, editors. *Cancer epidemiology and prevention*. Philadelphia: W.B. Saunders Company. pp. 536-553.
- Shen H, Sturgis E, Dahlstrom K, Zheng Y, Spitz M, Wei Q (2002). A variant of the DNA repair gene XRCC3 and risk of squamous cell carcinoma of the head and neck: a case-control analysis. *Int. J. Cancer.* 99: 869-872.
- Shiboski CH, Shiboski SC, Silverman S (2000). Trends in oral cancer rates in the United States, 1973-1996. *Community. Dent. Oral. Epidemiol.* 28: 249-256.
- Shimada T, Martin MV, Pruess-Schwartz D, Marnett LJ, Guengerich FP (1989). Roles of individual cytochrome P-450 enzymes in the bioactivation of benzo(a)pyrene, 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Res.* 49: 6304-6312.
- Tevfik P, Recep A, Arslan T, Kadri A, Mustafa C (2007). The relationship between occupations and head and neck cancers. *J. Natl. Med. Assoc.* 99(1): 68-71.
- Toefil L, Oana CT, Horea AA, Ovidiu M (2007). Head and neck cancer, epidemiology and histological aspects-Part 1: A decade's results 1993- 2002. *J. Cranio. Maxill. Surg.* 35(2): 120-125.
- Vierhapper H, Bieglmayer C, Heinze G, Baumgartner-Parzer SM (2004). Frequency of RET protooncogene mutations in patients with normal and with moderately elevated (50-100 pg/ml) pentagastrin-stimulated serum concentrations of calcitonin. *Thyroid.* 14: 580-583.
- Yu MC, Yuan JM (2002). Epidemiology of nasopharyngeal carcinoma. *Sem Cancer Biol.* 12: 421-429.