RESEARCH ARTICLE

Gene-gene and gene-environment interaction: An important predictor of oral cancer among smokeless tobacco users in Karachi

Muhammad Mohiuddin Alamgir[1], Qamar Jamal[2], Talat Mirza[3]

Abstract

Objective: To determine the risk for oral cancer caused by simultaneous occurrence of more than one of the tested cytochrome P450 CYP1A1 MspI, glutathione S-transferase M1 null gnd Glutathione S-transferases T1 null gene polymorphisms.

Method: The cross-sectional case-control study was conducted from December 2011 to October 2016 at the Ziauddin University, Karachi, in collaboration with Dow University of Health Sciences, Karachi, and comprised oral squamous cell carcinoma cases in group A and healthy tobacco habit-matched controls in group B. All investigations were done using standardised laboratory protocols. The outcomes were determined in terms of association of various combinations of cytochrome P450 1A1MspI, glutathione S-transferasesM1 null and glutathione S-transferases T1 null polymorphisms with oral cancer. Data was analysed using SPSS 20.

Results: Of the 238 subjects, 140(58.8%) were in group A and 98(41.2%) were in group B. Mean ages in group A and B were 47.1±12.22 and 41.6±14.58 years, respectively. Male/Female ratio in group A was 1.88:1 while 83.4% were using tobacco. When cytochrome P450 1A1MspI homozygous (m2/m2) and glutathione S-transferases M1 null variants occured simultaneously in an individual, an odds ratio of 12.8 (95% confidence interval: 1.20-135.5; p=0.03) among overall tobacco chewers was observed. For glutathione S-transferases M1 not null and glutathione S-transferases T1 null variant combination among overall tobacco users, the conferred odds ratio was 4.58 (95% confidence interval: 0.99-21.2; p=0.05). The other studied gene combinations did not reveal significant associations (p>0.05).

Conclusion: A higher risk of oral squamous cell carcinoma was found to be associated with combined-gene polymorphisms of phase I and phase II enzymes than that attributed to a single-gene polymorphism.

Keywords: Gene-polymorphisms, Tobacco indices, Odds ratios, Oral squamous cell carcinoma. (JPMA 72: 477; 2022)

DOI: https://doi.org/10.47391/JPMA.1806

Introduction

There are naturally-occurring chemical substances called xenobiotics. These include environmental agents, naturally-occurring compounds/drugs and carcinogens. Our biological system perceives these as foreign.[1,2] In fact, the human biological system is continuously exposed to xenobiotics contained in food, tobacco, alcoholic beverages, coffee, tea and smoke from burning wood etc. Xenobiotic intermediate metabolites once generated exert adverse effects via covalent interactions with genetic material or proteins.[3] Free radical mediated carcinogenesis is another mechanism related to many xenobiotics, including tobacco.[4] Catabolism of carcinogenic moieties generated by xenobiotic metabolising enzymes (XMEs) can be categorised into phase I and phase II reactions.[5] The coordinated expression and regulation of these XMEs determines the outcome of carcinogen exposure. Phase I biotransformation is the function of many enzyme systems. Among these, cytochrome P450 1A1 (CYP1A1) plays a vital role in the activation of polycyclic aromatic hydrocarbons (PAHs) to convert them to carcinogens. Enzymes coded by CYP1A1 gene are involved in the activation of pro-tobacco carcinogens in several human tissues.[6] Polymorphisms of CYP1A1 gene may result in increased enzyme activity which appear to play a pivotal role in deoxyribonucleic acid (DNA) adduct formation and cancer risk.[7] Phase II reactions comprise a wide range of reactions that either detoxify harmful compounds or facilitate their excretion from the body by making them more hydrophilic.[8] An important effect of phase II reactions is to conjugate these reactive moieties with endogenous molecules through reactions, such as glucouronidation, sulfation, methylation, acetylation, glutathione conjugation and amino acid conjugation.[9] This is the function of several enzyme families connected to phase II reactions, including Glutathione S-transferases (GSTs), Uridine 5'-diphospho-glucuronosyltransferase (UGTs), N-acetyltransferases (NATs), sulfotransferases (STs) and various methyltransferases.[1] GSTs are phase II enzymes responsible for detoxification of phase I-derived substrates via conjugation. Null genotypes for GSTs results in reduced detoxification and increases susceptibility to cancers.[10]

Tobacco, a xenobiotic, has been found to contain 30 known carcinogens in its various products. Three main classes

[1]Department of Pathology, Dow University of Health Sciences, Karachi, Pakistan; [2,3]Department of Pathology, Ziauddin University, Karachi, Pakistan.

Correspondence: Muhammad Mohiuddin Alamgir.
e-mail: mohiuddin.alamgir@duhs.edu.pk

include tobacco specific nitrosamines (TSNAs), PAHs and aromatic amines.[11] The individual ability to properly cope with xenobiotic stress can influence susceptibility to diseases. By virtue of their genotype for XMEs involved in carcinogen metabolism, certain individuals are more susceptible to cancer formation when exposed to these compounds.[8,12] This could probably be the reason behind the fact that only a small number of persons among those addicted to xenobiotics, like tobacco or alcohol, develop cancer in their lifetime.[13]

In humans there has been a variable expression profile for XMEs, as dictated by polymorphisms of their respective genes, hence, altering the cancer risk posed by tobacco-related carcinogens. Furthermore, studies have suggested that the presence of combined gene polymorphisms of phase I and phase II enzymes in an individual enhances the risk of cancer formation than that ascribed to single-gene polymorphism. These studies have shown the interaction of combined genotypes of carcinogen-metabolising genes with environmental factors, like tobacco, in modulating susceptibility to nasopharyngeal cancer formation.[5,14] To date, limited number of studies have analysed combined gene effects of all the investigated genes, i.e. CYP1A1MspI, GSTM1 and GSTT1 for oral carcinogenesis.[7,9,11]

A substantial proportion of Karachi's population comprises offspring of individuals who migrated from India. A case-control study done on Indian population showed that simultaneous presence of CYP1A1 homozygous variant with GSTM1 null genotype upgraded oral cancer risk by 1.6 fold.[9] A meta-analysis observed a combined effect for GSTM1 homozygous deletion and CYP1A1 heterozyugous genotypes on cancer risk.[15] A study on Pashtun subjects of Khyber Pakhtunkhwa (KP) province of Pakistan reported a weak and non-significant association of CYP1A1 polymorphism with oral squamous cell carcinoma (OSCC). However, when considered in combination with GSTM1 and GSTT1 gene alleles, a 16-fold increased risk of oral cancer compared to the controls was observed in the presence of other confounding risk factors.[11]

Although interactions between genotype and environment exposures have long been studied, the subject has assumed great significance for Pakistan in general and Karachi in particular because of the widespread tobacco use among its citizens and rising prevalence of oral cancer and pre-cancerous lesions. The current study was planned to probe the role played by molecular mechanisms in OSCC carcinogenesis in the target population.

Subjects and Methods

The cross-sectional case-control study was conducted at the Ziauddin University (ZU), Karachi, from December 2011 to October 2016 in collaboration with Dow University of Health Sciences (DUHS), Karachi. After approval from the ethics review boards of ZU and DUHS, the sample size was calculated using Epi-Info 6.0 with 95% confidence level, 10% precision and design effect 2, while keeping prevalence data mentioned in literature.[9,16] The sample was raised using non-probability purposive sampling technique in which researchers were able to choose the most appropriate cases in terms of case information and research material from the target group of OSCC and Precancerous lesions (PCL) cases, and habit-matched controls. The subjects were enrolled from the ZU Oncology Department, North Nazimabad, Karachi, the Otolaryngology Ward of Civil Hospital, Karachi (CHK), Dr. Ishratul Ebad Khan Institute of Oral Health Sciences (DIKIOHS) at the DUHS, and by setting up camps in different localities to enrol controls.

For both cases and controls, subjects, the subjects had to be aged >10 years. For the controls, there had to be no prior history of any cancer, including oral cancer, and the tobacco habit had to approximately match in duration and frequency those of the cases. Individuals with cancer of any site other than oral cavity, with any other serious disease, aged <10 years and those who did not give consent were excluded.

For the purpose of analysing the association between the nature of tobacco exposure and gene polymorphisms, the subjects were further divided into different tobacco-habit groups as exclusive chewers, exclusive smokers, mixed tobacco habitués, individuals who consumed tobacco as smokers as well as in the smokeless form, and habit-free individuals who reported a lack of former or current use of tobacco in any form. Lifetime tobacco exposure was calculated as chewing and smoking index among all tobacco users for both the cases and the controls as described in a study.[9]

Interactions between different combinations of three tested polymorphisms and various tobacco exposures were analysed based on the assumption that certain genotype combinations can insert a synergistic action on the risk to OSCC. Enhanced carcinogen potentiation by phase I enzymes with simultaneous reduction of detoxification by phase II enzymes can translate into increased risk than that caused by polymorphism of an isolated gene.[17] The same hypothesis was evaluated in the current study as regards to the type and quantity of tobacco used. The gene combinations analysed included four possible scenarios; CYP1A1 homozygous (m2/m2) and GSTM1 null, either CYP1A1 homozygous (m2/m2) or GSTM1 null, GSTM1 null and GSTT1 not null, and, finally, GSTM1 not null and GSTT1 null.

After getting informed consent from the subjects, 5ml venous blood samples were collected into sterile vacutainer tubes containing ethylenediaminetetra acetic acid (EDTA). Genomic DNA was extracted using the kit-method (PureLink® Genomic DNA Kits for DNA purification; Invitrogen Life Technologies, Carlsbad, United States. Cat. # K182001) from whole blood-lymphocytes samples and was stored at -80^{0}Celsius.

Polymorphisms of target genes were then tested.

For CYP1A1MspI polymorphism (gene location 15q24.1), a 340bp fragment of exon-7 of CYPIAI containing the polymorphic region was amplified by using polymerase chain reaction (PCR) as described previously.[7]

Primers sequence was:
Forward 5′-CAGTGAAGAGGTGTAGCCGCT-3′, and
Reverse 5′-TCCGTACTCTGTTCTGAGGATT-3′.

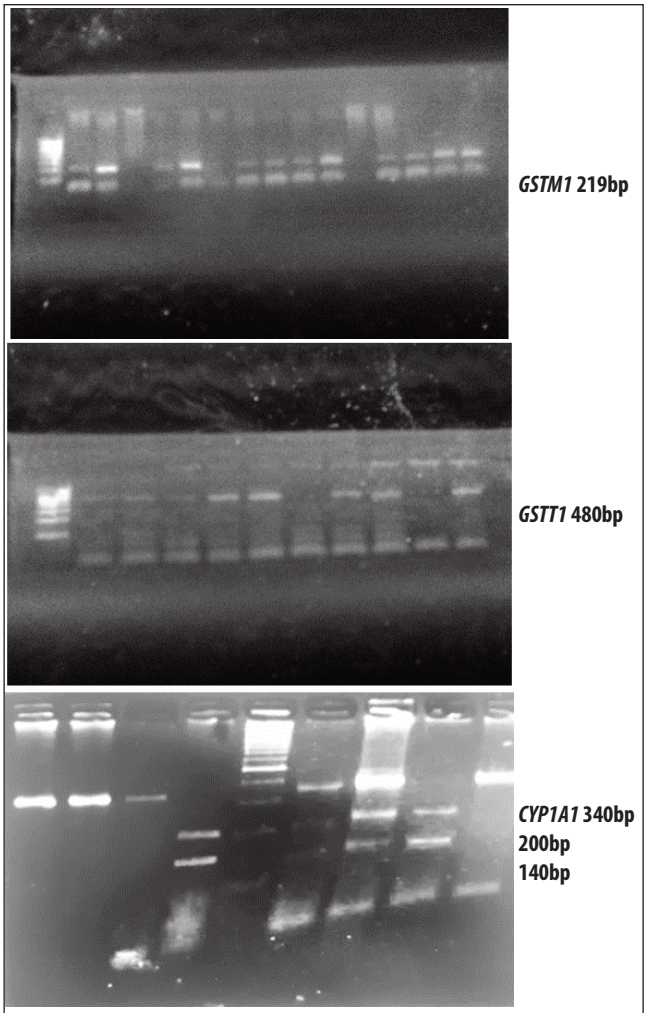

Figure-: Gel electrophoresis images for tested genes (current study and as reviewed from literature)..

The PCR product was digested with MspI (Hpall, Thermo Scientific) restriction enzyme for 12hrs at 37^{o}C.7 After digestion, the CYP1A1 amplified products were loaded on agarose gel stained with ethidium bromide and subjected to ultraviolet (UV) analysis. Presence of MspI restriction site resulted in splicing of the original 340bp CYP1A1 fragment into two 200bp and 140bp fragments (Figure). On the gel each of the three polymorphisms were identified as wild type (m1/m1) → only one 340bp band; herozygous variant (m1/m2) → three bands of 340bp, 200bp and 140pb, respectively; and homozygous variant (m2/m2) → only two bands of 200bp and 140bp.

For GSTM1 null polymorphism (gene location 1p13.3). the GSTM1 deletion polymorphism was identified by amplification of a 219bp fragment with specific primers as described previously.[7]

Primer sequence was:
Forward 5′-GAACTCCCTGAAAAGCTAAAGC-3′; and
Reverse 5′-GTTGGGCTCAAATATACGGTGG-3′.

For GSTT1 null polymorphism (gene location 22q11.23), the GSTT1 deletion polymorphism was identified by amplification of 480bp fragment with specific primers as described previously.[7,17]

Primer sequence was:
Forward 5′-TTCCTTACTGGTCCTCACATCTC-3′; and
Reverse 5′-TCACCGGATCATGGCCAGCA -3′.

After PCR amplification, the products of GSTM1 and GSTT1 were loaded on agarose gel stained with ethidium bromide for UV analysis. The presence or absence of a band at specified region corresponded to the presence or absence of GSTM1 and GSTT1.

Statistical analysis was done using SPSS 20. Genotype variants of CYP1A1, GSTM1 and GSTT1 were distributed among different habit groups and results were expressed as frequency and percentage of total number of cases from each category. The risk of oral cancer due to the studied genes was determined by binary logistic regression model with CYP1A1MspI wild type (m1/m1), GSTM1 not null and GSTT1 not null considered as the reference category. Odds ratios (ORs) were calculated while the precision of ORs was adjusted by 95% confidence interval (CI). Multi-variant analyses were performed to understand gene-environment interactions. Chi square test was applied for the determination of significance. The significance level was set as $p<0.05$.

Results

Of the 238 subjects, 140(58.8%) were the cases in group A and 98(41.2%) were controls in group B. In group A (OSCC

cases) age ranged from 20-78 years, mean age was 47.1 ±12.22 years while for group B (controls) age ranged from 15 to 87 years, mean age being 41.6±14.58 years. In group A, 65.3% were males and 34.7% were females. The male to female ratio was 1.88:1. In group B, 74.1% were males and 25.9% were females. Tobacco habit was found to be present in 83.4% of oral cancer patients in group A while 100% of controls were consuming tobacco in one form or other.

An enhancement of risk was observed in individuals having the studied genotypes in conjunction and who were also in the habit of using tobacco. The effect was not only an additive, but also multiplicative, indicating that when CYP1A1MspI is the only polymorphism present in a subject, it does not pose significant risk to oral cancer when the individual is exposed to tobacco in any of its forms and the same is true for GSTM1 null genotype (Tables 1 and 2). However, when same genotypes, CYP1A1 homozygous (m2/m2) and GSTM1 null variants, happen to occur simultaneously in an individual, they carry a significant rise in the OR up to 12.8 (95% CI: 1.20-135.5; p=0.03) among overall tobacco chewers (Table 2). This OR is quite higher compared to when either of the genes is present alone; OR 2.36 (95% CI: 1.0-6.2) for CYP1A1MspI homozygous variant, and OR 0.95 (95% CI: 0.51-2.22) for GSTM1 null.

The subjects carrying GSTM1 null and GSTT1 not null gene variants revealed an OR of 1.49 (95% CI: 0.71-3.13) among

Table-1: Genotype variants in overall OSCC cases and controls with corresponding ORs.

Genotype	Cancer	Control	OR	CI (95%)	*p*-value
CYP1A1					
m1/m1	26	26			
m1/m2	88	61	1.44	(0.71-2.52)	0.26
m2/m2	26	11	2.36	(1.0-6.20)	0.05
GSTM1					
Not null	97	67			
Null	43	31	0.95	(0.51-2.22)	0.91
GSTT1					
Not null	123	96			
Null	17	02	6.63	(1.49-29.4)	0.01

OSCC: Oral squamous cell carcinoma, ORs: Odds ratios; n: number of cases.

Table-2: Odds Ratio (OR) analysis for *CYP1A1* m2/m2 & *GSTM1* genotype combinations.

Gene variant combinations	Tobacco exposure	Control	Cancer	OR	CI (95%)	p-value
CYP1A1 m2/m2 or ***GSTM1*** null	Chewers	19	38			
Reference:	Ref.	55	60	1.83	0.94-3.55	0.07
m1/m1	<median	8	21			
or	Ref.	25	28	2.34	0.88-6.22	0.08
GSTM1 not null	>median	11	18			
	Ref.	30	32	1.53	0.62-3.77	0.35
	Smokers	5	4			
	Ref.	4	7	0.45	0.07-2.76	0.40
	Mixed habits	7	8			
	Ref.	17	24	0.80	0.24-2.65	0.74
	No habit	6	9			
	Ref.	5	19	0.39	0.09-1.64	0.20
	Total	37	59			
	Ref.	81	110	1.17	0.71-1.93	0.54
CYP1A1 m2/m2 & ***GSTM1*** null	Chewers	1	8			
Reference:	Ref.	8	5	12.8	1.20-135.5	0.03
CYP1A1 m1/m1 or	<median	1	3			
GSTM1 not null	Ref.	6	4	1.5	0.33-60	0.25
	>median	0	5			
	Ref.	2	1		NR*	
	Smokers	3	1			
	Ref.	1	0		NR*	
	Mixed habits	0	1		NR*	
	Ref.	2	5			
	No habit	1	0		NR*	
	Ref.	1	3			
	Total	5	10	1.84	0.48-6.97	0.37
	Ref.	12	13			

*NR: Not represented, n: number of studied subjects, Ref.: Reference cases; CI: Confidence interval.

Table-3: Odds Ratio (OR) analysis conferred by *GSTM1* & *GSTT1* genotype combinations.

Gene variant combinations	Tobacco exposure	Control	Cancer	OR	CI (95%)	p-value
CYP1A1 m2/m2 or ***GSTM1*** null	Chewers	16	26			
Reference:	Ref.	45	49	1.49	0.71-3.13	0.29
GSTM1 not null &	< median	6	15			
GSTT1 not null	Ref.	25	23	2.7	0.90-8.18	0.07
	> median	10	11			
	Ref.	20	26	0.84	0.3-2.48	0.76
	Smokers	4	4			
	Ref.	4	3	1.33	0.17-10.25	0.79
	Mixed habits	6	2			
	Ref.	13	18	0.24	0.04-1.38	0.11
	No habit	0	6			
	Ref.	0	15			
	Total	26	38			
	Ref.	64	85	1.10	0.60-1.99	0.76
GSTM1 not-null & ***GSTT1*** null	Chewers	2	6			
Reference:	Ref.	45	49	2.75	0.52-14.3	
GSTM1 not null &	< median	1	3			
GSTT1 not null	Ref.	25	23	3.2	0.31-33.6	
	>median	1	3			
	Ref.	20	26	2.3	0.22-23.8	
	Smokers	0	1			
	Ref.	4	3		NR*	
	Mixed habits	0	4			
	Ref.	13	18		NR	
	No habit	0	1			
	Ref.	3	15		NR	
	Total	2	12			
	Ref.	65	85	4.58	0.99-21.2	0.05

*NR: Not represented, n: number of studied subjects, Ref.: Reference cases, CI:: Confidence interval.

exclusive chewers, OR 2.7 (95% CI: 0.90-8.18) among below-median chewers, and OR 1.33 (95% CI: 0.17-10.25) among the smokers (Table 3). There was also a trend towards protection as depicted by OR 0.84 (95% CI: 0.3-2.48) among above-median chewers and OR 0.24 (95% CI: 0.04-1.38) among mixed habit groups. However, all the above observations lacked statistical significance ($p>0.05$).

The next gene combination of GSTM1 not null and GSTT1 null variants conferred an OR of 2.75 (95% CI: 0.52-14.3) among chewers, OR 3.2 (95% CI: 0.31-33.6) among below-median chewers, and OR 2.3 (95% CI: 0.22-23.8) among above-median chewers. However, these associations also lacked statistical significance (p>0.05). Among smokers and mixed habit groups, ORs could not be calculated due to lack of representation. Overall, this gene combination conferred an OR of 4.58 (95% CI: 0.99-21.2) and these values were near the level of significance ($p=0.05$).

Discussion

The interaction of combined genotypes for carcinogen-metabolising genes with environmental factors. like tobacco, may modulate susceptibility of head and neck cancers. Studies analysed the effects of combination of CYP1A1 and GSTs genes polymorphisms and compared them with the results when the CYP1A1 polymorphism existed alone.[5,18,19] One similar study on the association of null genotypes and mutations of metabolic neutralising genes in the presence of environmental habits, such as tobacco smoking or chewing, eating smoked meat and fermented fishes, even suggested the presence of these genotypes to be used as a possible biomarker for early detection and preventive measures for nasopharyngeal carcinomas.[14] Studies on lung cancer evaluating alterations in the action of phase I and phase II enzymes due to the presence of combinations of CYP1A1, GSTM1 and GSTT1 also suggested a higher risk when individuals had polymorphisms in more than one of these genes.[5,20]

However, there is overall paucity of studies that have evaluated the effects of aforementioned gene combinations on OSCC. To date, relatively fewer studies[7,9,11] have analysed the risk posed by simultaneous occurrence of two or more of the mutations tested in the current study.

A study in India reported that the presence of either CYP1A1MspI or GSTM1 null variant enhanced chances of oral cancer 1.6-fold, while the same genes in combination increased the risk 7.42 fold in the above-median tobacco chewer category.[9] In northern India, head and neck cancer risk enhancement due to the combined effect of CYP1A1, GSTs genes polymorphisms and smoking and tobacco-betel quid chewing revealed several fold increased risk of these cancers.[7]

In the current study, gene interactions also displayed a pattern of risk association which was quite different from single-gene effects. The results support the notion that "combined gene variants can lead to increased risk by acting synergistically".[9]

The current findings are also in concurrence with a study conducted in KP province of Pakistan comprising subjects of Pashtun ethnicity who happen to be heavily engaged in the consumption of Naswar, which is a raw tobacco-containing smokeless tobacco product. The same study reported that patients with either GSTM1 or GSTT1 null genotypes had significantly higher risk of oral cancer, which further increased when either one or both null genes were present in combination. GSTM1 and GSTT1 showed a 3-fold independent association, while CYP1A1 was weakly involved. Combined effects of both GST and CYP1A1 genes further increased the association to 8-fold in patients who had variant alleles of all three genes (OR: 8.143), emphasising the significance of CYP1A1 gene as well as the synergistic effect of gene combinations.[11]

In the current study, GSTT1 null polymorphism surfaced as an independent risk factor for oral cancer irrespective of any other examined genotype or tobacco exposure. When GSTT1 null polymorphism combined with GSTM1 not null variant, an overall increased risk was observed (OR: 4.58, 95% CI: 0.99-21.2, $p=0.05$). This observation reflected the contribution to increased risk by GSTT1 null genotype. This finding of ours differs from the study which documented the presence of GSTT1 null genotype as a form of protection against oral cancer in Indian population.[9]

The main limitation of the current study is the lack of representation of certain genotypes in certain categories of tobacco consumption. This could probably be addressed by a larger sample size having substantial enrollments to cover each of the tested domains.

Conclusion

A higher risk of OSCC seems to be associated with the presence of combined gene polymorphisms of phase I and phase II enzymes than that attributed to a single gene. Interaction of combined genotypes of carcinogen-metabolising enzymes with tobacco consumption might modulate susceptibility of oral cancer in high-risk communities.

Disclaimer: The approvals from the two ethics review committees of the two institutions were meant for a larger research project, and the current study covers one aspect of that larger study.

Conflict of Interest: None.

Source of Funding: The Higher Education Coomission (HEC), Pakistan, and Ziauddin University, Karachi.

References

1. Omiecinski CJ, Heuve VJP, Perdew GH, Peters JM. Xenobiotic metabolism, disposition, and regulation by receptors:From biochemical phenomenon to predictors of major toxicities. Toxicol Sci.2011; 120:S49-75.
2. Gundert-RemyU, BernauerU, BlomekeB, DoringB, FabianE, GoebelC, etal. Extrahepatic metabolism at the body's internal-external interfaces. Drug Metab Rev.2014; 46:291-324.
3. Taspinar M, Aydos SE, ComezO, Elban AH, Karabulut HG, Sunguroglu A. CYP1A1,GST gene polymorphisms and risk of chronic myeloid leukaemia.Swiss Med Wkly.2008; 138:12-7.
4. KatakwarP, MetqudR, NaikS, MittalR. Oxidative stress marker in oral cancer:A review. J Can Res Ther.2016; 12:438-46.
5. Peddireddy V, Badabagni SP, Gundimeda SD, Mamidipudi V, Penagaluru PR, Mundluru HP. Association of CYP1A1,GSTM1 and GSTT1 gene polymorphisms with risk of non-small cell lung cancer in Andhra Pradesh region of South India. [Online] [Cited 2021 Jan 13].Available from: URL:https://link.springer.com/article/10.1186/s40001-016-0209-x
6. Feki-Tounsi M , Khlifi R, Louati I, Fourati M, Mhiri MN, Chaffai AH, etal.Polymorphisms in XRCC1,ERCC2,and ERCC3DNA repair genes,CYP1A1 xenobiotic metabolism gene, and tobacco are associated with bladder cancer susceptibility in Tunisian population. Environ Sci Pollut Res.2017; 24:22476-84.
7. Choudhury JH, Singh SA, Kundu S, Choudhury B, Talukdar FR, Srivasta S, etal.Tobacco carcinogen-metabolizing genes CYP1A1,GSTM1, and GSTT1 polymorphisms and their interaction with tobacco exposure influence the risk of head and neck cancer in North east Indian population. Tumour Biol.2015; 36:5773-83.
8. CroccoP, Montesanto A, Dato S, Geracitano S, Iannone F, Passarino G, etal. Inter-individual variability in Xenobiotic-Metabolizing Enzymes:Implications for Human Aging and Longevity.Genes.2019; 10:403.
9. Anantharaman D,, Chaubal PC, Kannan S, Bhisey RA, Mahimkar MB. Susceptibility to oral cancer by genetic polymorphisms at CYP1A1,GSTM1 and GSTT1 loci among Indians:Tobacco exposure as a risk modulator.Carcinogenesis.2007; 28:1455-62.
10. Zhou T, Li HY, Xie WJ, Zhong Z, Zhong H, Lin ZJ. Association of Glutathione Stransferase gene polymorphism with bladder Cancer susceptibility. BMC Cancer [Online] [Cited 2020 Dec 23]. Available from: URL:https://bmccancer.biomedcentral.com/articles/10.1186/s12885-018-5014-1.
11. Zakiullah, Ahmadullah, Khisroon M, Saeed M, Khan A, Khunda F, etal. Genetic susceptibility to oral cancer due to combined effects of GSTT1,GSTM1 and CYP1A1 gene variants in tobacco addicted patients of Pashtun ethnicity of Khyber Pakhtunkhwa province of Pakistan. Asian Pac J Can Prev.2015; 16:1145-50.
12. Abbas M, Srivastava K, Imran M, Banerjee M. Association of CYP1A1 gene variantsrs4646903 (T>C) and rs1048943 (A>G) with cervical cancer in North Indian population.Eur J Obstet Gynecol Reprod Biol.2014; 176:68-74.
13. Zakiullah, Saeed M, Ali S, Khisroon M, Muhammad B, Khuda F,etal. Genetic susceptibility to esophageal cancer due to CYP1A1 gene variants 4646903 in tobacco addicted patients of Pashtun ethnicity: a case-control study in Khyber Pakhtunkhwa province of Pakistan. Asian Pac J Can Prev.2014;15:6715-20.
14. Singh SA, Ghosh SK. Metabolic PhaseI (CYPs) and PhaseII (GSTs) Gene Polymorphisms and Their Interaction with Environmental Factors in Nasopharyngeal Cancer from the Ethnic Population of Northeast India.Pathol Oncol Res.2019; 25:33-44.
15. Varela-LemaL,Taioli E, Ruano-RavinaA, Barros-Dios J, Anantharaman D, Benhamou S, etal. Meta-analysis and pooled analysis of GSTM1 and CYP1A1 polymorphisms and oral and pharyngeal cancers:a HuGE-GSEC review. Genet Med.2008; 10:369-84.
16. Daniel WW. Biostatistics:a foundation for analysis in Health Sciences,5thed. John Wiley & Sons,1987.
17. Ada AO, Yilmazer M, Suzen S, Demiroglu C, DemirbagAE, EfeS, etal. Cytochrome P450 (SYP) and glutathione S-transferases (SGT) polymorphisms (CYP1A1,CYP1B1,GSTM1,GSTP1,and GSTT1) and urinary levels of 1-hydroxypyrenein Turkish coke oven workers. Genet Mol Biol.2007; 30:511-19.
18. Li W, Song LQ, Tan J. Combined effects of CYP1A1MspI and GSTM1 genetic polymorphisms on risk of lung cancer:an updated meta-analysis.Tumor Biol. 2014; 35:9281-90.
19. Zhang WP, He XF, Ye XH. Association between the combined effects of GSTM1 present/null and CYP1A1MspI polymorphisms with lung cancer risk:an updated meta-analysis. [Online] 2020 [Cited 2021 January 13]. Available from: URL: https://portlandpress.com/bioscirep/ article/40/9/BSR20202275/ 226457/Association-between-the-combined-effects-of-GSTM1
20. Girdhar Y, Singh N, Behera D, Sharma S. Combinations of the Variant Genotypes of CYP1A1, GSTM1 and GSTT1are associated with an increased lung cancer risk in North Indian population:a case-control study. Pathol Oncol Res.2016; 22:647-52.