4.1 Background

The effects of environmental exposure to carcinogens in the form of BN, tobacco and smoking are neutralized by phase I and phase II biotransformation enzymes (e.g. cytochromes P450 and glutathione S-transferases, epoxide hydrolases and N-acetyl transferases) [1]. However, genetic polymorphisms are known to modulate the cancer susceptibility through differential ability to metabolize the carcinogenic compounds [2].

Microsomal epoxide hydrolase (mEH) (EPHX1) plays a dual function, both in detoxification and in the activation of pro-carcinogens depending on the types of exposed environmental compounds [3]. The EPHX1 gene is 35.48 kb in length with 8 intron and 9 exon, and is located on chromosome 1q42.1. It is expressed in most human tissues, including the lungs and upper aero-digestive tract [3,4] and it catalyzes the hydrolysis of reactive epoxide to trans-di-hydrodiols, which can be conjugated and excreted from the body [5]. Two distinct EPHX1 genetic polymorphisms are associated with differential enzyme activity and are considered risk factors for cancer; one in exon 3 (T>C, Tyr113His) which shows reduced enzyme activity by at least 50% (slow allele); and other in exon 4 (A>G, His139Arg) that shows increased activity by at least 25% (fast allele). Variation in enzyme activity due to EPHX1 gene polymorphism may lead to a variation in the susceptibility to cancers [1, 6]. Numerous epidemiologic studies reported the association of these two functional polymorphisms with HNC and other types of cancer. The EPHX1 113 His variant allele increases the risk of colorectal [4, 7], esophagus [8], and hepatocellular carcinoma [9], but reduce the risk of ovarian [10] and lung cancer [11]. However, EPHX1 139 variant allele increased the risk of oesophagus, [4] larynx, pharynx and oral cancer [11, 12].

The arylamine N- acetyltransferases (NAT) play an important role in detoxification and/or activation of several exogenous chemical compounds such as aromatic and heterocyclic amines which are potential carcinogens [13]. Two polymorphic isoenzymes, NAT1 and NAT2 are found on the chromosomal location 8p21.3-23.1 [14]. There are four major NAT1 genotypes present in 3`-untranslated region that can be identified by polymerase chain reaction (PCR)-restriction fragment length

polymorphism (RFLP) method [15,16], and allele-specific PCR as follows:*NAT1* ∗4 (wild type) which is usually the more frequent, NAT1*3, with a `T' at nucleotide 1088 and an `A' at 1095, NAT1*10, with an `A' at nucleotide 1088 and an `A' at 1095 [15, 16], and NAT1*11, with a deletion of 9 bp nucleotides between 1080 and 1088 [17]. Several studies have reported the associations of the *NAT1* ∗10 allele with larynx, lung, colon and bladder cancer risk [13, 15, 16]. However, there is no report existing on the association between polymorphism at NAT1 locus and risk of HNC in NE population. NAT1*3 and NAT1*11 are infrequent genotypes which do not perform to contribute to the risk of colorectal cancer [17].

NAT2 gene has a number of point mutations, which result in decreased NAT activity [18]. The three mutations that results in slow acetylation phenotype are NAT2 *5 (T at nucleotide 481), NAT*6 (A at nucleotide 590) and NAT2*7 (A at nucleotide 857) [13, 18]. Epidemiology studies demonstrated that NAT2 slow acetylation was linked to increased risk in rheumatoid arthritis and bladder cancer particularly among cigarette smokers [18, 19], and some studies reported reduced acetylation activity and decreased half-life of the protein. The slow acetylator alleles are observed more frequently in Caucasian population [13].

Genotyping of single-nucleotide polymorphisms (SNPs) alone may not always provide sufficient information for any convincing results. We have to understand the polymorphism with the help of haplotypes, which carry more information about the genotype–phenotype relationship [20]. To our knowledge, there are no published reports on the relationship between polymorphic EPHX1, NAT1 and NAT2 genes and association with HNC in North East population of India. The aim of this case-control study was to investigate the distribution of variant EPHX1, NAT1 and NAT2 genotype and haplotype status with environmental exposures (betel nut, tobacco, and smoking) and to evaluate their association with head and neck carcinogenesis in NE Indian population.

4.2 Material and Method

For this population based case-control study, HNC patients and healthy controls were enrolled from Dr. Bhubaneswar Borooah Cancer Institute (BBCI), Guwahati, India. The detail of the criteria adopted to recruit participants and methods of blood and information were mentioned in section 3.2.1.

As there were several types of chewing habits exist in NE population, we included the betel nut (fresh leaf, fresh areca nut, and slaked lime), smoking (cigarettes and beedi), tobacco chewing (khani with lime, gutkha, zarda) and Further, the habits of betel nut consumption, tobacco chewing and smoking were categorized as never, ≤10 unit per day and >10 unit per day.

4.2.1 DNA isolation

The genomic DNA was extracted from the peripheral blood by phenol-chloroform method as described in section 3.2.2.

4.2.2 Genotyping Analysis

The genotypic polymorphism of NAT1 (T1088A, G1095A and 9 bp deletion) and NAT2 (C481T, G590A, G857A) was identified by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The details of primer sequences and PCR conditions are mentioned in (Table. 14). PCR reactions were performed in final volume of 25- µl containing 100 ng of genomic DNA, 10X reaction buffer (New England biolabs, USA), 2.5 mM deoxyribonucleotide triphosphate mix (dNTP), 1.5 mM, Mgcl2, 15 pmol of each primer, 1 U Taq polymerase (New England biolabs, USA). The PCR products were digested with 5U PSY I (New England biolabs, USA) for EPHX1 exon 3 [21], and RsaI (New England biolabs, USA) for EPHX1 exon 4 (New England biolabs, USA), at 37ºC overnight as shown in figure 10 and 11 [21].

To analyze the NAT1 T1088A polymorphism and the 9 bp deletion, the PCR products were digested with 5U VspI (New England biolabs, USA), at 37ºC overnight. To analyze the NAT1 C1095A polymorphism and the 9 bp deletion, PCR product was

digested with 5U MboII (New England biolabs, USA), at 37 ºC overnight as shown in figure 12 and 13.

To analyze the NAT2 polymorphism of (C481T, G590A and G857A) genes, the PCR products were digested with 5U KpnI (New England biolabs, USA) for NAT2*5 (C481T) and 5 U BamHI for NAT2*7 (G857A) (New England biolabs, USA) at 35 ºC for overnight, and 5U TaqI for NAT2 for NAT2*6 (G590A) [13]. All the digested products were resolved on 2-3 % agarose gel (Sigma, India) stained with ethidium bromide (SRL, India) in Gel Doc system G-Box (SYNGENE, USA) and amplification was performed with a thermal Cycler C1000 (Bio-Rad, CA, USA) as shown in figure 14 and 15. A 20% randomly selected blinded samples were cross checked and the results obtained were found to be 100% concordant.

4.2.3 Statistical Analysis

The details of the statistical tools and software used were mentioned in 3.2.4.

4.3 Results

4.3.1 Distribution of EPHX113, EPHX139, NAT1, NAT2 genotypes in HNC cases and controls

The EPHX1-113, EPHX1-139, NAT1 and NAT2 genotypes distributed among the HNCs patients and healthy controls are presented in Table 15. The distribution of all polymorphism in cases and control were in agreement with Hardy-Weinberg equilibrium (data not shown). The results of the multivariate analysis demonstrated that CC of EPHX1 Tyr113His (OR^a, 2.73; 95 % CI 1.48–5.03p<0.001), CT of NAT2 C481T (OR^a, 1.57; 95 % CI 1.02– 2.42, p=0.03) and GA of NAT2 G590A (OR^a, 157; 95 % CI 1.01–2.43, p=0.04) polymorphisms were significantly associated with increased risk for HNC. Haplotyping analysis showed that the intermediate mEH activity of EPHX1 and slow acetylation phenotype of NAT2 were associated with increased risk of HNC (OR^a, 1.83; 95 % CI 0.96-3.47, p=0.05 and OR^a, 2.64; 95 % CI 1.45-4.82, p=0.02 respectively). However, no statistical significance for the EPHX1 His139Arg, NAT1 T1088A, NAT1 C1095A, NAT2 G857A genotypes and NAT1 acetylation activity were identified from our study (Table 15).

Table 14: Sequence of oligonucleotide primers and reaction conditions used for EPHX1, NAT1 and NAT2 genotyping

Genotype	Primers (5' to 3')	Alleles (bp)	PCR Condition
EPXH113	5-CTT GAG CTC TGTCCT TCC CAT CCC-3 5 -AAT CTT AGT CTT GAA GTG ACG GT-3	*TT- 232* *TC-232,21,21* *TT-211,21*	95°C/ 7 min, 35 cycles 94°C/ 30 sec 52°C/ 30 sec 72°C/ 30 sec 72°C/ 10 min
EPHX139	5-ACA TCC ACT TCA TCC ACG T-3 5 -ATG CCT CTG AGA AGC CAT-3	*AA-210* *AG-210,162,48* *GG-162,48*	95°C/ 7 min, 35 cycles 94°C/ 30 sec 52°C/ 30 sec 72°C/ 30 sec 72°C/ 10 min
NAT1	5-ACTCTGAGTGAGGTAGAAATA-3	*CC*- 176, 125, 24 ,19	94°C/ 4 min, 35 cycles
(C1095A)	5--ACAGGCCATCTTTAGAA-3	*CA*- 176, 144, 125, 24 ,19 *AA-176, 144, 24*	94°C/ 30 sec 45 °C/ 30 sec 72°C/ 45 sec 72°C/ 7 min
NAT2	5-GGAACAAATTGGACTTGG-3	***C481T***	
C481T	5-TCTAGCATGA ATCACTCTGC-3	*CC-660.443*	
G590A		*CT-1093,660,443*	
G857A		*TT-1093*	94°C/ 5 min, 35 cycles
		G590A	94°C/ 30 sec
		GG-380,317,226,170	50 °C/ 30 sec
		GA396,317,226,170	72°C/ 90 sec
		AA- 396,380,317	72°C/ 7 min
		G857A	
		GG-811,282	
		GA-1093,811,282	
		AA-1093	

4.3.2 Interaction of betel nut, tobacco, smoking habits with EPHX1, NAT1 and NAT2 genotypes for risk assessment in HNC

In our present study, we also evaluated the interaction of betel nut, tobacco chewing and smoking habits with various genotypes of EPHX1 and NAT. The multivariate analysis showed that CC of EPHX1 Tyr113His (OR^a, 3.37; 95 % CI 1. 1.68-6.74, p<0.01), AG of EPHX1His139Arg (OR^a, 3.42; 95 % CI 1.81-6.47, p<0.001) and CT of NAT2 C481T (OR^a, 1.58; 95 % CI 0.98-2.53, p=0.05) and TT of NAT2 C481T genotypes (OR^a, 2.80; 95 % CI 1.02-7.62, p=0.04) have increased risk for developing HNC in betel nut consuming subjects. Functional aspect of polymorphism showed a significantly increased risk for HNC with intermediate (OR^a, 3.35, 95 % CI 1.49-7.52, p<0.01) and

fast (OR[a], 3.00, 95 % CI 1.26-7.10, p=0.01), mEH activity (EPHX1) and slow Acetylation (NAT2) (OR[a], 2.23, 95 % CI 1.18-4.22, p=0.01) (Table 16).

Moreover, a subgroup analysis of the association among tobacco chewers, we observed that the EPHX1 Tyr113His genotypes have high impact association with HNC in both tobacco chewers having CC (OR[a], 3.85; 95 % CI 1.29-11.47, p=0.01) and non-chewers having CC genotype (OR[a], 3.36; 95%, CI 1.36-8.72, p<0.01) [Table 17]. Whereas, the AA of NAT2 G590A (OR[a], 2.72; 95 % CI 1.20-12.11, p=0.02) genotype and the slow acetylation of NAT2 gene have (ORa, 4.38; 95 % CI 1.41-13.60, p=0.01) increase risk association with HNC among non-chewers (Table 17).

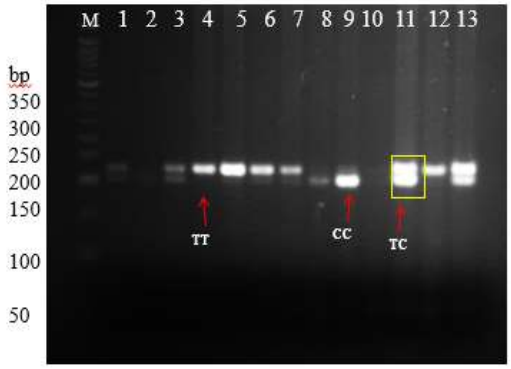

Figure 10: EPHX1-113 genotype analyzed by (PCR-RFLP) the PCR product digested with PsyI from thirteen samples. The yield were fragments with 232bp, for Tyr/Tyr; 232, 211, 21 bp for Tyr/His and His/His for Val/Val; lane M- 50 bp ladder.

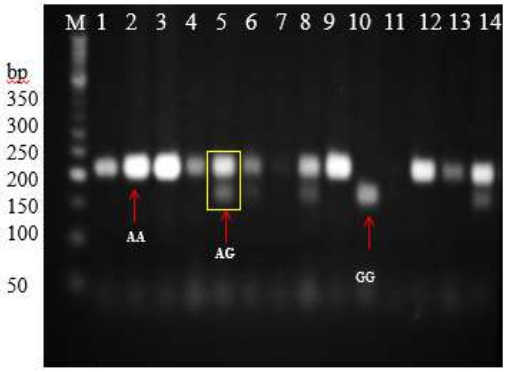

Figure 11: EPHX1-139 genotype analyzed by (PCR-RFLP) the PCR product digested with RsaI from fourteen samples The yield were fragments with 210 bp for His/His; 210,162,48 bp for His/Arg and 162, 48 bp for Arg/Arg, lane – M 50 bp ladder.

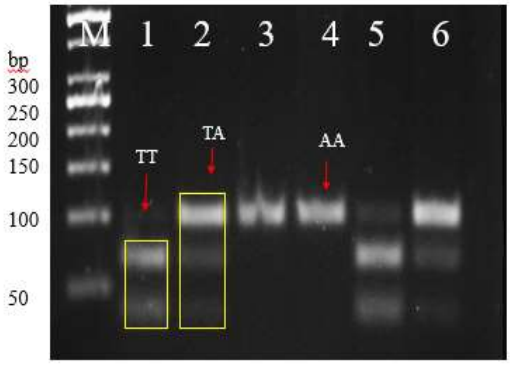

Figure 12: NAT1 T1088A genotype analyzed by (PCR-RFLP) the PCR product digested with VspI from six samples. The yield were fragments with 64, 32 bp for TT; 96, 64, 32 bp for TA and 96 bp for AA; lane – M, 50 bp ladder.

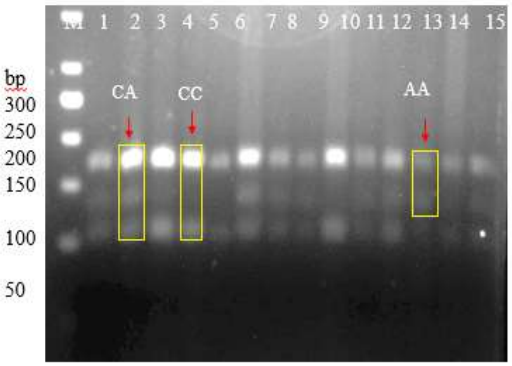

Figure 13: NAT1 G1095A genotype analyzed by (PCR-RFLP) the PCR product digested with MboII from fifteen samples. The yield were fragments with 176, 125, 24, 14 bp for CC; 176, 144, 125, 24, 19 bp for CA and 176, 144, 25 bp for AA; lane – 1, 50 bp ladder.

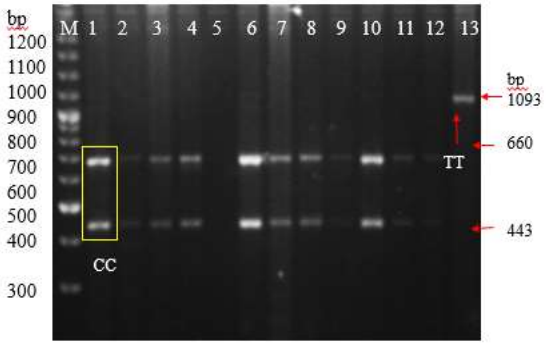

Figure 14: NAT2 C481T genotype analyzed by (PCR-RFLP) the PCR product digested with **Kpn I** from thirteen samples. The yield were fragments with 660, 433 bp for CC; and 1093 bp for TT; lane M - 100 bp ladder

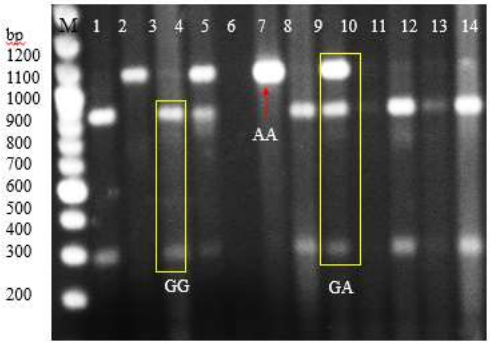

Figure 15: NAT2 G857A genotype analyzed by (PCR-RFLP) the PCR product digested with **TaqI** from fourteen samples. The yield were fragments with 811, 282 bp for GG; 1093, 811, 282 bp for GA and 1093, bp for AA; lane M - 100 bp ladder

Table 15: Distribution of EPHX113, EPHX139, NAT1, NAT2 genotypes in cases of HNC and healthy control

characteristics	Case (n=205) (n= %)	Control (n=210) (n= %)	OR [95% CI]	P- value	Adjusted OR [a] (95% CI)	P- value
EPHX1 113 (Exon 3)						
TT (Tyr/ Tyr)	66 (32.19)	99 (47.14)	Referent (1.0)		Referent (1.0	
TC (Tyr/Hi)s	91 (44.39)	88 (41.90)	1.55 [1.01- 2.37]	**0.04**	1.11 [0.69-1.79]	0.64
CC (His/His)	48 (23.41)	23 (10.95)	3.13 1.7-5.62]	**<0.001**	2.73 [1.48-5.03]	**0.001**
EPHX1 139 (Exon 4)						
AA (His/His)	137(66.82)	151 (71.90)	Referent (1.0)		Referent (1.0	
AG (His/Arg)	58 (28.29)	50 (23.81)	1.27[0.82- 1.99]	0.27	1.59 [0.95-2.63]	0.07
GG (Arg/Arg)	10 (4.87)	9 (4.28)	1.22[0.48- 3.10]	0.66	1.15 [0.43-3.41]	0.79
Predicted mEH activity						
slow	143 (69.75)	157 (78.10)	Referent (1.0)		Referent (1.0	
Intermediate	35 (17.07)	22 (10.94)	1.74 [0.97-3.11]	**0.05**	1.83 [0.96 -3.47]	**0.05**
Fast	27 (13.17)	31 (15.42)	0.96 [0.54- 1.67]	0.87	1.19 [0.62-2.29]	0.58
NAT1 T1088A						
TT	64 (31.21)	72 (35.82)	Referent (1.0)		Referent (1.0	
TA	111 (54.14)	109 (54.22)	1.14[0.74-1.75]	0.53	1.09 [0.69-1.72]	0.70
AA	30 (14.63)	29 (14.42)	1.16 [0.63-2.14]	0.62	1.06 [0.55-2.04]	0.85
NAT1 C1095A						
CC	91 (44.39)	106 (52.73)	Referent (1.0)		Referent (1.0	
CA	85 (41.46)	81(40.29)	1.22[0.80-1.84]	0.34	1.30 [0.83-2.03]	0.24
AA	29 (14.14)	23 (11.44)	1.44[0.79-2.71]	0.22	1.46 [0.76-2.80]	0.25
Acetylation phenotype						
slow	109 (53.17)	106 (50.47)	Referent (1.0)		Referent (1.0	
Intermediate	75 (36.58)	85 (40.47)	0.85 [0.57-1.29]	0.46	0.84 [0.54-1.30]	0.45
Fast	21 (10.24)	19 (9.04)	1.07 [0.54-2.11]	0.83	0.99 [0.48-2.08]	0.99
NAT2 C481T						
CC	101 (49.26)	127 (63.18)	Referent (1.0)		Referent (1.0	
CT	89 (43.41)	72 (35.82)	1.55 [1.03-2.33]	**0.03**	1.57 [1.02-2.42]	**0.03**
TT	15 (7,31)	11 (5.47)	1.71 [0.75-3.89]	0.19	1.90 [0.80-4.51]	0.14
NAT 2 G590A						
GG	89 (43.41)	118 (58.70)	Referent (1.0)		Referent (1.0	
GA	91 (44.39)	74 (36.81)	1.63 [1.07-2.46]	**0.02**	1.57 [1.01- 2.43]	**0.04**
AA	25 (12.19)	18 (8.95)	1.84 [0.94-3.58]	0.07	1.69 [0.84 -2.43]	0.14
NAT2 G857A						
GG	112 (54.63)	134 (66.66)	Referent (1.0)		Referent (1.0	
GA	76 (37.07)	65 (32.33)	1.39 [0.92-2.11]	0.11	1.45 [0.93-2.26]	0.09
AA	17 (8.29)	11 (5.47)	1.84 [0.83-4.11]	0.13	1.74 [0.76 -4.00]	0.18
Acetylation phenotype						
Fast	25 (12.19)	44 (21.89)	Referent (1.0)		Referent (1.0	
Intermediate	63 (30.73)	82 (39.04)	1.35 [0.74-2.44]	0.31	1.46 [0.78- 2.73]	0.23
Slow	117 (55.07)	84 (40)	2.45 [1.39-4.314]	**<0.01**	2.64 [1.45 -4.82]	**0.02**

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR [a] (Adjusted in multivariate logistic regression models) including, gender, age, dose of betel nut and dose of smoking

Table 16: Distribution of EPHX113, EPHX139, NAT1, NAT2 genotypes in betel nut and non-betel nut chewing cases of HNC and healthy control

Betel nut				**Non-betel nut**		
Genotype	**Cases/ Control (n=182/150)**	**OR [95% CI] P-value**	**Adjusted OR [a] (95% CI) P- value**	**Cases (n=23/60)**	**OR [95% CI] P- value**	**Adjusted OR [a] (95% CI) P- value**
EPHX1 113 (Exon 3)						
TT (Tyr/ Tyr)	57/67	**Referent (1.0)**	**Referent (1.0**	9/32	**Referent (1.0)**	**Referent (1.0**
TC (Tyr/Hi)s	80/68	1.38 [0.85- 2.23] 0.18	1.03 [0.61-1.74] 0.89	11/20	1.95 [0.68- 5.55] 0.20	1.54 [0.47 -5.02] 0.47
CC (His/His)	45/15	3.52 [1.78-6.98] **<0.001**	3.37 [1.68-6.74] **0.001**	3/8	1.33 [0.29-6.09] 0.71	0.40 [0.04-3.87] 0.43
EPHX1 139 (Exon 4)						
AA (His/His)	120/131	**Referent (1.0)**	**Referent (1.0**	17/20	**Referent (1.0)**	**Referent (1.0**
AG (His/Arg)	53/16	3.61 [1.96-6.66] **<0.001**	3.42 [1.81 - 6.47]**<0.001**	5/34	0.17 [0.05- 0.54] **<0.01**	0.21 [0.06-0.73] **0.01**
GG (Arg/Arg)	9/3	3.27 [0.86-12.38] 0.08	2.28 [0.56- 9.18] 0.24	1/6	0.19 [0.02- 1.79] 0.14	0.20 [0.01-2.91] 0.24
Predicted mEH activity						
slow	125/133	**Referent (1.0)**	**Referent (1.0**	18/24	**Referent (1.0)**	**Referent (1.0**
Intermediate	32/9	3.78 [1.73-8.24] **<0.001**	3.35 [1.49-7.52] **<0.01**	3/13	0.30 [0.07- 1.24] 0.09	0.47[0.40-1.96] 0.20
Fast	25/8	3.32 [1.44-7.64] **<0.01**	3.00 [1.26 -7.10] **0.01**	2/23	0.11 [0.02-0.55] **<0.01**	0.07 [0.02-0.43] **0.01**
NAT1 T1088A						
TT	57/49	**Referent (1.0)**	**Referent (1.0**	7/23	**Referent (1.0)**	**Referent (1.0**
TA	100/83	1.03 [0.64-1.67] 0.88	1.02 [0.62 -1.69] 0.91	11/26	1.39 [0.46- 4.18] 0.55	1.50 [0.43-5.23] 0.52
AA	25/18	1.19 [0.58 -2.44] 0.62	1.02 [0.49-2.15] 0.94	5/11	1.49 [0.38-5.78] 0.56	108 [0.22- 5.23] 0.91
NAT1 C1095A						
CC	82/76	**Referent (1.0)**	**Referent (1.0**	9/30	**Referent (1.0)**	**Referent (1.0**
CA	75/57	1.21 [0.76-1.94] 0.40	1.32.[0.81-2.15] 0.26	10/24	1.38 [0.48-3.96] 0.53	1.10 [0.32 -3.71] 0.87
AA	25/17	1.36 [0.68- 2.71] 0.37	1.33 [0.65-2.71] 0.43	4/6	2.22 [0.51-9.64] 0.28	2.54 [0.53- 12.30] 0.24
Acetylation phenotype						
slow	98/77	**Referent (1.0)**	**Referent (1.0**	11/29	**Referent (1.0)**	**Referent (1.0)**
Intermediate	65/61	0.83 [0.52-1.32] 0.44	0.80 [0.50-1.29] 0.37	10/24	1.09 [0.39-3.02] 0.85	0.90 [0.28-2.88] 0.86
Fast	19/12	1.24 [0.56-2.71] 0.58	1.07 [0.47-2.42] 0.87	2/7	0.75 [0.13-4.19] 0.74	0.90 [0.14-5.64] 0.91
NAT2 C481T						
CC	90/95	**Referent (1.0)**	**Referent (1.0**	11/32	**Referent (1.0)**	**Referent (1.0**
CT	77/49	1.65 [1.04-2.62] **0.03**	1.58 [0.98 -2.53] **0.05**	12/23	1.51 [0.57-4.03] 0.40	1.35 [0.45-4.04] 0.58
TT	15/6	2.63 [0.98-7.09] **0.05**	2.80 [1.02- 7.62] **0.04**	0/5	-	-
NAT 2 G590A						
GG	78/83	**Referent (1.0)**	**Referent (1.0**	11/35	**Referent (1.0)**	**Referent (1.0**
GA	82/51	1.71 [1.07-2.72] **0.02**	1.63 [1.01-2.63] 0.04	9/23	1.24 [0.44- 3.47] 0.67	1.36 [0.41- 4.49] 0.60
AA	22/16	1.46 [0.71-2.98] 0.29	1.33 [0.63- 2.81] 0.44	3/2	4.77 [0.70- 32.33] 0.10	7.79 [1.21- 45.34] 0.06
NAT2 G857A						
GG	100/94	**Referent (1.0)**	**Referent (1.0**	12/40	**Referent (1.0)**	**Referent (1.0**
GA	67/47	1.34 [0.83- 2.13] 0.21	1.39 [0.86- 2.65] 0.17	9/18	1.66 [0.59- 4.65] 0.33	1.90 [0.60 - 16.22] 0.27
AA	15/9	1.56 [0.65-3.75] 0.31	1.65 [0.67-4.03] 0.27	2/2	3.33 [0.42 - 26.24] 0.25	2.49 [0.15- 40.48] 0.52
Acetylation phenotype						
Fast	25/29	**Referent (1.0)**	**Referent (1.0**	0/15	**Referent (1.0)**	**Referent (1.0**
Intermediate	53/58	1.06 [0.55- 2.03] 0.86	1.09 [0.56-2.13] 0.78	10/24	-	--
Slow	104/63	1.91 [1.03- 3.55] 0.03	2.23 [1.18 -4.22] **0.01**	13/21	-	-

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR [a] (Adjusted in multivariate logistic regression models) including, gender, age, dose of betel nut and dose of smoking.

Table 17: Distribution of EPHX113, EPHX139, NAT1, NAT2 genotypes in tobacco and non-tobacco chewing cases of HNC and healthy control

	Tobacco			Non tobacco		
Genotype	**Cases/ Control (n=132/52**	**OR [95% CI] P- value**	**Adjusted OR [a] (95% CI) P- value**	**Cases/ Control (n=73/158**	**OR [95% CI] P- value**	**Adjusted OR [a] (95% CI) P- value**
EPHX1 113 (Exon 3)						
TT (Tyr/ Tyr)	45/28	**Referent (1.0)**	**Referent (1.0**	21/71	**Referent (1.0)**	**Referent (1.0**
TC (Tyr/Hi)s	58/19	1.89 [0.94-3.82] 0.07	1.64 [0.74 -3.60] 0.21	33/69	1.61 [0.85-3.06] 0.14	1.10 [0.51-2.37] 0.80
CC (His/His)	29/5	3.60 [1.25-10.41] **0.01**	3.85 [1.29-11.47] **0.01**	19/18	3.56 [1.59- 8.00] **<0.01**	3.36 [1.36-8.27] **<0.01**
EPHX1 139 (Exon 4)						
AA (His/His)	83/39	**Referent (1.0)**	**Referent (1.0**	54/112	**Referent (1.0)**	**Referent (1.0**
AG (His/Arg)	40/10	1.87 [0.85-4.14] 0.11	1.74 [0.73-4.16] 0.20	18/40	0.93 [0.49- 1.77] 0.83	1.22 [0.54-2.72] 0.62
GG (Arg/Arg)	9/3	1.40 [0.36- 5.49] 0.62	1.64 [0.36-7.32] 0.51	1/6	0.34 [0.04-2.94] 0.33	0.29 [0.01-6.67] 0.44
Predicted mEH activity						
slow	87/40	**Referent (1.0)**	**Referent (1.0**	56/117	**Referent (1.0)**	**Referent (1.0**
Intermediate	24/4	2.75 [0.89-8.47] 0.07	2.39 [0.72-7.86] 0.15	11/18	1.27 [0.56-2.88] 0.55	1.17 [0.44-3.12] 0.74
Fast	21/8	1.20 [0.49-2.95] 0.68	1.28 [0.48-3.43] 0.61	6/23	0.54 [0.21-1.41] 0.21	0.85 [0.25 - 2.87] 0.80
NAT1 T1088A						
TT	44/18	**Referent (1.0**	**Referent (1.0**	20/54	**Referent (1.0)**	**Referent (1.0**
TA	71/26	1.11 [0.54-2.26] 0.75	1.19 [0.57-2.46] 0.63	40/83	1.30 [0.68-2.45] 0.41	1.34[0.63-2.86] 0.44
AA	17/8	0.86 [0.31-2.37] 0.78	0.83 [0.27-2.48] 0.74	13/21	1.67 [0.70- 3.95] 0.24	1.53 [0.56- 4.12] 0.40
NAT1 C1095A						
CC	58/24	**Referent (1.0)**	**Referent (1.0**	33/82	**Referent (1.0)**	**Referent (1.0**
CA	58/20	1.20 [0.59- 2.40] 0.60	1.42 [0.68-2.95] 0.34	27/61	1.09 [0.59-2.01] 0.75	1.04 [0.50-2.17] 0.90
AA	16/8	0.82 [0.31-2.18] 0.70	0.84 [0.30-2.30] 0.73	13/15	2.15 [0.92-5.01] 0.07	2.30 [0.89 –5.94] **0.08**
Acetylation phenotype						
slow	76/26	**Referent (1.0)**	**Referent (1.0)**	33/80	**Referent (1.0)**	**Referent (1.0)**
Intermediate	42/23	0.62 [0.31-1.22] 0.17	0.58 [0.28-1.18] 0.13	33/62	1.29 [0.71-2.31] 0.39	1.40 [0.71-2.78] 0.32
Fast	14/3	1.59 [0.42-6.00] 0.48	1.16 [0.29-4.66] 0.83	7/16	1.06 [0.40-2.81] 0.90	1.08 [0.35-3.36] 0.88
NAT2 C481T						
CC	69/36	**Referent (1.0)**	**Referent (1.0**	32/91	**Referent (1.0)**	**Referent (1.0**
CT	56/13	2.24 [1.08- 4.64] **0.02**	2.04 [0.95-4.38] **0.06**	33/59	1.59 [0.88- 2.85] 0.12	1.13 [0.71- 2.51] 0.35
TT	7/3	1.21 [0.29- 4.99] 0.78	1.23 [0.29 -5.30] 0.77	8/8	2.84 [0.98-8.20] **0.05**	**3**.81[1.06-10.68] 0.39
NAT 2 G590A						
GG	57/26	**Referent (1.0)**	**Referent (1.0**	32/92	**Referent (1.0)**	**Referent (1.0**
GA	60/19	1.44 [0.71- 2.88] 0.30	1.40 [0.68-2.88] 0.35	31/55	1.62 [0.89-2.94] 0.11	1.63 [0.56-2.26] 0.71
AA	15/7	0.97 [0.35- 2.68] 0.96	1.03 [0.37- 2.87] 0.95	10/11	2.61 [1.01-6.73] **0.04**	2.72 [1.20-12.11] **0.02**
NAT2 G857A						
GG	70/27	**Referent (1.0)**	**Referent (1.0**	42/107	**Referent (1.0)**	**Referent (1.0**
GA	56/21	1.02 [0.52-2.00] 0.93	1.15[0.55-2.39] 0.70	20/44	1.15 [0.61-2.19] 0.65	1.55 [0.78- 3.08] 0.21
AA	6/4	0.57 [0.15- 2.21] 0.42	0.83 [0.29-2.38] 0.73	11/7	4.00 [1.45- 11.02] **<0.01**	2.03 [0.67-6.17] 0.20
Acetylation phenotype						
Fast	18/8	**Referent (1.0)**	**Referent (1.0**	7/36	**Referent (1.0)**	**Referent (1.0)**
Intermediate	40/21	0.84 [0.31- 2.26] 0.74	1.06 [0.53-2.12] 0.86	23/61	1.93 [0.75- 4.96] 0.16	1.27 [0.61-2.67] 0.51
Slow	74/21	1.56 [0.59- 4.10] 0.36	0.67 [0.17-2.65] 0.57	43/61	3.62 [1.47-8.90] **<0.01**	4.38 [1.41-13.60] **0.01**

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR [a] (Adjusted in multivariate logistic regression models) including, gender, age, dose of betel nut and dose of smoking

On the other hand, a significant association was observed between the smokers of AG of EPHX1 His139Arg (OR[a], 3.12; 95 % CI 1.33-7.33, p<0.01) and AA of NAT2 G590A (OR[a], 9.48; 95 % CI 1.14-78.85, p=0.02) genotypes (Table 18). We also analyzed the haplotype frequencies of EPHX1 genotype in the patients and the healthy group and observed that intermediate mEH activity (OR[a], 2.83; 95 % CI 1.04-7.69, p=0.04) was associated with increased risk for HNC. However, among never smokers with CC of EPHX1Tyr113His (OR[a], 3.41; 95 % CI 1.67-6.96, p<0.01), AA of NAT2 G590A (OR[a], 9.48; 95 % CI 1.14-78.85, p = 0.02) genotypes and slow acetylation of NAT2 (OR[a], 2.62, 95 % CI 1.26-5.46, p = 0.01) was found to have high impact association with HNC risk (Table 18).

4.3.3 Interaction of combined habits of betel nut, tobacco, smoking with EPHX1, NAT1 and NAT2 genotypes for risk assessment in HNC

Here, we also investigated the interaction of multiple habits (betel nut+ tobacco and betel nut+-tobacco+ smoking) with EPHX1, NAT1 and NAT2 gene polymorphism on HNC risk (Table 19). The result showed that betel nut-tobacco exposure on subjects having CC of EPHX1 Tyr113His **(**OR[a], 3.45; 95 % CI 1.14-10.39, p=0.02) and AG of EPHX1 His139Arg (OR[a], 3.00; 95 % CI 1.11-8.08, p=0.02) genotypes have higher risk associated with HNC. However, in case of triple habit, only AG of EPHX1 His139Arg (OR[a], 5.22; 95 % CI 1.40-19.50, p=0.01) genotype and fast mEH activity (OR, 4.32; 95% CI 1.03-18.18, p=0.04] were associated with HNC risk.

4.3.4 Interaction of EPHX1, NAT1 and NAT2 genotypes with doses of betel nut and tobacco chewing on HNC risk

In table 20, we showed that the lower and higher dose of BN increased the risk for HNC in subjects carrying CC of EPHX1 Tyr113His (9.59 fold) and also for TC (11.85 fold) genotypes and these risk is synergistically increased when the subjects consumed tobacco (for lower dose OR[a], 25.40; 95 % CI 7.00-92.18, p<0.001 and for higher dose OR[a], 59.97; 95 % CI 13.22-2.71, p<0.001) in addition to BN. Similar pattern of synergistic increased risk was also observed in subjects carrying EPHX1 His139ArgAG genotypes. Interestingly, higher dose of tobacco when combined with BN is exposed on subjects carrying AG of EPHX1 His139Arg genotype the risk become 29.95 fold higher

(95 % CI 3.78-237.2, p<0.01). Haplotypic analysis indicated that intermediate and fast activity of mEH among lower dose consumer of betel nut; and fast activity among lower dose consumer of tobacco in dual habit increases the risk for HNC. However, in case of dual habit the higher dose have profound risk for both intermediate (OR^a, 29.84; 95 % CI 3.77-235.8, <0.01) and fast (OR^a, 9.25; 95 % CI 1.04-82.27), p=0.04) mEH activity.

In table 21, there is evidence of risk for HNC in subjects carrying TA of NAT1 T1088A genotype exposed to lower (3.16 fold) and higher (5.48 fold) doses of BN, which is further increased to 4.69 fold (4.69 95 % CI 2.27-9.72, p<0.001) and 10.13 fold (95 % CI 3.03-33.08, p<0.001) when tobacco is added. Similarly, the risk is increased from 3.46 fold (lower dose of BN) to 23.68 fold (95 % CI 2.84-197.1, p<0.01) when subjects added higher dose of tobacco in addition to BN. Further, we observed similar synergistic risk increased in subjects carrying CA and AA of NAT1 C1095A for both lower and higher doses of BN and tobacco.

In case of phenotype, intermediate and fast acetylation with lower and higher dose of BN increased the risk for HNC. Furthermore, we observed that the addition of tobacco (lower and higher dose) with BN with intermediate acetylation has increased the risk for HNC.

We also analyzed the NAT2 polymorphism and their interaction with various doses of BN and tobacco and the results are presented in (table 22). The NAT2 C481T genotypic variation (CT, TT) increases the risk for HNC in subjects having both BN and tobacco habits. The estimated risk for HNC is increased with increase in doses of BN and that of tobacco in combination with BN. The higher doses of tobacco with BN in subjects with CT genotype of NAT2 C481T possess highest risk for HNC (OR^a, 21.19; 95 % CI 4.55-18.36, p<0.001). Similarly, we observed risk for HNC with NAT2 G590A having BN and tobacco chewing habits. Highest risk for HNC is observed in subjects with AA genotype of NAT2 G590A having higher dose BN consuming habit (OR^a, 15.78; 95 % CI 1.61- 154.3, p<0.01). The GA genotype of NAT2 G857A gene also exhibit risk for HNC in subjects with lower dose of BN habit and this risk is further increased when the dose of BN is increased and also along with addition of higher dose of tobacco. Our haplotype analysis indicated that the intermediate and slow acetylation activity of NAT2 gene increases the risk for HNC in both BN and tobacco chewing subjects and the risk

is increased when the dose increases. The higher dose of BN in subjects having slow acetylation activity increases the risk about 56.19 fold (95 % CI 5.62-561), p<0.01).

Table 18: Distribution of EPHX113, EPHX139, NAT1, NAT2 genotypes in smoking and non-smoking chewing cases of HNC and healthy controls

	Smoking			Non smoking		
Genotype	**Cases/ Control (n=87/48**	**OR [95% CI], P- value**	**Adjusted OR [a] (95% CI) P- value**	**Cases/ control (n=118/162)**	**OR [95% CI] P- value**	**Adjusted OR [a] (95% CI) P- value**
EPHX1 113 (Exon 3)						
TT (Tyr/ Tyr)	25/10	**Referent (1.0)**	**Referent (1.0**	41/89	**Referent (1.0)**	**Referent (1.0)**
TC (Tyr/Hi)s	46/34	0.54 [0.22-1.27] 0.16	0.42 [0.16-1.05] 0.06	45/54	1.80 [1.052-3.10] **0.03**	1.15 [0.80-2.84]0.12
CC (His/His)	16/4	1.60 [0.42- 5.98] 0.48	1.29 [0.33 -5.02] 0.71	32/19	3.65 [1.85-7.19] **<0.001**	3.41 [1.67-6.96]**<0.01**
EPHX1 139 (Exon 4)						
AA (His/His)	42/33	**Referent (1.0)**	**Referent (1.0**	95/118	**Referent (1.0)**	**Referent (1.0)**
AG (His/Arg)	37/11	2.64 [1.17-5.95] **0.01**	3.12[1.33- 7.33] **<0.01**	21/39	0.66 [0.36-1.21] 0.18	1.33 [0.66-2.71] 0.41
GG (Arg/Arg)	8/4	1.57 [0.43-5.67] 0.49	1.11 [0.28-4.31] 0.87	2/5	0.49 [0.09-2.61] 0.40	1.41 [0.21-9.42] 0.72
Predicted mEH activity						
slow	44/34	**Referent (1.0)**	**Referent (1.0**	99/123	**Referent (1.0)**	**Referent (1.0)**
Intermediate	23/7	2.53 [0.97- 6.61] **0.05**	2.83 [1.04 -7.69**] 0.04**	12/15	0.99 [0.44-2.22] 0.98	1.66 [0.67-4.11] 0.27
Fast	20/7	2.20 [0.83- 5.82] 0.10	2.16 [0.79 -5.88] 0.13	7/24	0.36 [0.44-2.22] **0.02**	0.82 [0.30 -2.23] 0.70
NAT1 T1088A						
TT	23/15	**Referent (1.0)**	**Referent (1.0**	41/57	**Referent (1.0)**	**Referent (1.0)**
TA	48/26	1.20 [0.53-2.69] 0.65	1.15 [0.49-2.71] 0.73	63/83	1.05 [0.62-1.77] 0.83	1.13 [0.64-1.97] 0.66
AA	16/7	1.49 [0.49-4.48] 0.47	1.42 [0.45- 4.50] 0.54	14/22	0.88 [0.40- 1.93] 0.75	0.86 [0.37-2.00] 0.73
NAT1 C1095A						
CC	40/23	**Referent (1.0)**	**Referent (1.0**	51/83	**Referent (1.0)**	**Referent (1.0)**
CA	36/20	1.03 [0.48-2.19] 0.92	1.09 [0.48-2.43] 0.83	49/61	1.57 [0.95-2.59] 0.07	1.13 [0.76 -2.27] 0.32
AA	11/5	1.26 [0.39-4.09] 0.69	1.25 [0.37- 4.20] 0.71	18/18	1.62 [0.77-3.41] 0.19	1.59 [0.72- 3.50] 0.24
Acetylation phenotype						
slow	45/24	**Referent (1.0)**	**Referent (1.0**	64/82	**Referent (1.0)**	**Referent (1.0)**
Intermediate	32/20	0.85 [0.40-1.80] 0.67	0.80 [0.36-1.75] 0.58	43/65	0.84 [0.51-1.40] 0.52	0.89 [0.52-1.52] 0.67
Fast	10/4	1.33 [0.37-4.70] 0.65	1.20 [0.31-4.56] 0.78	11/15	0.94 [0.40-2.18] 0.88	0.93 [0.37-2.33] 0.88
NAT2 C481T						
CC	39/28	**Referent (1.0)**	**Referent (1.0**	62/99	**Referent (1.0)**	**Referent (1.0)**
CT	43/17	1.81 [0.86-3.81] 0.11	2.14 [0.95-4.83] 0.06	46/55	1.33 [0.80-2.11] 0.26	1.24 [0.73-2.12] 0.41
TT	5/3	1.19 [0.26- 5.42] 0.81	1.41 [0.30-6.58] 0.66	10/8	1.39 [0.74- 5.33]0.16	2.30 [0.79-6.66] 0.12
NAT 2 G590A						
GG	36/25	**Referent (1.0)**	**Referent (1.0**	53/93	**Referent (1.0)**	**Referent (1.0)**
GA	36/22	1.13 [0.54-2.37] 0.73	1.21 [0.54- 2.70] 0.62	55/52	1.85 [1.11-3.08] **0.01**	1.93 [1.13-3.31] **0.01**
AA	15/1	10.41 [1.29-84.01] **0.01**	9.48 [1.14-78.85] **0.03**	10/17	1.03 [0.44-2.41] 0.94	1.04 [0.43-2.55] 0.91
NAT2 G857A						
GG	51/27	**Referent (1.0)**	**Referent (1.0**	61/107	**Referent (1.0)**	**Referent (1.0**
GA	30/18	0.89 [0.41- 1.86] 0.74	0.86 [0.38-1.91] 0.71	46/47	1.71 [1.02- 2.87] **0.03**	1.91 [1.10- 3.2**] 0.02**
AA	6/3	1.05 [0.24- 4.56] 0.93	0.91 [0.19-4.33]0.90	11/8	2.41 [0.92-6.32] 0.07	1.92 [0.71-5.20] 0.19
Acetylation phenotype						
Fast	10/8	**Referent (1.0)**	**Referent (1.0**	15/36	**Referent (1.0)**	**Referent (1.0**
Intermediate	28/19	1.17 [0.39-3.53] 0.76	1.66 [0.50-5.49] 0.40	35/63	1.33 [0.64- 2.76] 0.44	1.12 [0.52-2.42] 0.76
Slow	49/21	1.86 [0.64-5.39] 0.24	2..33[0.76-7.17] 0.13	68/63	2.59 [1.29- 5.18] **<0.01**	2.62 [1.26-5.46] **0.01**

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR [a] (Adjusted in multivariate logistic regression models) including, gender, age, dose of betel nut and dose of smoking

Table 19: Distribution of EPHX113, EPHX139, NAT1, NAT2 genotypes in combined effect off betel nut-tobacco and betel nut –tobacco- smoking habits cases of HNC and healthy control

	Betel nut - tobacco			Betel nut - tobacco - smoking		
Genotype	**Cases/ control (n=122/49)**	**OR [95% CI], P- value**	**Adjusted OR [a] (95% CI) P- value**	**Cases/ Control (n=52/22)**	**OR [95% CI] P- value**	**Adjusted OR [a] (95% CI) P- value**
EPHX1 113 (Exon 3)						
TT (Tyr/ Tyr)	42/25	**Referent (1.0)**	**Referent (1.0**	15/6	**Referent (1.0)**	**Referent (1.0**
TC (Tyr/Hi)s	51/19	1.59 [0.77-3.29] 0.20	1.37 [0.60-3.11] 0.44	29/13	0.89 [0.28-2.82] 0.84	0.51 [0.14- 1.86] 0.3
CC (His/His)	29/5	3.45 [1.18- 10.06] **0.02**	3.45 [1.14 -10.39] **0.02**	8/3	1.06 [0.20-5.44] 0.93	0.90 [0.15-5.16] 0.90
EPHX1 139 (Exon 4)						
AA (His/His)	76/39	**Referent (1.0)**	**Referent (1.0**	18/14	**Referent (1.0)**	**Referent (1.0**
AG (His/Arg)	38/7	2.78 [1.13-6.80] **0.02**	3.00[1.11-8.08] **0.02**	28/5	4.35 [1.33-14.18] **0.01**	5.22 [1.40-19.50] **0.01**
GG (Arg/Arg)	8/3	1.36 [0.34-5.45] 0.65	2.00 [0.43 -9.16] 0.37	6/3	1.55 [0.32- 7.34] 0.57	2.52 [0.44-14.38] 0.29
Predicted mEH activity						
slow	80/40	**Referent (1.0)**	**Referent (1.0**	20/15	**Referent (1.0)**	**Referent (1.0**
Intermediate	22/4	2.75 [0.88- 8.52] 0.07	2.65[0.78-8.98] 0.11	17/3	4.25 [1.05-17.20] **0.04**	4.13 [0.87-19.52] 0.07
Fast	20/5	2.00 [0.69-5.72] 0.19	2.70 [0.84 -8.61] 0.09	15/4	2.81 [0.77-10.21] 0.11	4.32 [1.03-18.18] **0.04**
NAT1 T1088A						
TT	40/18	Referent (1.0)	**Referent (1.0**	15/6	**Referent (1.0)**	**Referent (1.0**
TA	67/25	1.20 [0.58- 2.48] 0.61	1.22 [0.57- 2.58] 0.59	29/13	0.89 [0.28-2.82] 0.84	0.90 [0.27-3.04] 0.87
AA	15/6	1.12 [0.37-3.37] 0.83	0.97 [0.30-3.11] 0.96	8/3	1.06 [0.20-5.44] 0.93	0.95 [0.15-5.70] 0.95
NAT1 C1095A						
CC	54/23	**Referent (1.0)**	**Referent (1.0**	27/10	**Referent (1.0)**	**Referent (1.0**
CA	53/18	1.25 [0.60-2.58] 0.53	1.59 [0.72 -3.35] 0.25	18/8	0.83 [0.27-2.51] 0.74	1.28 [0.37-4.32] 0.69
AA	15/8	0.80 [0.29-2.14] 0.65	0.82 [0.29-2.29] 0.70	7/4	0.64 [0.15-2.69] 0.55	0.68 [0.14-3.23] 0.62
Acetylation phenotype						
slow	70/24	**Referent (1.0**	**Referent (1.0**	26/10	**Referent (1.0**	**Referent (1.0**
Intermediate	39/22	0.60 [0.30-1.22] 0.16	0.57 [0.27-1.19] 0.14	19/10	0.73 [0.25-2.10] 0.56	0.65 [0.20-2.08] 0.47
Fast	13/3	1.48 [0.39-5.66] 0.56	1.05 [0.28-4.55] 0.86	7/2	1.34 [0.23-7.61] 0.73	0.80 [0.11-5.56] 0.82
NAT2 C481T						
CC	65/34	**Referent (1.0)**	**Referent (1.0**	27/15	**Referent (1.0)**	**Referent (1.0**
CT	52/12	2.26 [1.06- 4.81] **0.03**	1.95[0.88-4.30] 0.09	22/5	2.44 [0.76-7.78] 0.13	2.20 [0.61-7.85] 0.22
TT	5/3	0.87 [0.19-3.86] 0.85	1.21 [0.28 -5.22] 0.79	3/2	0.83 [0.12-5.55] 0.85	0.82 [0.11 -6.09] 0.84
NAT 2 G590A						
GG	53/24	**Referent (1.0)**	**Referent (1.0**	21/10	**Referent (1.0)**	**Referent (1.0**
GA	55/19	1.31 [0.64-2.66] 0.45	1.00 [0.47-2.12] 0.99	22/11	0.95 [0.33- 2.70] 0.92	0.65 [0.19-2.24] 0.50
AA	14/6	1.05 [0.36- 3.08] 0.91	1.01[0.28-2.68] 0.82	9/1	4.28 [0.47-38.63] 0.19	3.49 [0.35-33.90] 0.28
NAT2 G857A						
GG	65/25	**Referent (1.0)**	**Referent (1.0**	29/9	**Referent (1.0)**	**Referent (1.0**
GA	52/20	1.00 [0.50- 1.99] 1.00	1.01 [0.49-2.07] 0.97	22/11	0.62 [0.21-1.75] 0.36	0.56 [0.18-1.74] 0.32
AA	5/4	0.48 [0.11- 1.93] 0.30	0.54 [0.13-.2.26] 0.40	1/2	0.15 [0.01- 1.91] 0.14	0.18 [0.01-2.57] 0.20
Acetylation phenotype						
Fast	18/10	**Referent (1.0)**	**Referent (1.0**	8/4	**Referent (1.0)**	**Referent (1.0**
Intermediate	36/15	1.33 [0.50-3.55] 0.56	1.34 [0.47-3.77] 0.57	13/5	1.30 [0.26-6.32] 0.74	1.89 [0.29-12.13] 0.49
Slow	68/24	1.57 [0.63-3.88] 0.32	1.31 [0.51-3.40] 0.56	31/13	1.19 [0.30- 4.66] 0.80	1.08 [0.22-.16] 0.92

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR [a] (Adjusted in multivariate logistic regression models) including, gender, age, dose of betel nut and dose of smoking

Table 20: Interaction of EPHX1 genotypes with doses of betel nut habits for head and neck cancer risk factor

		Betel nut Betel nut doses			Betel nut-tobacco Tobacco doses		
Dose/ day	**Genotype**	**Cases /control (n=205/210)**	**Adjusted OR[a] [95%CI]**	**P- value**	**Cases /control (n=205/210)**	**Adjusted OR[a] [95%CI]**	**P- value**
EPHX1 EXON 3	*TT (Tyr/ Tyr)*	9/32	**Referent (1.0)**		24/74	**Referent (1.0)**	
Never	*TC (Tyr/His)*	11/20	1.70 [0.58-4.95]	0.32	40/69	1.58 [0.84-2.96]	0.14
	CC (His/His)	3/8	1.02 [0.21-4.87]	0.97	19/18	2.68 [1.19-6.01]	**0.01**
≤10 times /day	*TT (Tyr/ Tyr)*	57/63	2.62 [1.13-6.06]	0.02	42/17	6.99 [3.32-14.73]	**<0.001**
	TC (Tyr/Hi)s	52/62	2.11 [0.89-4.97]	0.08	6/17	0.81[0.26-2.45]	0.71
	CC (His/His)	43/13	9.59 [3.60-25.55]	**<0.001**	27/3	25.40 [7.00-92.18]	**<0.001**
>10 times /day	*TT (Tyr/ Tyr)*	0/4	-	**-**	0/8	-	-
	TC (Tyr/His)	28/6	11.85 [3.66-38.30]	**<0.001**	45/2	59.97 [13.22-271]	**<0.001**
	CC (His/His)	2/2	2.18 [0.25-18.79]	0.47	2/2	4.31 [0.34-54.05]	0.25
EPHX1 EXON 4							
Never	*AA (His/His)*	17/20	**Referent (1.0)**		61/112	**Referent (1.0)**	
	AG (His/Arg)	5/34	0.19 [0.06-0.60]	**<0.01**	20/43	0.87 [0.46-1.64]	0.66
	GG (Arg/Arg)	1/6	0.23 [0.2-21.6]	0.20	2/6	0.67[1.27-3.57]	0.64
≤10 times /day	*AA (His/His)*	101/119	1.00 [0.49-2.03]	0.99	54/30	3.43 [1.96-5.98]	<0.001
	AG (His/Arg)	46/16	2.94 [1.20-7.19]	**0.01**	19/6	5.45 [1.98-15.00]	**<0.01**
	GG (Arg/Arg)	5/3	1.29 [0.25-6.59]	0.75	2/1	2.13 [0.17-15.45]	0.55
>10 times /day	*AA (His/His)*	19/12	1.77 [0.66-4.78]	0.25	22/9	4.88 [2.08-11.43]	**<0.001**
	AG (His/Arg)	7/0	-	-	19/1	29.95 [3.78-237.2]	**<0.01**
	GG (Arg/Arg)	4/0	-	-	6/2	4.21 [0.77-23.00]	0.09
Predicted mEH activity	slow	18/24	**Referent (1.0)**		63/117	**Referent (1.0)**	
Never	*Intermediate*	3/13	0.33 [0.08-1.36]	0.12	13/18	1.25 [0.56.277]	0.57
	Fast	2/23	0.13 [0.02-0.64]	**0.01**	7/26	0.55 [0.22-1.36]	**0.19**
≤10 times /day	slow	106/121	1.15 [0.58-2.26]	0.67	58/30	3.68 [2.13-6.37]	**<0.001**
	Intermediate	25/9	3.08 [1.12-8.51]	**0.02**	3/3	1.95 [0.36-10.59]	0.43
	Fast	21/8	3.86 [0.99-8.20]	**0.05**	14/4	5.12 [1.55-16.93]	**<0.01**
>10 times /day	slow	19/11	2.00 [0.76-526]	0.16	22/10	4.26 [1.87-9.68]	**<0.01**
	Intermediate	7/0	-		19/1	29.84 [3.77-235.8]	**<0.01**
	Fast	4/1	5.17 [0.51-0.49-.23]	0.18	6/1	9.25 [1.04-82.27]	**0.04**

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR **[a]** (Adjusted in multivariate logistic regression models) including, gender, age, doses of betel nut, doses of smoking.

Table 21: Interaction of NAT1 genotypes with doses of betel nut habits for head and neck cancer risk factor

		Betel nut Betel nut doses			Betel nut-tobacco Tobacco doses		
Dose/ day	**Genotype**	**Cases /control (n=205/210)**	**Adjusted OR[a] [95%CI]**	**P- value**	**Cases /control (n=205/210)**	**Adjusted OR [a] [95%CI]**	**P- value**
	TT	7/23	**Referent (1.0)**		24/54	**Referent (1.0)**	
NAT1 T1088A	TA	11/26	1.41 [0.46-4.33]	0.54	44/84	1.16 [0.62-2.17]	0.63
Never	AA	5/11	1.32 [0.33-5.24]	0.69	15/23	1.34 [0.58-3.09]	0.48
≤10 times /day	TT	46/47	2.73 [1.06-7.07]	**0.03**	24/11	5.12 [2.13-12.25]	<**0.001**
	TA	87/77	3.16 [1.26-7.92]	**0.01**	47/21	4.69 [2.27-9.72]	<**0.001**
	AA	19/14	3.46 [1.13-10.54]	**0.02**	4/5	1.39 [0.32-6.06]	0.65
>10 times /day	TT	11/2	15.10 [2.61-87.30]	<**0.01**	16/7	4.44 [1.57-12.53]	<**0.01**
	TA	13/6	5.48 [1.46-20.61]	**0.01**	20/4	10.13 [3.03-33.086]	<**0.001**
	AA	6/4	3.91 [0.83-18.48]	0.08	11/1	23.68 [2.84-197.1]	<**0.01**
NAT1 C1095A **Never**	*CC*	9/30	**Referent (1.0)**		37/83	**Referent (1.0)**	
	CA	10/24	1.30 [0.44-3.78]	0.62	32/63	1.12 [0.61-2.03]	0.70
≤10 times /day	*AA*	4/6	2.20 [0.50-9.67]	0.29	14/15	2.12 [0.91-4.93]	0.07
	CC	64/70	2.52 [1.08-5.85]	**0.03**	28/16	4.08 [1.93-8.62]	<**0.001**
	CA	70/53	3.69 [1.58-8.61]	<**0.01**	40/14	5.90 [2.81-12.38]	<**0.001**
>10 times /day	*AA*	18/15	3.36 [1.19-9.49]	**0.02**	7/7	2.13 [0.66-6.82]	0.20
	CC	18/6	8.03 [2.38-27.07]	<**0.01**	26/7	6.97 [2.68-18.07]	<**0.001**
	CA	5/4	3.06 [0.64-14.46]	0.15	13/4	7.40 [2.21-24.75]	<**0.01**
Acetylation phenotype ***Never***	*AA*	7/2	9.54 [1.61-56.58]	**0.01**	8/1	17.80 [2.09-151.5]	<**0.01**
	Slow	11/29	**Referent (1.0)**		39/82	**Referent (1.0)**	
≤10 times /day	*Intermediate*	10/24	1.11 [0.40-3.06]	0.83	36/63	1.15 [0.65-2.04]	0.62
	Fast	2/7	0.78 [0.13-4.41]	0.78	8/16	0.95 [0.36-2.49]	0.92
	Slow	85/74	3.06 [1.43-6.57]	<**0.01**	48/16	5.97 [2.93-12.18]	<**0.001**
>10 times	*Intermediate*	54/54	2.69 [1.22-5.95]	**0.01**	24/18	2.55 [1.20-5.38]	**0.01**
	Fast	13/10	3.45 [1.16-10.21]	**0.02**	3/3	2.08 [0.39-11.00]	0.38
	Slow	13/3	11.85 [2.81-49.94]	<**0.01**	22/8	4.49 [1.78-11.31]	<**0.01**
	Intermediate	11/7	4.31[1.32-14.03]	**0.01**	15/4	6.46 [1.95-21.38]	<**0.01**
	Fast	6/2	7.90 [1.37-45.37]	**0.02**	10/0	-	-

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR **[a]** (Adjusted in multivariate logistic regression models) including, gender, age, doses of betel nut, doses of smoking.

Table 22: Interaction of NAT2 genotypes with doses of betel nut habits for head and neck cancer risk factor

		Betel nut Betel nut doses			Betel nut-tobacco Tobacco doses		
Dose/ day	**Genotype**	**Cases/control (n=205/210)**	**Adjusted OR[a] [95%CI]**	**P-value**	**Cases/control (n=205/210)**	**Adjusted OR[a] [95%CI]**	**P-value**
	CC	11/32	**Referent (1.0)**		36/93	**Referent (1.0)**	
NAT2 C481T	CT	12/23	1.45 [0.54-3.91]	0.45	39/60	1.54 [0.87-2.74]	0.13
Never	TT	0/5	-	-	8/8	2.82 [0.97-8.19]	**0.05**
	CC	77/88	2.16 [1.00-4.65]	**0.04**	36/24	3.65 [1.88-7.10]	**<0.00**
≤10 times /day	CT	60/45	3.29 [1.48-7.33]	**<0.01**	32/10	8.02 [3.53-18.23]	**<0.001**
	TT	15/5	7.43 [2.15-25.64]	**<0.01**	7/3	5.57 [1.32-23.42]	**0.01**
>10 times /day	CC	13/7	4.34 [134.14.07]	**0.01**	29/10	6.70 [2.88-15.55]	**<0.001**
	CT	17/4	9.86 [2.65-34.65]	**<0.01**	18/2	21.19 [4.55-18.36]	**<0.001**
	TT	0/1	-		0/0	-	-
NAT2 G590A	*GG*	10/35	**Referent (1.0)**		35/94	**Referent (1.0)**	
Never	*GA*	9/23	1.39 [0.48-4.00]	0.53	36/55	1.74 [0.97-3.14]	0.06
	AA	4/2	5.02[0.78-32.89]	**0.01**	11/12	2.37 [0.93-6.07]	0.07
	GG	67/77	2.73 [1.34-5.99]	**0.01**	36/18	5.18 [2.55-10.51]	**<0.001**
≤10 times /day	*GA*	69/46	4.43 [1.96-9.98]	**<0.001**	33/15	5.77 [2.74-12.15]	**<0.001**
	AA	1615	3.26 [1.16-9.10]	**0.02**	6/4	3.84 [0.98-15.04]	**0.05**
	GG	11/6	5.54 [1.59-19.24]	**<0.01**	17/6	6.94 [2.45-19.69]	**<0.001**
>10 times /day	*GA*	13/5	7.24 [203-25.87]	**<0.01**	22/4	12.58 [3.96-39.93]	**<0.001**
	AA	6/1	15.78 [1.61- 154.3]	**<0.01**	8/2	11.20 [2.19-57.32]	**<0.01**
	GG	12/40	**Referent (1.0)**		47/109	**Referent (1.0)**	
NAT2 G857A	*GA*	9/18	1.76 [0.62-5.01]	0.28	24/45	1.33 [0.71-2.46]	0.36
Never	AA	2/2	2.21[0.25-19.20]	0.47	12/7	3.98 [1.44-10.99]	**<0.01**
	GG	81/87	2.64 [1.27-5.50]	**<0.01**	35/16	5.17 [2.56-10.44]	**<0.001**
	GA	59/43	3.96 [1.81-8.66]	**<0.01**	36/17	4.97 [2.47-9.99]	**<0.001**
≤10 times /day	AA	12/8	4.62 [1.50.14.21]	**<0.01**	4/4	2.26 [0.52-9.88]	0.27
	GG	19/7	7.33 [2.39-22.44]	**<0.001**	30/9	7.08 [3.03-16.54]	**<0.001**
	GA	8/4	5.44 [1.35-21.97]	**0.01**	16/3	12.89 [3.49-47.61]	**<0.001**
>10 times /day	AA	3/1	8.72 [0.79-96.09]	0.07	1/0	-	-
	Fast	1/15	**Referent (1.0)**		7/34	**Referent (1.0)**	
Acetylation phenotype	*Intermediate*	9/24	5.19 [0.59-45-54]	0.13	27/67	1.88 [0.74-4.81]	0.18
Never	*Slow*	13/21	8.18 [0.95-70.11]	**0.05**	49/60	3.82 [1.54-9.47]	**<0.01**
	Fast	19/25	9.27 [1.11-77.22]	**0.04**	10/7	6.22 [1.73-22.41]	**<0.01**
≤10 times /day	*Intermediate*	49/54	11.27 [1.42-89.17]	**0.02**	21/10	9.67 [3.15-29.66]	**<0.001**
	Slow	84/59	18.19 [2.321.42]	**<0.01**	44/20	9.59 [3.59-25.59]	**<0.001**
>10 times /day	*Fast*	6/4	17.25 [1.55-1.99]	**0.02**	8/3	10.06 [2.06-49.04]	**<0.01**
	Intermediate	4/4	14.42 [1.22-170]	**0.03**	15/5	12.51 [3.36-46.57]	**<0.001**
	Slow	20/4	56.19 [5.62-561]	**<0.01**	24/4	26.92 [6.93-104.1]	**<0.001**

P<0.05 is consider to be significance, OR (odds ratio), CIs (confidence Intervals), OR **[a]** (Adjusted in multivariate logistic regression models) including, gender, age, doses of betel nut, doses of smoking

4.4 Discussion

In this present study, we investigated the association between seven potential genetic variants (EPHX1Tyr113His, EPHX1His139Arg, NAT1T1088A, NAT1 C1095A, NAT2 C481TA, NAT2 G590A and NAT2 G857A) with BN and tobacco chewing habits in order to estimate the risk for HNC in NE population of India. In our study we found that the EPHX1Tyr113His, intermediate mEH activity, NAT2 C481T, NAT2 G590A genetic variants with slow acetylation phenotype was significantly associated with increased risk of HNC. Previous investigations on the association between EPHX1 polymorphism (Tyr113His and His139Arg) and cancer risk have yielded inconsistent results. His/His genotype of EPHX1 Tyr113His polymorphism is a risk factor for developing cancer for Asian and mixed population while no evidences were found for the association of His139Arg polymorphism and cancer risk [21]. Earlier studies have also demonstrated that polymorphism in exon 3 of EPHX1 113 gene increases risk for several types of cancers including oropharyngeal [22], ovarian cancer [23], and acute leukemia [24]. The association between EPHX1 Tyr113His polymorphism and lung cancer and breast cancer risk has been reported in many recent studies but the results were inconsistent. Recent meta-analysis conducted by Tan et al. indicated that Tyr113His polymorphism may be a risk factor for lung cancer in Asians whereas it may decrease the risk among Caucasians [5], but no association was found with breast cancer risk [5]. Our study on the North East subjects also demonstrated strong association of His/His genotype of EPXH1 Tyr113Arg polymorphism with HNC risk, however, we could not observe His139Arg polymorphism as a risk factor in our subject. EPHX1 His139Arg polymorphism have been reported to be associated with higher risk of esophageal cancer in high risk area of India and lung cancer in Asian population [4, 25].Recent meta-analysis with 10 case-control studies also indicated that EPHX1 Tyr113His polymorphism may be a risk factor for HNC while His139Arg polymorphism was not associated with the risk of HNC [3].

Reports from *Jain et al*. demonstrated a lower risk for esophagus cancer in low-risk Areas of India [1]. Further studies or meta-analysis suggested that EPHX1 Tyr113His polymorphism might be a risk factor of HNC, whereas, no evidence was found for the

association between the EPHX1 His139Arg polymorphism with HNC [3], esophageal [26] and breast cancer [27].

The polymorphism in NAT1 is reported to have increased the susceptibility to cancers occurring in the colon [28], bladder [29], pancreas [30], prostate [31], breast [32] and gastric cancer [13]. However, we did not find any association of NAT1 gene polymorphism, similar to previous researcher who worked on Caucasians [33], and Indian population [34]. Our observation on NAT2 C481T and G590A variant demonstrated a high impact in HNC in NE population of India. In context of East India population NAT2 C481T and G857A variant has increased risk of oral submucosa fibrosis and G590A variant didn't show any impact [35].

Meta-analysis carried out in 14 case-control studies demonstrated that NAT2 G590A may be a risk factor while the NAT2 G857A polymorphism was associated with a decreased risk of cancer and likely to act as a protective factor against cancer development [36]. In our study population NAT2 G857A was not associated with HNC risk. The NAT2 occurs as NAT*5, NAT*6 and NAT*7 alleles and are classified as rapid, intermediate, or slow acetylators phenotype respectively [37]. Pervious study demonstrated that NAT2 slow genotype was significantly associated with a higher risk for colon [38], larynx [39], and bladder cancer [37]. We confirmed that among our study cohort the slow acetylator NAT2 has significantly increased risk for HNC as compared with healthy controls. Recent meta-analysis carried out with 23 case-control studies also demonstrated NAT2 might be a low penetrant risk factor for HNC among Asians and in subgroup analysis according to ethnicity, slow acetylators were observed to be associated with increased susceptibility for HNC among the Asians but not among Caucasians or population with mixed ethnicities [40]. In the present study, we also assessed the association and interaction of polymorphism in xenobiotic metabolism genes with exposure to chemical carcinogens such as betel nut, tobacco and smoking habits, as previous studies have showed that these habits play a contributory role in development of HNC [41, 42].

In our study we demonstrated that chewing of betel nut enhanced the risk for HNC in combination with tobacco chewing. In this region betel nut chewing is a unique,

widespread and cultural tradition prevalent in many peoples. The basic component of betel nut is a chewing mixture composed of betel nut wrapped with betel leaf spread with slaked lime, in addition with tobacco (zarda, gutkha, khaini) or without tobacco (pan masala) [43]. BN is known to contain various types of compounds including phenolic, alkaloids, and tannins; besides which nitrosamines are formed from an *in vivo* reaction of betel arecoline, nitrite and thiocyanate, all of which act as potent carcinogens [44, 45]. Numerous studies have reported that the constituents of BN/betel quid (BQ) chewing have significant association with increased risk for oral, oropharyngeal [42], stomach [46], esophageal [4] and breast cancer [47]. Tobacco contains highly reactive electrophiles such as benzo-a- pyrene (BaP), polycyclic hydrocarbon (PAHs) and naphthalene-derivative compounds[48].The nitration of arecoline produces a variety of betel quid-specific nitrosamines (BQSN) which interact with protein, DNA and other biomolecules and forms adducts to exert its carcinogenic activity [49]. Therefore, we can assume that the environmental exposure of BQSN might have an association with the increased risk for HNC prevalent in this region [45].

In consistent with earlier studies, it is quite clear that the genetic polymorphisms and their interaction with carcinogenic agents show a significant individualistic risk response. *Ihsan* et *al*. reported that betel quid chewing and smoking habit among EPHX1 113Tyr/His allele have moderate effect and tobacco chewers and betel quid users having 139His/Arg possess significant risk for esophageal cancer in NE population [4]. Conflicting results have been found that showed 113 His/His allele plays a protective role for esophageal cancer among tobacco smokers and betel nut chewers, as compared with the 113Tyr/Tyr wild-type genotype in Taiwanese population [50].

However, our study observed that 113 His/His variant with BN and tobacco chewing habits had increased the risk of HNC. On the other hand, among smokers the fast mEH activity phenotype was associated with an increased risk of colorectal adenomas [51]. Our results indicated that the intermediate and fast mEH activity phenotype is associated with increased risk for HNC with only BN chewing habit and not with tobacco chewers. A comprehensive meta-analysis have demonstrated that putative low EPHX1 activity may have a protective role on tobacco related carcinogenesis of lung

and upper aero-digestive tract cancers whereas putative higher activity may have harmful effect, suggesting that smoking status may influence association of EPHX1 activity and cancer risk [52] and His/Arg genotype at Exon 4 in prostate cancer risk in the Slovak Population [53]. In our study population we observed the association of intermediate activity phenotype with HNC risk among the smoker.

We also did not observe any association of NAT1 gene polymorphism with betel nut, tobacco chewing and smoking habits. Furthermore, our study is the first to report that higher doses of tobacco chewing act synergistically with BN in our subjects. There are few studies that demonstrated association of smoking [54], and tobacco chewing [55], with NAT1 polymorphism in developing HNC. Also other study reported the associated risk for HNC with NAT1 polymorphism within the smoking stratified subgroup in Caucasians population [33].

Our result also revealed the association between NAT2 polymorphism and tobacco smoking habits as a risk factor for oral cancer in South African population [56]. The slow acetylatorNAT2 genotype might modify the effect of carcinogenic agents present in betel quid tobacco, smoking habits, and their intermediate and slow acetylator genotype may be responsible for increased risk of cancer [57, 58]. Several other studies reported that NAT2 slow acetylation with smoking plays a vital role in increasing the risk of several cancers including colorectal [59], and bladder cancer [60], and chewing of betel quid increased the risk for oral and pharyngeal cancer in Taiwan population [57]. In this context, to best of our knowledge, this study is the first one to investigate that betel nut chewing habit with NAT2 slow acetylation activity exhibit higher risk for HNC in NE population. Previous study suggested that NAT2 C481T genotype increased the risk for HNC among Taiwan population with betel-quid and smoking habits [58], which supports our observation that NAT2 C481T (CT) allele showed higher impact among betel nut, tobacco chewers and smoking habits. In case of NAT2 C481T (TT) for betel nut and NAT2G590A (AA) allele for smoking habit, higher impact on HNC were observed. In contrast, earlier studies on NAT2 polymorphism did not show significant association with environmental factor like tobacco as for esophagus cancer risk in India population [1]. NAT2 A857G showed high synergistic interaction in increasing the risk

for oral sub mucous fibrosis (OSF) exposed to betel nut/areca nut-tobacco among eastern Indian population [35]. However, we found the doses of BN in slow acetylator was associated with higher impact on HNC (56.19 fold) and we also found that higher exposure of tobacco with BN increased the risk (26.92 fold) of HNC in NE population.

In conclusion, our finding showed that EPHX1 and NAT2 polymorphism enhances the risk of HNC either alone or in combination. However, the study identified diverse forms of interaction in betel nut and tobacco habits. Our study suggests that higher exposure of tobacco chewing have synergy with BN in developing HNC in the NE population. Further, the study sample size limits the polymorphism analysis and with large cohorts are warranted to find the risk factor associated with the effect of genetic variation of the EPHX1 and NAT genes of this study.

4.5 Bibliography

1. Jain, M., Tilak, A. R., Upadhyay, R., Kumar, A and Mittal, B.Microsomal epoxide hydrolase (EPHX1), slow (exon 3, 113His) and fast (exon 4, 139Arg) alleles confer susceptibility to squamous cell esophageal cancer. *Toxicol Appl Pharmacol*, 230(2): 247-251, 2008.
2. Boccia, S., Cadoni, G., Sayed-Tabatabaei, F. A., Volante, M., Arzani, D., De Lauretis, A., Cattel, C., Almadori, G., van Duijn, C. M., Paludetti, G and Ricciardi, G. CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4, and NAT2 polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer. *J Cancer Res ClinOncol*, 134(1): 93–100, 2008.
3. Chen, H., Ge, L., Sui, Q and Lin, M. Systematic Review and Meta-Analysis of the Relationship between EPHX1 Polymorphisms and the Risk of Head and Neck Cancer, *journal pone*, 0123347, 2015.
4. Ihsan, R., Chattopadhyay, I., Phukan, R., Mishra, A. K., Purkayastha, J., Sharma, J., Zomawia, E., Verma, Y., Mahanta, J., Saxena, S and Kapur, S. Role of epoxide hydrolase 1 gene polymorphisms in esophageal cancer in a high-risk area in India, *Journal of Gastroenterology and Hepatology*, 25(8):1456–1462, 2010.
5. Tan, X., Wang, Y. Y., Chen, X. Y., Xian, L., Guo, J. J., Liang, G. B and Chen, M. W. Quantative assessment of the effect of the ephx1 tyr113 his polymorphism on lung breast cancer genetic and molecular cancer. *Genet Mol Res*, 13 (3):7437-7446, 2014.
6. Mittal, R. D and Srivastava, D. L. Cytochrome P4501A1 and Microsomal Epoxide Hydrolase Gene Polymorphisms: Gene–Environment Interaction and Risk of Prostate Cancer, *DNA Cell Biol*, 26(11):791-79, 2007.
7. Kiss, I., Orsós, Z., Gombos, K., Bogner, B., Csejtei, A., Tibold, A., Varga, Z., Pázsit, E., Magda, I., Zölyomi, A and Ember, I. Association between allelic polymorphisms of metabolizing enzymes (CYP 1A1, CYP 1A2, CYP 2E1, mEH) and occurrence of colorectal cancer in Hungary. *Anticancer Res*, 27(4): 2931–2937, 2007.
8. Casson, A. G., Zheng, Z., Porter, G. A., and Guernsey, D. L. Genetic polymorphisms of microsomal epoxide hydroxylase and glutathione S-transferases

M1, T1 and P1, interactions with smoking, and risk for esophageal (Barrett) adenocarcinoma. *Cancer Detect Prev*, 30(5): 423–431, 2006.

9. McGlynn, K. A., Rosvold, E. A., Lustbader, E. D., Hu, Y., Clapper, M. L., Zhou, T., Wild, C. P., Xia, X. L., Baffoe-Bonnie, A and Ofori-Adjei, D. Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. *Proc Natl AcadSci USA*, 92(6): 2384–2387, 1995.
10. Lancaster, J. M., Brownlee, H. A., Bell, D. A., Futreal, P. A., Marks, J. R., Berchuck, A., Wiseman, R. W and Taylor, J. A. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol Carcinog*, 17(3): 160–162, 1996.
11. Gsur, A., Zidek, T., Schnattinger, K., Feik, E., Haidinger, G., Hollaus, P., Mohn-Staudner, A., Armbruster, C., Madersbacher, S., Schatzl, G., Trieb, K., Vutuc, C and Micksche M.Association of microsomal epoxide hydrolase polymorphisms and lung cancer risk. *Br J Cancer*, 89(4):702–706, 2003.
12. Jourenkova-Mironova, N., Mitrunen, K., Bouchardy, C., Dayer, P., Benhamou, S and Hirvonen, A. High-activity microsomal epoxide hydrolase genotypes and the risk of oral, pharynx, and larynx cancers. *Cancer Res*, 60(3): 534–536, 2000.
13. Demokan, S., Suoglu, Y., Gözeler, M., Demir, D and Dalay N. N acetyltransferase 1 and 2 gene sequence variants and risk of head and neck cancer, *Mol Biol Rep*, 37(7): 3217–3226, 2010.
14. Khlifi, R., Ben, Salah, G., Chakroun, A., Hamza-Chaffai, A and Rebai, A. Inter-ethnic differences in genetic polymorphisms of xenobiotic-metabolizing enzymes (CYP1A1, CYP2D6, NAT1 and NAT2) in healthy populations: correlation with the functional in silico prediction. *Mol Biol Rep*, 41(9):5735–5743, 2014.
15. Gu, J., Liang, D., Wang, Y., Lu, C and Wu, X. Effects of N-acetyl transferase 1 and 2 polymorphisms on bladder cancer risk in Caucasians. *Mutation Research*, 581(1-2): 97–104, 2005.
16. Bell, D. A., Badawi, A. F, Lang, N. P., Ilett, K. F., Kadlubar, F. F and Hirvonen, A. Polymorphism in the N-acetyltransferase 1 (NAT1) polyadenylation signal: association ofNAT1 * 10 allele with higher N-acetylation activity in bladder and colon tissue. *Cancer Res*, 55(22): 5226–5229, 1995.

17. Fukutome, K., Watanabe, M., Shiraishi, T., Murata, M., Uemura, H., Kubota, Y., Kawamura, J., Ito, H and Yatani, R.N-Acetyltransferase 1 genetic polymorphism influences the risk of prostate cancer development. *Cancer Letters*, 136(1): 83-87, 1999.
18. Hsieh, F. I., Pu, Y. S., Chern, H. D., Hsu, L. I., Chiou, H. Y and Chen, C. J.Genetic polymorphisms of N-acetyltransferase 1 and 2 and risk of cigarette smoking-related bladder cancer. *British Journal of Cancer*, 81(3): 537–541, 1999.
19. Ladero, J. M., Andres, M. P., Bañares, A., Fernández, B., Hernández, C and Benítez, J.Acetylator polymorphism in rheumatoid arthritis. *Eur J ClinPharmacol*, 45(3):279-281, 1993.
20. Srivastava, D. S., Mandhani, A and Mittal, R. D. Genetic polymorphisms of cytochrome P450 CYP1A1 (*2A) and microsomal epoxide hydrolase gene, interaction with tobacco-users, and susceptibility to bladder cancer: a study from North India. *Arch Toxicol*, 82(9): 633–639, 2008.
21. Yang, X., Wang, Y and Wang, G. Quantitative assessment of the influence of EPHX1 gene polymorphisms and cancer risk: a meta-analysis with 94,213 subjects. *J Exp Clin Cancer Res*, 28: 33-82, 2014.
22. Amador, A. G., Righi, P. D., Radpour, S., Everett, E. T., Weisberger, E., Langer, M., Eckert, G. J., Christen, A. G., Campbell, S. Jr., Summerlin, D. J., Reynolds, N and Hartsfield, J. K. Jr. Polymorphisms of xenobiotic metabolizing genes in oropharyngeal carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 93(4): 440-445, 2002.
23. Goode, E. L., White, K. L., Vierkant, R. A., Phelan, C. M., Cunningham, J. M., Schildkraut, J. M., Berchuck, A., Larson, M. C., Fridley, B. L., Olson, J. E., Webb, P. M., Chen, X., Beesley, J., Chenevix-Trench, G and Sellers, T. A. Xenobiotic-Metabolizing gene polymorphisms and ovarian cancer risk. *Mol Carcinog*, 50(5):397-402, 2011.
24. Tumer, T. B., Sahin, G and Arinç, E.Association between polymorphisms of EPHX1 and XRCC1 genes and the risk of childhood acute lymphoblastic leukemia, *Archives of Toxicology*, 86(3): 431–439, 2012.

25. Yu, W., Lin, Z and Qu, B. Impact of epoxide hydrolase 1 polymorphisms on lung cancer susceptibility in Asian populations. *Cell Biochem Biophys*, 71(2): 81320, 2015.
26. Yan, Y. L., Chen, X., Liang, H. J., Wang, J., Li, T. J., Li, R. L., Li, S and Qin, X. Lack of association between EPHX1 polymorphism and esophageal cancer risk: evidence from meta-analysis. *Dis Esophagus*, 28(2): 164-171, 2015.
27. Gong, W. F., He, W., Zhang, Q. M., Xiang, B. D., Ma, L., Huang, S., Bai, T., Zhong, J. H and Li, L. Q.Try113His and His139Arg polymorphisms in the microsomal epoxide hydrolase gene are not associated with risk of breast cancer. *TumourBiol*, 35(8): 8087-8093, 2014.
28. Ishibe, N., Sinha, R., Hein, D. W., Kulldorff, M., Strickland, P., Fretland, A. J., Chow, W. H., Kadlubar, F. F., Lang, N. P and Rothman, N. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. *Pharmacogenetics*, 12(2):145–150, 2002.
29. Katoh, T., Inatomi, H., Yang, M., Kawamoto, T., Matsumoto, T and Bell, D. A. Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) genes and risk of urothelial transitional cell carcinoma among Japanese. *Pharmacogenetics*, 9(3): 401–404, 1999.
30. Li, D., Jiao, L., Li, Y., Doll, M. A., Hein, D. W., Bondy, M. L., Evans, D. B., Wolff, R. A., Lenzi, R., Pisters, P. W., Abbruzzese, J. L and Hassan, M. M.Polymorphisms of cytochrome P4501A2 and N-acetyltransferase genes, smoking, and risk of pancreatic cancer. *Carcinogenesis*, 27(1): 103–111, 2006.
31. Hein, D. W., Leff, M. A., Ishibe, N., Sinha, R., Frazier, H. A., Doll, M. A., Xiao, G. H., Weinrich, M. C and Caporaso, N. E. Association of prostate cancer with rapid N-acetyltransferase 1 (NAT1*10) in combination with slow N-acetyltransferase 2 genotypes in a pilot case control study. *Environ Mol Mutagen*, 40(3): 161-167, 2002.
32. Krajinovic, M., Ghadirian, P., Richer, C., Sinnett, H., Gandini, S., Perret, C., Lacroix, A., Labuda, D and Sinnett, D. Genetic susceptibility to breast cancer in French-Canadians: role of carcinogen metabolizing enzymes and gene-environment interactions. *Int J Cancer*, 92(2): 220-225, 2001.

33. Fronhoffs, S., Brüning, T., Ortiz-Pallardo, E., Bröde, P., Koch, B., Harth, V., Sachinidis, A., Bolt, H. M., Herberhold, C., Vetter, H and Ko, Y. Realtime PCR analysis of the N-acetyltransferase NAT1 allele *3, *4, *10, *11, *14 and *17 polymorphism in squamous cell cancer of head and neck. *Carcinogenesis*, 22(9):1405-12, 2001
34. Majumder, M., Ghosh, S and Roy, B. Association between polymorphisms at N-acetyltransferase 1 (NAT1) & risk of oral leukoplakia & cancer, *Indian J Med Res*, 136(4): 605-613, 2012.
35. Mukherjee, S., Bhowmik, A. D., Roychoudhury, P., Mukhopadhyay, K., Ray, J. G and Chaudhuri, K. Association of XRCC1, XRCC3, and NAT2 polymorphisms with the risk of oral submucous fibrosis among eastern Indian population, *J Oral Pathol Med*, 41(4): 292-302, 2014.
36. Tian, F. S., Shen, L., Ren, Y. W., Zhang, Y., Yin, Z. H and Zhou, B. S.N-acetyltransferase 2 gene polymorphisms are associated with susceptibility to cancer: a meta-analysis. *Asian Pac J Cancer Prev*, 15(14): 5621-5626, 2014.
37. Cui, X., Lu, X., Hiura, M., Omori, H., Miyazaki, W and Katoh, T. Association of genotypes of carcinogen-metabolizing enzymes and smoking status with bladder cancer in a Japanese population. *Environ Health Prev Med*, 18(2): 136–142, 2013.
38. Frazier, M. L., O'Donnell, F. T., Kong, S., Gu, X., Campos, I., Luthra, R., Lynch, P. M and Amos, C. I. Age-associated risk of cancer among individuals with N-acetyltransferase 2 (NAT2) mutations and mutations in DNA mismatch repair genes. *Cancer Res*, 61(4): 1269–1271, 2001.
39. Unal, M., Tamer, L., Akbaş, Y., Pata, Y. S., Vayisoglu, Y., Degirmenci, U and Camdeviren, H. Genetic polymorphism of N-acetyltransferase 2 in the susceptibility to laryngeal squamous cell carcinoma. *Head Neck*, 27(12): 1056–1060, 2005.
40. Zhang, L., Xiang, Z., Hao, R., Li, R and Zhu, Y. N-acetyltransferase 2 genetic variants confer the susceptibility to head and neck carcinoma: evidence from 23 case-control studies. *TumourBiol*, 35(4): 3585-3595, 2014.
41. Boccia, S., Cadoni, G., Sayed-Tabatabaei, F. A., Volante, M., Arzani, D., De Lauretis, A., Cattel, C., Almadori, G., van. Duijn, C. M., Paludetti, G and Ricciardi,

G. CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4 and NAT2 polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer. *J Cancer Res ClinOncol*, 134(1): 93–100, 2008.

42. Chung, C. H., Yang, Y. H., Wang, T. Y., Shieh, T. Y and Warnakulasuriya, S. Oral precancerous disorders associated with areca quid chewing, smoking and alcohol drinking in southern Taiwan. *J Oral Pathol Med*, 34(8):460-466, 2005.
43. Gupta, P. C and Ray, C. S. Areca Nut Use and Cancer in India. *Biomed Res J*, 2(2):140–165, 2015.
44. Sharan, R. N., Mehrotra, R., Choudhury, Y and Asotra, K. Association of Betel Nut with Carcinogenesis: Revisit with a Clinical Perspective. *PLoS One*. 7(8): e42759, 2012.
45. Ihsan, R., Chauhan, P. S., Mishra, A. K., Yadav, D. S., Kaushal, M., Sharma, J. D., Zomawia, E., Verma, Y., Kapur, S and Saxena, S. Multiple Analytical Approaches Reveal Distinct Gene- Environment Interactions in Smokers and Non Smokers in Lung Cancer. *PLoS ONE*, 6 (12): e29431, 2011.
46. Ihsan, R., Devi, T. R., Yadav, D. S., Mishra, A. K., Sharma, J., Zomawia, E., Verma, Y., Phukan, R., Mahanta, J., Kataki, A. C., Kapur, S and Saxena, S. Investigation on the role of p53 codon 72 polymorphism and interactions with tobacco, betel quid, and alcohol in susceptibility to cancers in a high-risk population from North East India. *DNA Cell Biol*, 30(3): 163–171, 2011.
47. Kaushal, M., Mishra, A. K., Raju, B. S., Ihsan, R., Chakraborty, A., Sharma, J., Zomawia, E., Verma, Y., Kataki, A., Kapur, S and Saxena, S. Betel quid chewing as an environmental risk factor for breast cancer. *Mutat Res*, 703(2): 143–148, 2010.
48. Srivastava, D. S., Mandhani, A and Mittal, R. D. Genetic polymorphisms of cytochrome P450 CYP1A1 (*2A) and microsomal epoxide hydrolase gene, interactions with tobacco-users, and susceptibility to bladder cancer: a study from North India. *Arch Toxicol*, 82(9): 633–639, 2008.
49. Itsuo Chiba, Prevention of Betel Quid Chewers' Oral Cancer in the Asian- Pacific Area, *Asian Pacific Journal of Cancer Prevention*, 2(4): 263-269, 2001.

50. Lin, Y. C., Wu, D. C., Lee, J. M., Hsu, H. K., Kao, E. L., Yang, C. H and Wu, M. T. The association between microsomal epoxide hydrolase genotypes and esophageal squamous- cell-carcinoma in Taiwan: interaction between areca chewing and smoking. *Cancer Lett*, 237(2): 281–288, 2006.
51. Nisa, H., Budhathoki, S., Morita, M., Toyomura, K., Nagano, J., Ohnaka, K., Kono, S., Ueki, T., Tanaka, M., Kakeji, Y., Maehara, Y., Okamura, T., Ikejiri, K., Futami, K., Maekawa, T., Yasunami, Y., Takenaka, K., Ichimiya, H and Terasaka, R. Microsomal epoxide hydrolase polymorphisms, cigarette smoking, and risk of colorectal cancer: the Fukuoka Colorectal Cancer Study, *Mol Carcinog*, 52(8): 619-626, 2013.
52. Wang, D., Zhai, J. X., Zhang, L. M., Liu, D. W and Liu, X. H. EPHX1 Tyr113His polymorphism contributes to hepatocellular carcinoma risk: evidence from a meta-analysis. *MolBiol (Mosk),* 49(2):351-361, 2015.
53. Sivonova, M. K., Dobrota, D., Matakova, T., Dusenka, R., Grobarcikova, S., Habala, V., Salagovic, J., Tajtakova, M., Pidanicova, A., Valansky, L., Lachvacs, L., Kliment, J. Jr., Nagy, V and Kliment, J. Microsomal epoxide hydrolase polymorphisms, cigarette smoking and prostate cancer risk in the Slovak population, *Neoplasma*, 59(1): 79-84, 2012.
54. Khlifi, R., Messaoud, O., Rebai, A., Hamza-Chaffai, A. Polymorphisms in the Human Cytochrome P450 and Arylamine N-Acetyltransferase: Susceptibility to Head and Neck Cancers. *Biomed Res Int,* 2013:582768, 2013.
55. McKay, J. D., Hashibe, M., Hung, R. J., Wakefield, J., Gaborieau, V., Szeszenia-Dabrowska, N., Zaridze, D., Lissowska, J., Rudnai, P., Fabianova, E., Mates, D., Foretova, L., Janout, V., Bencko, V., Chabrier, A., Hall, J., Boffetta, P., Canzian, F and Brennan, P. Sequence variants of NAT1 and NAT2 and other xenometabolic genes and risk of lung and aerodigestive tract cancers in Central Europe. *Cancer Epidemiol Biomarkers Prev*, 17(1): 141-714, 2008.
56. Matejcic, M., Vogelsang, M., Wang, Y and Iqbal, P. M. NAT1 and NAT2 genetic polymorphisms and environmental exposure as risk factors for oesophageal squamous cell carcinoma: a case-control study. *BMC Cancer*, 18(15):150, 2015.

57. Tsukino, H., Kuroda, Y., Nakao, H., Imai, H., Inatomi, H., Osada, Y and Katoh, T. Cytochrome P450 (CYP) 1A2, sulfotransferase (SULT) 1A1, and N-acetyltransferase (NAT) 2 polymorphisms and susceptibility to urothelial cancer. *J Cancer Res ClinOncol*, 130(2): 99-106, 2004.
58. Hou, Y. Y., Ou, H. L., Chu, S. T., Wu, P. C., Lu, P. J., Chi, C. C, Leung, K. W., Lee, C. Y., Wu, P. H., Hsiao, M and Ger, L. P. NAT2 slow acetylation haplotypes are associated with the increased risk of betel quid-related oral and pharyngeal squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral RadiolEndod*, 112(4):484-492, 2011.
59. Nöthlings, U., Yamamoto, J. F., Wilkens, L. R., Murphy, S. P., Park, S. Y., Henderson, B. E., Kolonel, L. N and Le. Marchand, L. Meat and heterocyclic amine intake, smoking, NAT1 and NAT2 polymorphisms, and colorectal cancer risk in the multiethnic cohort study. *Cancer Epidemiol BiomarkersPrev*, 2009, 18(7): 2098–2106, 2009.
60. Brockmöller, J., Cascorbi, I., Kerb, R and Roots, I. Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferase M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder-cancer risk. *Cancer Res*, 56(17): 3915–3925, 1996.