6.1 Background

Exogenous and endogenous carcinogens have been established to be associated with increased cancer risk. The DNA repair genes play a vital role to tackle the carcinogen induced DNA damage and maintaining genomic stability and integrity of the cell [1, 2]. Molecular epidemiology studies have shown that inheritance of genetic variation in DNA repair genes and/or failure of DNA repair mechanism increase the genetic instability and carcinogenesis [3, 4].

Three major DNA repair mechanism have been identified*:* base-excision repair (BER), Nucleotide Excision Repair (NER) and direct repair have been studied for their individualistic variation in relation to susceptibility towards cancer [5]. The X-ray repair cross-complementing group 1 (XRCC1) gene involved in Base-excision repair (BER) of single strand breaks in DNA. The XRCC1 re-joins of DNA strand breaks by interacting with poly (ADP-ribose) polymerase and DNA ligase III and removes base adduct agents produced during oxidative damage, reduction or fragmentation [6-8]. Three XRCC1 polymorphism have been identified *viz.* 194 (Arg>Trp) in exon 6 and 399 (Arg>Gln) in exon 10 [9]. Previous studies demonstrated that the SNPArg194Trp and Arg399Gln have additive and/or synergistic impacts on the risk of development of oral [10], lung [11], breast [12] and ovarian cancers [10-13].

The xeroderma pigmentosum complementing groups (XPD) protein plays a role in NER pathway [14]. It is responsible for removal of bulky DNA lesions arising from smoking related DNA adduct to development of carcinogens. The function of NER genes encoded by the XPD gene, is a part of transition factor BTF2/TFIIH complex, which is multi-protein and perform function such as transcription, NER, transcription-coupled repair, apoptosis, and cell cycle regulation [1, 15]. Two numbers of polymorphism in codon 751 of exon 23 and codon 156 of exon 6 are known for XPD gene. Studies have reported the association of XPD polymorphism with tobacco exposure in upper aero-digestive tract cancer [16-17].

The O^6-Methylguanine -DNA methyltransferase (MGMT) is involved in direct repair of O^6-alkylguanine adducts and protects cells from cellular injury from carcinogenic and mutagenic effect [18, 19]. The chronic exposure to alkylating/ methylating agents

can lead to increased MGMT activity. The alkyltransferase binds to and removes alkyl groups from the O6 position of guanine in a single step [20]. Inactivation of MGMT increases efficacy of alkylating agent against cancer [20]. The MGMT polymorphism gene have also been associated with increased risk of cancer [19].

The earlier studies have reported that polymorphism in XRCC1, XPD, MGMT are associated with head and neck [5, 21], lung [22], urinary bladder [23], nasopharynx [24] and aero-digestive cancer [25]. However, none of the study define the polymorphism in these genes induces risk for HNC in high betel nut, tobacco, smoking and alcohol exposure population of North East India. Therefore, we have made an attempt to investigate the prevalence of HNC cases with deciphering the role of XRCC1, XPD and MGMT, and their interaction with intake of betel nut, tobacco, smoking and alcohol consumption in HNC patients of NE region. In the present study we have established the relation between genetic polymorphism with joint effects, gene–gene and gene–environment interactions with synergistic effect of BN, tobacco, smoking and alcohol consumption on the risk of HNC in NE India population.

6.2 Material and Methods

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 and 4.2.

6.2.1 DNA isolation

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

6.2.2 Genotyping Analysis

Polymerase chain reaction (PCR) with restriction fragment length polymorphism evaluate was used for the genotyping. The XRCC1 Arg194Trp, XPD Arg156Arg, MGMT Trp65Cys and MGMT Leu84Phe polymorphisms were described [5]. The details of primers sequences and PCR conditions are mentioned in (Table 1). The restriction digestion carried out are as follow XRCC1 Arg194Trp exon 6 (C→T),

digested with Pvu II enzyme 5 U (New England Biolabs) at 37º C 12- 16 hrs, XPD Arg156Arg (C→ A), digested with 6 Tfi I, enzyme 5 U (New England Biolabs) at 37º C 12- 16 hrs., MGMT Trp65Cys, (G→ C), digested with Mva I, enzyme 5 U (New England Biolabs) and MGMT Leu84Phe, (C→ T), digested with Ear I enzyme 5 U (New England Biolabs) at 37º C 12- 16 hrs [5]. All PCR-RFLP products were visualized in 2- 3 % agarose gel (Sigma, USA) stained with ethidium bromide (Sigma, USA) in Gel Doc system G-Box (SYNGENE, USA), as shown in figure 19-21. 20 % results were cross checked and the results obtained were found to be 100 % concordant

6.2.3 Statistical analysis

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

Table 30: Sequence of oligonucleotide primers and reaction conditions used for XRCC1 Arg194Trp, XPD (ERCC2) exon 6, MGMT Trp65Cys, and MGMT 84 Leu84Phe

Genotype	Primers (5' to 3')	Alleles (bp)	PCR Condition
XRCC1	5'-GCCAGGGCCCCTCCTTCAA-3'	CC: 485	
Arg194Trp	5'- TACCCTCAGACCCACGAGT- 3'	CT: 485, 396, 89	95°C/ 5 min, 35 cycles
		TT: 396, 89	94°C/ 20 sec
			54°C/ 20 sec
			72°C/ 20 sec
			72°C/ 5 min
XPD (ERCC2)	5'-TGGAGTGCTATGGCACGATCTCT-3'	CC: 587, 57	95°C/ 5 min, 35 cycles
exon 6	5'-CCATGGGCATCAAATTCCTGGGA-3'	CA: 587, 474, 113, 57	94°C/ 20 sec
		AA: 474, 113, 57	62°C/ 20 sec
			72°C/ 20 sec
			72°C/ 5 min
MGMT	5'- CTAAGCCCCTGTTCTCACTTTT- 3'	GG: 110, 91	95°C/ 9 min, 32 cycles
Trp65Cys	5'- ACACCGCAGATGGCTTAGTTAC-3'	GC: 201, 110, 91	95°C/ 20 sec
		CC: 201	55°C/ 20 sec
			72°C/ 20 sec
			72°C/ 5 min
MGMT 84	5'- CTAAGCCCCTGTTCTCACTTTT-3'	CC: 128, 53, 20	95°C/ 5 min, 32 cycles
Leu84Phe	5'- ACACCGCAGATGGCTTAGTTAC-3'	CT: 128, 73, 53, 20	94°C/ 20 sec
		TT: 128, 73	55 C/ 20 sec
			72°C/ 20 sec
			72°C/ 5 min

6.3 Results

In continuous with earlier study that indicated risk associated with BN, tobacco, smoking and alcohol habits, we analyzed their risk in relation to starting age. As shown in (Table 31).We observed that chewing of tobacco and smoking habits impose risk for HNC, irrespective of starting age. However, among BN consumer, started only before 20 years exhibit HNC risk. The combined habit of BN consumption with smoking and BN with tobacco and smoking increased risk of HNC, if started only before 20 years,

however, combination of BN and tobacco increases risk for HNC, irrespective of starting age.

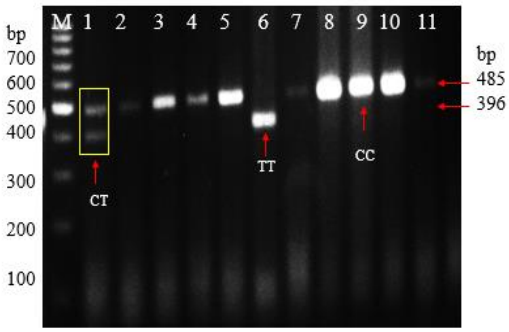

Figure 19: XRCC1 Arg194Trp genotype analyzed by (PCR-RFLP) the PCR product digested with **PvuII** from eleven samples and three genotypes was seen, the yield were fragments with 485 bp for CC; 485, 396, 89 for CT and 396, 89 bp for TT; lane M- 100 bp ladder

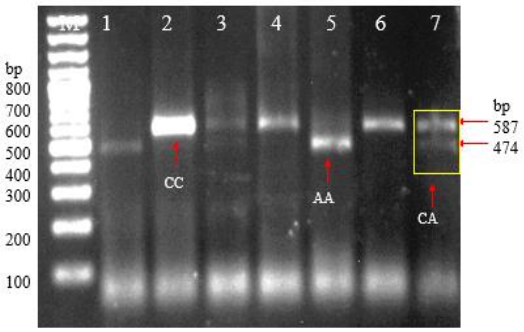

Figure 20: XPD genotype analyzed by (PCR-RFLP) the PCR product digested with **TfiI** from seven samples and three genotypes was seen, the yield were fragments with 587, 57 bp for CC; 587, 474, 113, 57 for CA and 474, 113, 57 bp for AA; lane M – 100 bp ladder

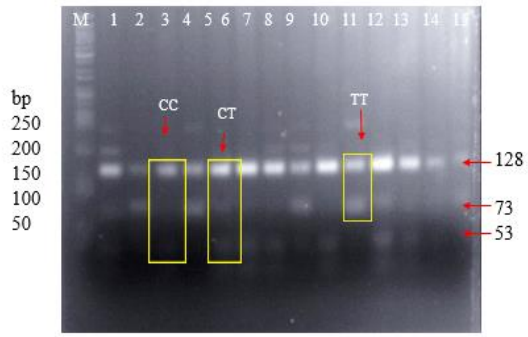

Figure 21: MGMT Leu84Phe genotype analyzed by (PCR-RFLP) the PCR product digested with **EarI** from fifteen samples and three genotypes was seen, the yield were fragments with 128, 53, 20 bp for CC; 128, 73, 53 for CT and 128, 73 bp for TT; lane M – 50 bp marker

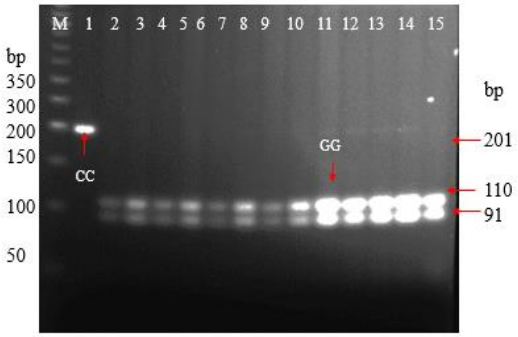

Figure. 22: MGMT Trp65cys genotype analyzed by (PCR-RFLP) the PCR product digested with **MvaI** from fifteen samples and two genotypes has seen the yielded were fragments with 110, 91 bp for GG; and 201 bp for CC; lane M- 50 bp marker

Table 31: Characteristic and Duration of dietary habits of HNC cases and healthy control

Duration with starting age	Cases (205) (%)	Control (210) (%)	Crude OR [95% CI]	P – value	Adjusted ORa [95% CI]	P – value
Betel nut chewers						
Never	21 (10.24	60 (28.57)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	152 (74.14	89 (42.38)	4.88 [2.78-8.55]	**<0.001**	3.31 [1.79-6.19]	**<0.001**
>20 years	32 (15.60)	61 (29.04)	1.49 [0.77-2.88]	0.22	1.00 [0.71-2.45]	0.56
Tobacco chewers						
Never	73 (35.60)	158 (75.23)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	103 (50.24)	32 (15.23)	6.96 [4.29-11.30]	**<0.001**	5.01 [2.96-8.45]	**<0.001**
>20 years	29 (14.14)	20 (9.52)	3.13 [1.66-5.91]	**<0.001**	4.18 [1.93-9.07]	**<0.001**
Smoking						
Never	118 (57.56)	162 (77.14)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	6 (29.75)1	33 (15.71)	2.53 [1.56-4.12]	**<0.001**	2.35 [1.35-4.10]	**<0.01**
>20 years	26 (12.68)	15 (7.14)	2.38 [1.20-4.68]	**<0.01**	2.53[1.12-5.75]	**0.02**
Alcohol						
Never	132 (64.39)	143 (68.09)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	45 (21.95)	40 (19.04)	1.21 [0.74-1.98]	0.42	1.40 [0.79-2.47]	0.24
>20 years	28 (13.65)	27 (12.85)	1.12 [0.63-2.00]	0.69	0.74 [0.37-1.46]	0.39
Betel nut +Tobacco						
Never	105 (51.21)	164 (78.09)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	80 (39.02)	34 (16.19)	3.67 [2.29-5.88]	**<0.001**	3.72 [2.32-5.97]	**<0.001**
>20 years	20 (9.75)	12 (5.71)	2.60 [1.22-5.54]	**0.01**	2.55 [1.19-5.47]	**0.01**
Betel nut + smoking						
Never	140 (68.29)	191 (90.95)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	53 (25.85)	12 (5.71)	6.02 [3.10-11.69]	**<0.001**	6.26 [3.21-12.21**]**	**<0.001**
>20 years	12 (5.85)	7 (3.33)	2.33 [089-6.09]	0.08	2.19 [0.83-5.76]	0.11
Betel nut + tobacco + smoking						
Never	172 (83.90)	195 (92.85)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	27 (13.17)	10 (4.76)	3.06 [1.44-6.50]	**<0.01**	3.19 [1.49-6.84]	**<0.01**
>20 years	6 (2.92)	5 (2.38)	1.36 [0.40-4.53]	0.61	1.26 [0.37-4.27]	0.70
Betel nut + alcohol						
Never	163 (79.51)	183 (87.14)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	34 (16.58)	19 (90.04)	2.00 [1.10-3.66]	**0.02**	2.02 [1.10-3.68]	**0.02**
>20 years	8 (3.90)	8 (3.80)	1.12 [0.41-3.05]	0.82	1.04 [0.38-2.87]	0.98
Betel nut + tobacco+ alcohol						
Never	181 (88.29)	198 (94.28)	**1.0 (referent)**		**1.0 (referent)**	
≤20 years	13 (6.34)	6 (2.85)	2.37 [0.88-6.36]	0.08	2.44 [0.90-6.58]	0.07
>20 years	11 (5.36)	6 (2.85)	2.00 [072-5.53]	0.17	1.97 [0.71-5.47]	0.18

P<0.05 is considering being significance by chi-square ($\chi 2$), OR (odds ratio), CIs (confidence Intervals), OR**[a]** (Adjusted in multivariate logistic regression models) including age, gender, betel nut, tobacco, smoking

6.3.1 Genotype distribution and risk assessment

The distribution of the genotype of XRCC1 *Arg194Trp*, XPD Arg156Arg, MGMT *Trp65cys and* MGMT *Leu84Phe* polymorphism among the cases and controls are showed in (Table 32). The distribution of genotypes was found to follow the Hardy Weinberg equilibrium (data not shown). When the CC genotype of XRCC1

Arg194Trp and XPD Arg156Arg was considered as reference group, the CT (OR^a, 1.68, 95%CI 1.04-2.73, P=0.03), TT (OR^a, 3.58, 95%CI 1.01-12.65, P=0.04), and CA (OR^a, 1.70, 95%CI 1.02-2.84, P=0.04), AA (OR^a, 3.56, 95%CI 1.83-6.91, P<0.001] genotype of XRCC1 and XPD, respectively have greater risk for HNC. The reference XRCC1 CC genotype in interaction with mutant XPD (AA) genotype and MGMT 84 (TT) genotype has increase the risk of HNC. The study demonstrated interaction of mutant genotypes of XRCC1, XPD and MGMT-84 with each other. The XRCC1 (CC) genotype exhibited strong interaction with mutant XPD (AA) and MGMT-84 (TT) and resulted in 3.92 and 3.52 fold increase in the risk for HNC. Interestingly we observed heterozygous XRCC1 (CT) genotype interacted with XPD (CA, AA) and MGMT-84 (CC) and increased the risk for HNC 4.09, 4.09 and 2.63 folds respectively. However, wild genotype of MGMT-84 (CC) association with XRCC1 (TT) and XPD (AA, CA) genotypes showed 12.95, 4.96 and 2.40 fold increased HNC risk. The heterozygous genotype of XPD (CA) also exhibited strong association with MGMT-84 (CT) and mutant XPD exon 6 (AA) genotypes increased the risk for HNC 2.49 and 4.18 folds respectively.

6.3.2 Interaction of betel nut, tobacco, smoking and alcohol habit with genotypes of XRCC1 Arg194Trp, XPD exon 6 and MGMT Leu84Phe of HNC risk.

In our present analysis, we also evaluated the interaction and association between betel nut, tobacco, smoking and alcohol habits and the genetic polymorphism of XRCC1 Arg194Trp, XPD Arg156Arg and MGMT Leu84Phe for HNC development (Table 33). The results showed that XPD Arg156Arg having AA (mutant) had the higher risk for HNC with BN (OR^a, 3.05, 95%CI 1.49-6.23, P<0.01), tobacco (OR^a, 3.55, 95%CI 1.27-9.89, P=0.01), smoking (OR^a, 3.88, 95%CI 0.92-16.36, P=0.03) and alcohol (OR^a, 5.14, 95%CI 2.20-12.01, P<0.001) habits respectively. However, the heterozygous (CA) genotype of XPD Arg156Arg was associated with BN (OR^a, 1.82 95%CI 1.05-3.17, P=0.03) and tobacco (OR^a, 2.50 95%CI 1.18-5.23, P=0.01) had increased risk of HNC. Contrastingly, XRCC1 Arg194 Trp (CT) genotype had showed increased risk for HNC with smoking (OR^a, 2.48 95%CI 1.00-6.14 P=0.04) and alcohol (OR^a = 2.16 95%CI 1.65-4.03 P=0.01) habit. In case of XRCC1 the mutant allele (TT) exhibited association with alcohol consumption (OR^a = 4.49

95%CI 1.10-26.62 P=0.03) and heterozygous allele (CT) of MGMT-84 (OR[a] = 2.42 95%CI 1.00-5.87, P=0.04) was associated with smoking habits showed increased risk for HNC development.

Table 32: Distribution of Polymorphism and combined genotype of DNA repair genes in HNC patient and healthy control

Genotype	Case (n=205) (%)	Control (n=210) (%)	Crude OR [95% CI]	p- value	Adjusted OR[a] [95% CI]	p- value
XRCC1 Arg194Trp						
CC	123 (60)	154 (73.33)	**1.0 (referent)**			
CT	69 (34)	52 (24.76)	1.66[1.06-2.62]	**0.02**	1.68 [1.04-2.73]	**0.03**
TT	13 (6)	4 (1.90)	4.07[1.20-15.20]	**0.01**	3.58 [1.01-12.65]	**0.04**
XPD (ERCC2) EXON 6						
CC	47 (22.92)	79 (37.61)	**1.0 (referent)**			
CA	106 (51.21)	102 (48.57)	1.74 [1.11-2.74]	**0.01**	1.70 [1.02-2.84]	**0.04**
AA	52 (25.85)	29 (13.80)	3.01 [1.68- 5.38]	<**0.001**	3.56 [1.83-6.91]	<**0.001**
MGMT Trp65Cys						
GG	204 (99.51)	210 (100)	**1.0 (referent)**			
GC	1 (0.48)	0 (0.00)	-	-	-	-
CC	0 (00)	0 (0.00)	-	-	-	-
GC+ CC	1(0.49)	0 (0.00)	-	-	-	-
MGMT Leu84Phe						
CC	120 (58.53)	130 (61.90)				
CT	70 (34.14)	68 (32.38)	1.11 [0.73-1.69]	0.60	1.23 [0.77-1.96]	0.37
TT	15 (7.31)	12 (5.71)	1.35 [0.60- 3.00]	0.45	1.59 [0.66-3.82]	0.29
XRCC1 +XPD						
CC+CC	27 (13.17)	54 (25.71)	**1.0 (referent)**			
CC+CA	67 (32.68)	82 (39.04)	1.63 [0.93-2.87]	0.08	1.53 [0.81-2.89]	0.18
CC+AA	29 (14.14)	18 (8.57)	3.22 [1.52-6.80]	<**0.01**	3.92 [1.68-3.16]	<**0.01**
CT+CC	16 (7.80)	23 (10.95)	1.39 [0.63-3.05]	<**0.001**	1.23 [0.50.2.98]	**0**.64
CT+CA	34 (16.58)	18 (8.57)	3.77 [1.81-787]	<**0.01**	4.09 [1.80-9.32]	<**0.01**
CT+AA	19 (9.26)	11 (5.23)	3.45 [1.44-8.28]	0.12	4.09 [1.55-10.81]	<**0.01**
TT+CC	4 (1.95)	2 (0.95)	4.00 [0.68-23.22]	0.06	4.13 [0.59-28.95]	0.15
TT +CA	5 (2.43)	2 (0.95)	5.00 [0.91-27.47]	0.99	4.80 [0.72-31.95]	0.10
TT +AA	4 (1.95)	0 (0.00)	-	-		
XRCC1+MGMT 84						
CC+CC	67 (32.68)	99 (47.14)	**1.0 (referent)**			
CC+CT	43 (20.97)	47 (23.38)	1.35 [0.80-2.26]	0.25	1.74 [0.96-3.16]	0.06
CC+TT	13 (6.34)	8 (3.80)	2.40 [0.94-6.10]	0.06	3.52 [1.25-9.88]	**0.01**
CT+CC	43 (20.97)	30 (14.28)	2.11 [1.21-3.70]	<**0.01**	2.63 [1.39-4.96]	<**0.01**
CT+CT	24 (11.70)	20 (9.52)	1.77 [0.90-3.36]	0.09	1.85 [0.86-3.99]	0.11
CT+TT	2 (0.97)	2 (0.95)	1.47 [0.20-10.78]	0.70	1.03 [0.11-9.56]	0.97
TT+CC	10 (4.87)	1 (0.47	14.77 [1.84-1118.1	**0.01**	12.95 [1.45-115.5]	**0.02**
TT+CT	3 (1.46)	1 (0.47	4.43 [0.45-43.52]	0.20	6.14 [0.55-67.74]	0.13
TT+TT	0 (0.00)	2 (0.95)	-	-	-	-
XPD+MGMT84						
CC+CC	25 (12.19)	50 (23.80)	**1.0 (referent)**			
CC+CT	18 (8.78)	24 (11.42)	1.50 [0.69-3.36]	0.30	2.07 [0.85-5.11]	0.10
CC+TT	4 (1.95)	5 (2.38)	1.60 [0.39-6.48]	0.51	3.09 [0.64-14.92]	0.15
CA+CC	67 (32.68)	66 (31.42)	2.03 [1.12-3.65]	**0.01**	2.40 [1.22-4.72]	**0.01**
CA+CT	32 (15.60)	29 (13.80)	2.20 [1.10-4.42]	**0.02**	2.49 [1.12-5.52]	**0.02**
CA+TT	7 (3.41)	7 (3.33)	2.00 [0.63-6.33]	0.23	2.01 [0.55-7.33]	0.28
AA+CC	28 (13.66)	14 (6.66)	4.00 [1.75-8.91]	<**0.01**	4.96 [1.97-12.44]	<**0.01**
AA+CT	20 (9.75)	5 (2.38)	2.66 [1.17-6.07]	**0.02**	4.18 [1.60-10.89]	<**0.01**
AA+TT	4 (1.95)	0 (0.00)	--	-	-	-

P<0.05 is considering being significance by chi-square ($\chi 2$), OR (odds ratio), CIs (confidence Intervals), OR[a] (Adjusted in multivariate logistic regression models) age, gender, betel nut, tobacco, smoking

6.3.3 Stratification of BN, tobacco and alcohol consumption and HNC risk

The distribution of XRCC1 Arg194Trp, XPD Arg156Arg and MGMT Leu84Phe genotypes with varying combinations of BN, tobacco and alcohol consumption were analyzed to estimate the risk for HNC and the findings are presented in (Table 34 and 35). The findings demonstrated that the consumer of BN in lower dose (L) having XRCC1 CT genotype exhibit 4.71 fold risk for HNC, which is further increased to 6.78 and 19.12 fold for addition of tobacco lower and higher dose, respectively. The risk for the same genotype with BN (L) habit is also increased to 6.49 fold, when alcohol (L) is added. Similarly, the risk for HNC is increased in case of XRCC1 TT genotype exposed to lower doses of BN (13.09 fold) and higher dose of tobacco in combination with BN (19.12 fold). Interestingly synergistic increase were also found with higher doses of BN (9.69 fold) and tobacco (19.12 fold) with the mutant allele (TT) of XRCC1.

The estimated risk for HNC is found to be increased to 5.29 fold in subjects carrying XPD CA genotype having BN (L) consuming habit. This risk is again increased along with increase in dose of BN itself. Furthermore, addition of lower and higher dose of tobacco increases the risk to 7.04 and 17.31 respectively. In case of XPD AA genotype risk associated with BN is also found to be enhanced from 8.70 to 11.81 fold, with higher doses of BN.

We found from the present study that MGMT -84 TT and CT genotype exhibited higher risk for HNC. The risk (4.07 fold) associated with mutant allele CT MGMT-84 in BN (L) chewing habits which increased, in combination with lower dose of tobacco (10.00 fold) and higher dose of alcohol (5.61 fold).

Table 33: Distribution frequency of DNA repair genotype and effect of betel nut, tobacco, smoking, alcohol habits in cases of HNC patients and healthy controls

	Betel nut			Tobacco			Smoking			Alcohol		
Genotype	**Cases /Control (n=182/150)**	**Adjusted OR [95%CI]**	**P- value**	**Cases /Control (n=132/52)**	**Adjusted OR[a] [95%CI]**	**P- value**	**Cases /control (n=87/48)**	**Adjusted OR[a] [95%CI]**	**P- value**	**Cases /Control (n=76/64)**	**Adjusted OR[a] [95%CI]**	**P- value**
XRXCC1 Arg194 Trp												
CC	110/108	**1.0 (referent)**		80/40	**1.0 (referent)**		47/36	**1.0 (referent)**		50/45	**1.0 (referent)**	
CT	62/39	1.58 [0.94-2.66]	0.08	44/11	2.00 [0.92-4.35]	0.07	30/11	2.48 [1.00 -6.14]	**0.04**	22/18	2.16 [1.65-4.03]	**0.01**
TT	10/3	2.94 [0.71-12.20]	0.13	8/1	3.08 [0.35-27.18]	0.31	10/1	7.66 [0.87-67.26]	0.06	4/1	4.49 [1.10-26.62]	**0.03**
XPD												
CC	41/53	**1.0 (referent)**		29/22	**1.0 (referent)**		24/15	**1.0 (referent)**		15/23	**1.0 (referent)**	
CA	96/75	1.82 [1.05-3.17]	**0.03**	72/23	2.50 [1.18-5.23]	**0.01**	43/30	0.94 [0.40-2.21]	0.79	45/32	1.79 [0.91-3.51]	0.08
AA	45/22	3.05 [1.49-6.23]	**<0.01**	31/7	3.55 [1.27-9.89]	**0.01**	20/3	3.88 [0.92-16.36]	0.03	16/9	5.14 [2.20-12.01]	**<0.001**
MGMT Leu84Phe											s	
CC	108/96	**1.0 (referent)**		82/33	**1.0 (referent)**		49/33	**1.0 (referent)**		43/38	**1.0 (referent)**	
CT	60/46	1.19 [0.71-1.99]	0.48	42/16	1.09 [0.53-2.24]	0.81	34/11	2.42 [1.00- 5.87]	**0.04**	2621	1.35 [0.73-2.48]	0.32
TT	14/8	1.75 [0.66-4.64]	0.26	8/3	1.34 [0.32-5.67]	0.68	4/4	0.85 [0.17-4.14]	0.84	7/5	2.73 [0.80-9.24]	0.10

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, betel nut, tobacco, smoking

Table 34: Interaction of DNA repair genotypes with doses of betel nut and tobacco HNC risk in betel nut- tobacco associated cases.

		Betel nut Betel nut dose					betel nut-tobacco Tobacco doses				
Dose/ day	**Genotype**	**Cases /Control (n=205/210)**	**Crude OR [95%CI]**	**P- value**	**Adjusted OR[a] [95%CI]**	**P- value**	**Cases /Control (n=205/210)**	**Crude OR [95%CI]**	**P- value**	**Adjusted OR[a] [95%CI]**	**P- value**
XRCC1 Arg194 Trp	CC	13/46	**1.0 (referent)**				48/117	**1.0 (referent)**		**1.0 (referent)**	
Never	CT	7/13	1.90 [0.63-5.75]	0.25	1.85 [0.611-5.61]	0.27	28/41	1.66 [0.92-2.99]	0.08	1.62 [0.89-2.96	0.11
	TT	3/1	10.61 [101-110.7]	0.04	10.53 [100-110.7]	**0.04**	7/3	5.68 [1.41-22.91]	**0.01**	4.95 [1.20-20.40]	0.02
≤10 times /day	CC	94/97	3.42[1.74-6.75]	**<0.001**	3,45 [1.75-6.82]	**<0.001**	49/26	4.59 [2.56-8.22]	**<0.001**	4.63 [2.56-8.38]	**<0.001**
	CT	51/39	4.62 [2.20-9.73]	**<0.001**	4.71 [2,23-9.93]	**<0.001**	24/9	6.50 [2.81-15.00]	**<0.001**	6.78 [2.89-15.88]	**<0.001**
	TT	7/2	12.38[2.29-66.96]	**<0.01**	13.09 [2.40-71.24]	**<0.01**	2/1	4.87 [0.43-55.03]	0.20	3.56 [0.30-41.25	0.31
>10 times /day	CC	16/11	5.14[1.92-13.76]	**<0.01**	5.37 [1.99-14.45]	**<0.01**	26/11	5.76 [2.63-12.58]	**<0.001**	5.68 [2.52-12.70]	**<0.001**
	CT	11/0	-	**-**	-	**-**	17/2	20.71[4.60-93.15]	**<0.011**	19.12 [41.9-87.31]	**<0.001**
	TT	3/1	10.61[1.01-110.7]	**0.04**	9.69 [0.92-101.5]	**0.05**	4/0	-	**-**	-	-
XPD	CC	6/26	**1.0 (referent)**				21/57	**1.0 (referent)**		**1.0 (referent)**	
Never	CA	10/27	1.60 [0.51-5.05]	0.41	1.62 [0.51-5.12]	0.40	40/82	1.32 [0.702.47]	0.38	1.21 [0.63-2.30]	0.56
	AA	7/7	4.33 [1.09-17.10]	**0.03**	4.53 [1.14-18.00]	**0.03**	22/22	2.71 [1.25-.5.88]	**0.01**	3.00 [1.35-6.65]	0.07
≤10 times /day	CC	35/51	2.97 [1.10-7.97]	**0.03**	3.06 [1.14-8.25]	**0.02**	15/15	2.71 [1.13-6.50]	**0.02**	2.39 [0.97-5.93]	**0.05**
	CA	80/68	5.09 [1.98-13.11]	**<0.01**	5.29 [2.05-13.66]	**<0.01**	38/16	6.44 [2.98-13.91]	**<0.001**	7.04 [3.20-15.46]	**<0.01**
	AA	37/19	8.43 [2.96-24.01]	**<0.001**	8.70 [3.04-24.85]	**<0.001**	22/5	11.94 [4.00-36.60]	**<0.001**	11.97 [3.92-36.50]	**<0.01**
>10 times /day	CC	6/2	13.00 [2.08-81.04]	**0.01**	13.67 [2.18-85.84]	**<0.01**	11/7	4.26 [1.46-12.45]	**<0.01**	4.06 [1.33-12.42]	**0.01**
	CA	16/7	9.90 [2.82-34.77]	**<0.001**	10.44 [2.95-36.94]	**<0.001**	28/4	19.00 [5.95-60.66]	**<0.001**	17.31[5.30-56.46]	**<0.001**
	AA	8/3	11.55 [2.34-57.03]	**<0.01**	11.81 [2.38-58.56]	**<0.01**	8/2	10.85 [2.13-55.31]	**<0.01**	10.11[1.89-53.85]	**<0.01**
MGMT84	CC	12/34	**1.0 (referent)**				45/99	**1.0 (referent)**		**1.0 (referent)**	
Never	CT	10/22	1.28 [0.47-3.48]	0.61	1.31 [0.49-3.63]	0.57	19/35	1.19 [0.61-2.31]	0.59	1.18 [0.6-2.32]	0.62
	TT	1/4	0.70 [0.07-6.98]	0.76	0.69 [0.07-6.91]	0.75	7/9	1.71 [0.60-4.88]	0.31	1.60 [0.55-4.66]	0.38
≤10 times /day	CC	85/88	2.73 [1.32-5.63]	**<0.01**	2.85 [1.37-5.91]	**<0.01**	37/25	3.25 [1.75-6.03]	**<0.001**	3.34 [1.78-6.29]	**<0.001**
	CT	58/42	3.91 [1.81-8.43]	**<0.01**	4.07 [1.87-8.83]	**<0.001**	37/8	10.17 [4.38-23.60]	**<0.001**	10.0 [4.30-23.68]	**<0.001**
	TT	9/8	3.18 [1.00-10.14]	**0.05**	3.20 [1.00-10.23]	**0.04**	1/3	0.73 [0.07-7.24]	0.79	0.75 [0.07-7.53]	0.81
>10 times /day	CC	23/8	8.14 [2.88-23.03]	**<0.01**	8.84 [2.98-24.16]	**<0.001**	38/6	13.93 [5.49-35.32]	**<0.001**	12.65 [4.91-32.58]	**<0.001**
	CT	2/4	1.41 [0.22-8.74]	0.70	1.38 [0.22-8.67]	0.72	2/7	0.62 [0.12.-3.14]	0.57	0.58 [0.11-3.01]	0.51
	TT	5/0		-	-	-	7/0	-		-	--

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, smoking

Table 35: Interaction of DNA repair genotypes and doses of alcohol on the risk for HNC in betel nut – alcohol and betel nut- tobacco associated cases

		Betel nut Alcohol doses					Betel nut –Tobacco Alcohol doses				
Dose/ day	**Genotype**	**Cases /Control (n=205/210)**	**Crude [95%CI]**	**P- value**	**Adjusted OR[a] [95%CI]**	**P- value**	**Cases /Control (n=205/210)**	**Crude OR [95%CI]**	**P- value**	**Adjusted OR[a] [95%CI]**	**P- value**
XRCC1	CC	77/129	**1.0 (referent)**				71/94	**1.0 (referent)**		**1.0 (referent)**	
Never	CT	47/41	1.92 [1.15-3.18]	**0.01**	1.95 [1.16-3.27]	**0.01**	32/37	1.14 [0.65-2.01]	0.63	1.09 [0.61-1.95]	0.76
	TT	10/3	5.58 [1.49-20.92]	**0.01**	4.64 [1.21-17.71]	**0.02**	8/2	5.29 [1.09-25.70]	**0.03**	5.37 [1.07-26.90]	**0.04**
≤120 ml/ day	CC	8/12	1.11 [0.43-2.85]	0.81	1.34 [0.51-3.50]	0.54	36/39	1.22 [0.70-2.11]	0.47	1.34 [0.76-2.36]	0.30
	CT	6/7	1.43 [0.46-4.42]	0.52	1.47 [0.4604.68]	0.50	19/14	1.79 [0.84-3.82]	0.12	1.74 [0.80-3.79]	0.15
	TT	1/0	-	-	-	-	3/0	-	-	-	-
>120 ml /day	CC	38/13	4.89 [2.45-9.76]	**<0.001**	5.11 [2.50-10.43]	**<0.001**	22/15	1.94 [0.94-4.09]	0.07	1.96 [0.93-4.16]	0.07
	CT	16/4	6.70 [2.16-20.77]	**<0.01**	6.49 [2.04-20.64]	**<0.01**	10/9	1.47 [0.56-3.81]	0.42	1.18 [0.44-3.15]	0.74
	TT	2/1	3.35 [0.29-37.56]	0.32	2.30 [0.19-26.66]	0.50	4/0	-	-	-	-
XPD	CC	33/66	**1.0 (referent)**				35/43	**1.0 (referent)**		**1.0 (referent)**	
Never	CA	65/84	1.54 [0.91-2.62]	0.10	1.55 [0.90-2.67]	0.11	57/65	1.07 [0.60-1.90]	0.79	1.17 [0.65-2.11]	0.58
	AA	36/22	3.27 [1.66-6.42]	**<0.01**	3.50 [1.75-6.98]	**<0.001**	19/25	0.93 [0.44-1.96]	0.85	0.94 [0.43-2.02]	0.87
≤120 ml/ day	CC	0/7	-	-	-	-	12/18	0.81 [0.34-1.92]	0.64	0.91[0.38-2.21]	0.85
	CA	12/7	3.42 [1.23-9.52]	**0.01**	3.42 [1.20-9.71]	**0.02**	26/27	1.14 [0.56-2.28]	0.71	1.26 [0.61-2.57]	0.52
	AA	3/5	1.20 [0.27-5.33]	0.81	1.45 [0.31-6.64]	0.62	20/8	3.51 [1.33-9.25]	0.11	3.84 [1.42-10.38.]	**<0.01**
>120 ml /day	CC	14/5	5.60 [1.85-16.87]	**<0.01**	5.27 [1.69-16.47]	**<0.01**	8/10	0.98 [0.35-2.75]	0.97	1.18 [0.40-3.44]	0.75
	CA	29/11	5.27 [2.34-11.85]	**<0.001**	5.51 [2.39-12.71]	**<0.001**	21/11	2.34 [0.99-5.51]	**0.05**	2.14 [0.89-5.15]	0.08
	AA	13/2	13.00 [2.77-61.01]	**<0.01**	13.25 [2.75-63.82	**<0.01**	7/3	2.86 [0.69-11.91]	0.14	3.01 [0.70-12.93]	0.13
MGMT84	CC	80/111	**1.0 (referent)**				60/84	**1.0 (referent)**		**1.0 (referent)**	
Never	CT	46/54	1.18 [0.72-1.92]	0.50	1.21 [0.73-2.00]	0.44	42/42	1.40 [0.81-2.40]	0.22	1.46 [0.83-2.57]	0.18
	TT	8/8	1.38 [0.50-3.85]	0.53	1.28 [0.45-3.65]	0.63	9/7	1.80 [0.63-5.10]	0.26	1.79 [0.61-5.22]	0.28
≤120 ml/ day	CC	10/8	1.73 [0.65-4.58]	0.26	1.80 [0.66-4.89]	0.24	22/40	0.77 [0.41-1.42]	0.40	0.84 [0.44-1.58]	0.59
	CT	2/9	0.30 [0.06-1.46]	0.13	0.35 [0.07-1.73]	0.20	33/11	4.20 [1.96-8.96]	**<0.001**	4.41 [2.01-9.65]	**<0.001**
	TT	3/2	2.08 [0.34-12.74]	0.42	2.71[0.43-16.72]	0.28	2/2	1.40 [0.19-10.21]	0.74	1.98 [0.26-14.96]	**0.05**
>120 ml /day	CC	30/11	3.78 [1.79-7.99]	**<0.001**	3.94 [1.82-8.52]	**<0.01**	27/17	2.22 [1.11-4.43]	**0.02**	2.24 [1.10-4.57]	**0.02**
	CT	22/5	6.10 [2.21-16.80]	**<0.001**	5.61 [1.99-15.77]	**<0.001**	3/6	0.70 [0.16-2.91]	0.62	0.58 [0.13-2.55]	0.47
	TT	4/2	2.77 [0.49-15.52]	0.24	3.02 [0.50-18.09]	0.22	6/1	8.40 [0.98-71.59]	**0.05**	7.61 [0.86-66.98]	0.06

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, smoking

6.4 Discussion

In the present study, we evaluated the risk of HNC associated with gene polymorphisms, including BER (XRCC1 Arg194Trp), NER (XPD Arg156Arg) and MGMT Leu84Phe genes. The finding of our study demonstrated that cancer susceptibility can be affected by the polymorphism in DNA repair genes with exposure of environmental xenobiotics including betel quid, smoking, alcohol consumption which play a vital role for cancer development [5, 26]. To the best of our knowledge, this is the first report on polymorphisms of XRCC1 Arg194Trp, XPD Arg156Arg and MGMT Leu84Phe genes and their association with BN, tobacco, smoking and alcohol habits, from the high HNC prevalent North-Eastern Region of India.

The prevailing concept is that defect in one or more step of DNA repair may lead to carcinogenesis [6]. DNA repair pathway plays primary defensive role and is responsible for maintaining the genome integrity, in response to environmental assaults, mutations and replication error [27]. The present study demonstrated that XRCC1 Arg194Trp and XPD Arg156Arg genotype shows a significance risk of HNC in NE population of India (Table 32). Similar observation was found for XRCC1 Arg194Trp in lung cancer among Romanian population [28] and colorectal cancer among Kashmiri population [29]. Many studies have reported the association of XRCC1 Arg194Trp polymorphism with lung cancer risk but the results have remained controversial [30]. Recent reports suggested that XRCC1 Arg194Trp had no effect or protective effect in modulating lung cancer risk in North Indian population [31], and carcinoma of oral cavity in Brazilian patients but contributes to the risk for lung cancer in east Chinese Han population [32] and glioma in northwest China population [33]. Meta- analysis of published case-control study data have demonstrated that XRCC1 Arg194Trp polymorphism might contribute to individual susceptibility to lung cancer in Caucasians [30], and bladder and HNC in Asian populations [34, 35]. However, studies from *Catana et al, and Liang et al.* suggested that the XPD Arg156Arg genotype contributed to lung cancer in Romanian population [36], and breast cancer in Chinese population [37]. The reports have also indicated that XRD Arg156Arg might play an important role in the etiology of

bladder cancer [38] and is risk factor in basal cell carcinoma [39]. A study among the Thai population have demonstrated that variant genotypes of XRCC1 194Trp and XPD Arg156Arg contributes to the OSCC development [5]. Recent report have shown that the XPD Asp312Asn polymorphism increases risk for HNC in association with smoking and/or tobacco chewing in the northeast population of India [1], but no report on the role of XPD Arg156Arg on the risk of HNC were found. We are first to report the association of XPDArg156Arg polymorphism with HNC risk in the NE population of India.

Surprisingly, the MGMT variants found at codon 84 is not involved in esophageal cancer in Kashmiri population [18]. Meta-analysis have demonstrated that the variant allele of MGMT (rs12917) were protective in colorectal cancer [40]. Even though large number of case-control studies were conducted to explore the association between MGMT Leu84Phe polymorphism and cancer risk, their results were not consistent. Recent meta-analysis of published case-control studies have suggested that MGMT-84 polymorphism might contribute to the risk for certain cancers such as lung cancer in Caucasians, biliary tract cancer in Chinese population etc. [41, 42]. Gene-gene interaction was established as risk possession for esophageal cancer in Chinese population [43], and HNC in north east India [1]. However, none of the studies describe intra- and-inter genic interaction of XRCC1, XPD and MGMT along with exposure habits.

In this study, we have also observed the effect of betel nut, tobacco, smoking and alcohol consumption on risk for HNC and their association with XRCC1 Arg194Trp, XPD Arg156Arg polymorphisms (Table 33). In previous study XRCC1 and XPD polymorphism was advocated for significant positive association with betel quid chewing habit on lung cancer in NE India [44], and oral cancer in South India [45], tobacco in NE India [1]. Our study indicated similar result for XRCC1 194 (Arg>Trp) polymorphism which is associated with smoking, alcohol drinking in the risk of prostate cancer [46] and thyroid in Chinese population [47]. In meta-analysis XRCC1 Arg194Trp polymorphism with smoking habits possessed risk for HNC [48]. There are

only a few reports on the potential influence of XPD Arg156Arg and cancer risks. The XPD Arg156Arg genotype was found to have association with increased risk of lung adenocarcinoma in smokers of Romanian population [36]. Pervious finding among smoking habits of MGMT Leu84Phe polymorphism showed independent effects on gastric cancer [49].

According to a systematic epidemiology study, it has been reported that alcohol consumption is one of the risk factors associated with colorectal adenomas, laryngeal, esophagus and HNC [15, 50, 51] and nasopharyngeal in Malaysia population [52]. Alcohol intake has been correlated with the production of reactive oxygen species (acetaldehyde, oxygen radicals), which cause DNA damage that can however be removed by the DNA base-excision repair pathway [53, 54]. Our study support the earlier finding that XRCC1 Arg194Trp genotype in association with smoking and alcohol habits, XPD Arg156Arg with BN, tobacco, smoking and alcohol and MGMT codon 84 with smoking habit increased risk for HNC.

Previous study demonstrated that alcohol consumption and areca nut chewing may have synergistic effect on esophageal cancer [55]. We, therefore, stratified our data into light and heavy dose of BN and BN + tobacco dose and combination with BN + alcohol dose and BN+ tobacco +alcohol doses consumption and analyzed the risk modifying effect of the genotype in both groups. In the present study, we demonstrated that the association between various doses of BN, tobacco and alcohol consumption and increased risk for HNC.

In other studies it was reported that consumption of more than 400 gm (higher dose) of alcohol per week had higher risk for cancer in western countries [56, 57]. Our observation of greater risk for HNC among heavy consumer is similar to that of Japanese population with XRCC1 194 (Arg>Trp) [58] and Taiwan population with XPD gene polymorphism [59]. The O^6 -alkylguanine DNA alkyltransferase (MGMT) favorably removes O6 -guanine alkyl adducts caused by carcinogenic agent such as 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone found in tobacco smoke. MGMT-

mediated repair of alkylated DNA is reduced in presence of ethanol or its primary metabolite, acetaldehyde [60]. In our study, we observed that heavy doses of alcohol (>120ml/day) have synergistic effect on HNC development with BN in individuals having MGMT codon 84. Previous reports support our finding that the MGMT codon 84 SNP is associated with alcohol intake for development of colorectal [61] and HNC [60].

In conclusion, our result demonstrated that the XRCC1, XPD and MGMT polymorphism have strong association with various dietary habits in increasing risk for HNC. To the best of our knowledge, this is the first report that decipher the interaction of XRCC1 Arg194Trp, XPD Arg156Arg and MGMT Leu84Phe polymorphism interact alone and/or with each other and exhibit dose –dependent and synergistic risk for HNC in subjects having dual habit of BN + tobacco in Northeast (NE) Indian population. It would be interesting to further examine whether the gene interact with betel nut – alcohol to increase the risk for HNC. Additional studies including large samples are needed to illuminate the effects of these polymorphisms on HNC risk in the population of NE region of India.

6.5 Bibliography

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