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Association of Non-Hodgkin lymphoma risk with *CYP1A1*, *GSTM1* and *GSTT1 gene variants*, in tobacco addicted patients of Pashtun ethnicity of Khyber Pakhtunkhwa, Pakistan

Zakiullah[1], Muhammad Saeed[1], Muhammad Ovais[2], Fazli Khuda[1*], Nabila Javed[3], Sajid Ali[4] and Muhammad Khisroon[5]

[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Department of Biotechnology Quaid-i-Azam University, Islamabad, Pakistan
[3]Institute of Radiotherapy & Nuclear medicine, Peshawar, Pakistan
[4]Department of Biotechnology, Abdul Wali Khan, University Mardan, Pakistan
[5]Department of Zoology, University of Peshawar, Peshawar, Pakistan

Abstract: Associations of *GSTM1, GSTT1* and *CYP1A1* gene variants with risk of developing Non-Hodgkin lymphoma were evaluated in this case-control study involving183 NHL cases along with 151 population-based healthy controls of Pashtun ethnicity from Khyber Pakhtunkhwa province of Pakistan. Socio-demographic data were obtained and blood samples were collected with informed consent for analysis. Specific RT-PCR and conventional PCR methods were used to detect *CYP1A1*and *GSTs*, respectively, andresults were analyzed through SPSS version 20.Study showed that variant type (C/C) had weak association with NHL compared with wild genotype (T/T) (OR: 1.212); while heterozygous variant (T/C) had a significant association (OR: 2.742). Null genotypes of *GSTM1* and *GSTT1* genes were having weak or no association, respectively, with the increased risk of NHL cancer compared with wild type. Association of *GSTM1* Null/*GSTT1* combination was significant contrary to *GSTM1*/*GSTT1* Null. Both GST null genotypes were having non-significant association. Similarly *CYP1A1* gene variant showed significant association with NHL. Presence of all three gene variants showed strong and significant association. In conclusion, this study showed that the *CYP1A1, GSTM1* and *GSTT1* genes play role in the pathogenesis of NHL, especially in the presence of local confounding factor like tobacco use, although individual gene effects are complex and inconsistent.

Keywords: Non-Hodgkin lymphoma risk, *GSTM1, GSTT1*, *CYP1A1*, Pashtun population.

INTRODUCTION

Non-Hodgkin lymphoma (NHL) is the 10th most common cancer in the world, with approximately 386,000 newly diagnosed cases every year. It is also the 11th most common cause of cancer deaths worldwide with more than 199,000 deaths annually. High incidence of NHL is seen in North America while in South Central Asia incidence rate is lowest (Ferlay *et al*., 2010). In Pakistan NHL is the 3rd most commonly occurring malignancy (Pervez, 2012).

NHL is a heterogeneous disease and with possible risk factors such as infectious agents, environmental exposure, multiple genetic events, chemicals, sunlight and auto-immune diseases (Cartwright *et al*., 1994, Firdhouse *et al*., 2013). Among Infectious agents Epstein Barr virus (EBV), Herpesvirus 8 (HHV8), Human T Lymphotropic Virus type I (HTLV-I), Human Immunodeficiency Virus (HIV) and Hepatitis C virus (HCV) are perhaps the closest possible regarded causes of the disease (Cartwright *et al*., 1994; Engels 2007).

Immunosuppression, another NHL risk factor aids in enhancing cancer causing activity of infectious agents. Due to heterogeneous nature of NHL all subtypes of it can't be etiologically related to infection only. Extensive researches conducted for assessing tobacco use as risk factor for NHL also shows variable results. Some have reported no association of tobacco use with NHL while some reported statistically significant association of NHL especially follicular lymphoma with smoking tobacco (Stagnaro *et al*., 2004, Bracci and Holly*et al*., 2005, Schollkopf *et al*., 2005). Another study suggests cigarette use to be the risk factor for follicular lymphoma but have shown no risk for other NHL subtypes (Morton *et al*., 2005).Genetic susceptibility, gene expression profiling and genetic polymorphism studies might help to clarify the possible cause of lymphoma-genesis in near future which is on rise since mid of 20th Century (Staudt., 2002, Hegde *et al*., 2001).

In current study we focused on two families of genes CYPs (phase I cytochromes P450) and GSTs (phase II glutathione-S-transferase), responsible for encoding enzymes involved in carcinogen processing and oxidative stress. CYPs especially *CYP1A1* gene located on chromosome 15q22-q24 is known to be involved in the activation process of pro-carcinogens i.e., Poly Aromatic Hydrocarbons (PAHs), Tobacco Specific Nitrosamines (TSNAs) and aromatic amines into carcinogenic species

**Corresponding author:* e-mail: fazlikhuda2012@upesh.edu.pk

while GSTs are involved in their detoxification and excretion from the body(Khan *et al*., 2015). *GSTT1* locus has been mapped on chromosome 22q11.2 while *GSTM1* loci exist on chromosome 1p13.3 (Bin *et al*., 2013).

Variation in response to cancer therapy in NHL patients clearly indicates the heterogeneity in tumor, and /or in patients. Genetic polymorphism in genes encoding xenobiotic metabolizing enzymes mostly leads to heterogeneity in patients. Hence susceptibility to particular cancer type can be determined by evaluating polymorphism in genes encoding carcinogens metabolizing enzymes (Nebert*et al*., 1996). Increased risk of cancer will be in individuals having active form of carcinogens activating enzymes or less efficient alleles of detoxifying enzymes, while those individuals having vice versa of it will be at low risk (Al-Dayel *et al*., 2008). The argument can be further strengthened by the fact that among those individuals exposed to carcinogens (e.g., tobacco products) under similar environmental conditions, only few develop cancer. Variation in alleles of *CYP1A1* along with environmental factors such as tobacco use highly influences an individual susceptibility to different types of cancer ultimately resulting in increased risk of carcinogenesis. Likewise, deletion of *GSTT1* and *GSTM1* ultimately results in accumulation of carcinogenic species, paving way for cancer development (Zakiullah *et al*., 2014).

Association of genetic polymorphisms of the above mentioned genes with NHL has not been so far reported in Pashtun population of Khyber Pakhtunkhwa province of Pakistan. Therefore, a case-control study was carried out to evaluate the potential role of *CYP1A1* (T>C, rs4646903), *GSTM1* and *GSTT1* gene polymorphisms in the susceptibility to NHL in Pashtun population of Khyber Pakhtunkhwa province of Pakistan. This will help to adopt pro-active approaches for early detection and preventive life style modification strategies to decrease the incidence of the disease in the target population.

MATERIALS AND METHODS

Sample collection

Study sample comprised of 183Non-Hodgkin lymphoma patients and 151 healthy control subjects between 30 and 70 years of age as per exclusion/inclusion criteria. Patients were registered at the Institute of Radiotherapy and Nuclear Medicine (IRNUM), Peshawar, Khyber Pakhtunkhwa; while eligible control samples were collected from various districts of the same province. The study period was from July, 2012- July, 2013. All the patients were having histo-pathologically confirmed oral cancer.

Inclusion criteria (patients): Histo-pathologically proven oral cancer patients having age between 30 and 70 years with Pashtun ethnicity, and not less than 20 years of tobacco exposure in any form.

Exclusion criteria (patients): Patients with non-Pashtun ethnicity and/or having more than 70 years of age Criteria for selection of control subjects: Normal healthy age-matched subjects of similar ethnicity with not less than 20 years of exposure to tobacco in any form, and free from cancer.

Study was approved from the Ethical Committee of the Department of Pharmacy, University of Peshawar (No. 440, dated 17.12.2011). Informed consent and thorough interview was taken by expert in the relevant field before blood collection on a carefully designed proforma that contained information regarding age, place, occupation, socioeconomic status, cancer type and tobacco use habits etc. Three milliliter of whole blood was collected from all subjects in properly labeled EDTA tubes and genomic DNA was subsequently extracted by using standard DNA Isolation kit **(**Pure link Genomic DNA kit In vitrogen, USA) as per manufacturer's recommendations. The DNA quality and quantity were determined using a double beam spectrophotometer (Perkin Elmer series 200 system, Norwalk, USA).

Genotyping of CYP1A1 (T>C, rs4646903)

The *CYP1A1* (T>C, rs4646903) polymorphisms were analyzed using a previously reported highly specific Real Time Polymerase Chain Reaction (RT-PCR) (Khan *et al*., 2015). Light SNiP rs4646903 (primers and probes) and Fast Start DNA Master Hyprobe kit (Product No. 03003248001) were purchased from Tib-Molbiol (Germany) and Roche Diagnostics (Germany), respectively. Reaction was performed as per supplier's recommendation. Reaction mix comprised of Reagent Mix (1µL), FastStart DNA Master (2µL), Magnesium chloride (25MM, 1.6µL), and water (14.4µl). Finally, DNA (1µl, 100-150ng) was added to the reaction mix to make the final volume 20µL. Thermocycler **(**Step One Plus Real-Team PCR System Applied Bio systems, USA) conditions were: Denaturation at 95ºC for 10 minutes; Cycling for 45 cycles of 95ºC for 10 seconds, 45ºC for 60 seconds and 72ºC for 15 seconds; followed by melting curves analysis at 95ºC for 10 seconds, 40ºC for 2 minutes through 75ºC for 0 seconds. Duplicate samples were used as control. Melting peaks at 51-52ºC represented wild type (T/T) allele; the one at 59-60ºC represented variant (C/C) allele, while samples giving two peaks at 51ºC and 59ºC were heterozygous (T/C) allele (fig. 1).

Genotyping assay for GSTM1 and GSTT1

For the determination of homozygous null polymorphisms of GSTM1 and GSTT1 our previously reported method was used (Zakiullah *et al*., 2014). Primer sets used were5′- CATGTGACAGTATTCTTATTTC- 3′,&5′-ACTCAATCTCAGCATCACAGC- 3′ for GSTM1 and 5′- ATCTGTGGTCCCCAAATCAG-3′&5′- GGGGGTT GTCTTTTGCATAG-3′, for GSTT1, respectively.

Table 1: Demographic characteristics of lymphoma subjects

S. No.	Variables	Control N (% within group)	Lymphoma N (% within group)	P-value
1				
	Geographic Area (district)			
	Tribal	45 (29.80 %)	32 (17.48 %)	
	Charsadda	15 (9.93 %)	16 (8.74 %)	
	Bannu DI Khan	12 (7.94 %)	8 (4.37 %)	
	Nowshera	11 (7.28 %)	8 (4.37%)	0.948
	Peshawar	19 (12.58 %)	32 (17.48%)	
	Swabi	9 (5.96%)	7 (3.82%)	
	Swat malakand	16 (10.59 %)	16 (8.74%)	
	Mardan	15 (9.93 %)	32 (17.48%)	
	Kohat	1 (0.66 %)	0 (0 %)	
	Dirchitral etc.	8 (5.29 %)	32 (17.48%)	
2	Age (yrs)			
	20-40	24 (15.89 %)	48 (26.22 %)	
	41-50	42 (27.81 %)	48 (26.22 %)	0.955
	51-60	41 (27.15 %)	40 (21.85 %)	
	60+	44 (29.13 %)	47 (25.68 %)	
3	Occupation			
	Coal labour	2 (1.32 %)	0 (0 %)	
	Driver	9 (5.96 %)	16 (8.74 %)	
	Farmer	45 (29.80 %)	56 (30.60 %)	0.000
	Labour	58 (38.41 %)	64 (34.97 %)	
	Odd jobs (laborious)	25 (16.55 %)	40 (21.85 %)	
	Farmer/labour (tobacco)	12 (7.94 %)	7 (3.82 %)	
4	Tobacco type used			
	Smoker	68 (45.03 %)	72 (39.34 %)	0.56
	Naswar user	132 (87.41 %)	175 (95.62 %)	0.591
	Other tobacco users	46 (30.46 %)	39 (21.31%)	0.853
5	Age at 1st exposure (yrs.)			
	10-15	66 (43.70 %)	88 (48.08 %)	
	16-20	57 (37.74 %)	63 (34.42 %)	0.38
	21-25	11 (7.28 %)	32 (17.48 %)	
	25+	17 (11.25 %)	0 (0 %)	
6	Daily use			
	Mild	109 (72.18 %)	167 (91.25%)	0.853
	Moderate	9 (5.96 %)	0 (0 %)	
	Heavy	33 (21.85 %)	16 (8.74%)	

Table 2: Genotypic, allelic and carriage rate frequencies of CYP1A1 m1 (T>C) gene polymorphism in controls and NHL cancer cases

Genotype/allele	Case N (%)	Control N (%)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
T/T	99(49.5)	140 (71.42)	Ref.		Ref.	
C/C	6 (3)	7 (3.57)	1.212 (.395-3.716)	.736	1.1469 (.344-3.816)	.825
T/C	95 (47.5)	49 (25)	2.742 (1.783-4.216)	.000	2.885 (1.796-4.634)	.000
*T allele	293(73.25)	329(83.92)	Ref.			
*C allele	107 (26.75)	63 (16.07)	.835 (.381-1.829)	.652	.663 (.286-1.534)	.337
Allele carriage rate						
T (+)	194 (97)	189 (96.42)	Ref.			
T (-)	6 (3)	7 (3.57)	.835 (.276-2.530)	.750	.663 (.202-2.172)	.497
C (+)	101 (50.5)	56 (28.57)	Ref.			
C (-)	99 (49.5)	140 (71.42)	.392 (.259-.594)	.000	.815 (.647-1.028)	.084

*Alleles, total number of chromosomes in control and cases

Table 3: Crude and adjusted Odds Ratios (OR) of GSTs for NHL

Genotype/allele	Genetic polymorphism	Cases N (%)	Control N (%)	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
GSTM1	Wild type	56 (30.60)	65 (43.04)	Ref.			
	Null	127 (69.39)	86 (56.95)	1.714 (1.093-2689)	.019	1.664 (.976-2.838)	.061
GSTT1	Wild type	143 (78.14)	116 (76.82)	Ref.			
	Null	40 (21.85)	35 (23.17)	.927 (.554-1.552)	.773	.1.569 (.852-2.890)	.149
Combinations 2 genes							
	GSTM/GSTT both wild type	40 (21.85)	51 (33.77)	Ref.			
	GSTM1 Null/GSTT1	103 (56.28)	65 (43.04)	2.020 (1.204-.390)	.008	1.968 (1.087-3.563)	.025
	GSTM1 /GSTT1Null	16 (8.74)	14 (9.27)	1.457 (.637-3.336)	.373	2.632 (.951-7.280)	.062
	GSTM/GSTT both null	24 (13.11)	21 (13.90)	1.457 (.711-2.985)	.304	2.352 (1.011-5.470)	.047

Table 4: Crude and adjusted Odds Ratios (OR) of GSTs and CYP1A1 gene combination for NHL Cancer

Genetic polymorphism	Control N (%)	Cases N (%)	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
GSTMGSTT and CYP1A1 wild type	40	16	Ref.			
All 3 gene polymorphisms	7	12	4.286 (1.430-12.846)	.009	5.288(1.593-17.551)	.007
GSTM1 Null and CYP1A1	33	49	3.712 (1.791-7.693)	.000	3.156(1.436-6.937)	.004
GSTT null and CYP1A1	4	12	7.500 (2.103-26.745)	.002	9.123(2.316-35.943)	.002
Both GSTs and CYP1A1	11	24	5.455 (2.175-13.680)	.000	4.974(1.831-13.514)	.002
GST both null and CYP1A1 wild type	15	11	1.833 (.695-4.838)	.221	2.870(.980-8.401)	.054
GSTM null and CYP1A1 wild type	32	55	4.297 (2.081-8.874)	.000	5.125(2.274-11.549)	.000
GSTT null and CYP1A1 wild type	10	4	1.000 (.274-3.656)	1.000	1.371(.329-5.716)	.665

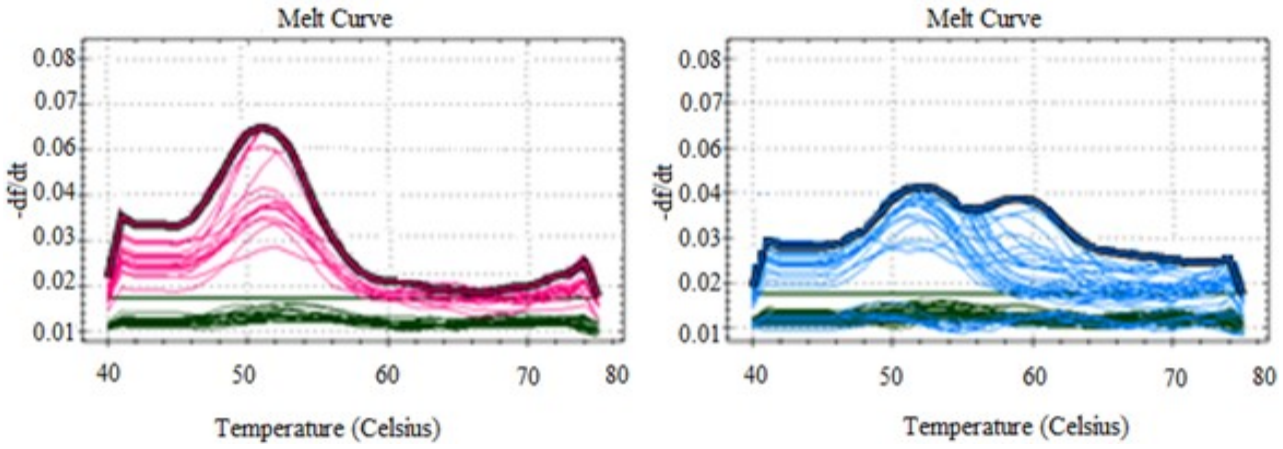

Fig. 1: Representative melting peaks of RT-PCR of CYP1A1 rs4646903 polymorphism. Pink peaks represent wild type, while blue peaks are of variant alleles.

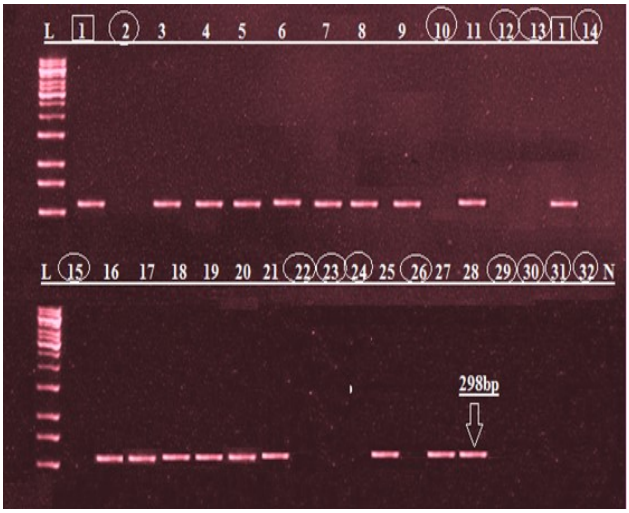

Fig. 2: Representative Electropherogram of GSTM1 analysis: L is molecular weight marker (1kb); N represent negative control; 1-32 are NHL samples; Duplicate sample 1 was used as positive control (shown in rectangles); GSTM1 band size is 298bp; GSTM1 null samples are encircled.

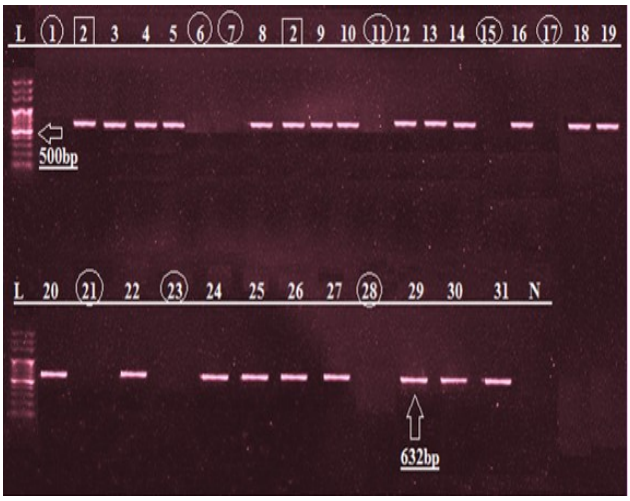

Fig. 3: Representative Electropherogram of GSTT1 analysis: L is molecular weight marker (100bp); N represent negative control; 1-31 are NHL samples; Duplicate sample 2 was used as positive control (shown in rectangles); GSTT1 has been shown at 632bp (vertical arrows); GSTT1 null samples are encircled.

Duplicate samples were used as positive control for both genes. PCR was separately performed for both genes in a 25µl reaction mixture containing 20mM Tris-HCl pH 9.0, 50Mm KCl, 2mM $MgCl_2$, 200µM dNTPs (Promega, USA), primers (Macrogen, South Korea) 10 pmol of each set individually, 0.5 units of Taq DNA polymerase (Bio-Labs, UK), and 50-100ng of genomic DNA. PCR was individually performed in the Gene Amp PCR system 9700 (Applied Bio systems, USA).

After an initial denaturation at 95°C for 4 minutes, amplification was carried out for 40 cycles at 95°C for 30 seconds, 52°C for 45 seconds and 72°C for 1 minute for GSTM1 and 55°C for 45 seconds and 72°C for 1 minute for GSTT1, followed by final elongation at 72°C for 10 minutes. The PCR products were electrophoresed in a 1% agarose gel for analysis. GSTM1 and GSTT1 genotype were identified by the presence of a band at 298 and 632 bp respectively (fig. 2 and 3).

STATISTICAL ANALYSIS

Chi-square ($\chi 2$) test was used to detect whether there were significant ($\alpha = 0.05$) differences in frequencies of genes. Odds Ratios (OR) for each polymorphism using binary logistic regression model were estimated with 95% confidence intervals (CIs), and the difference in genotype prevalence and association between case and control group were assessed independently as well as adjusted for confounding factors.

Age, gender, place of residence, tobacco type used, amount of tobacco used per day and age at first exposure were included as covariates as well as all the possible genotypes studied. *GSTM1* and *GSTT1* were categorized on the basis of presence and absence (null genotype) of the gene, while *CYP1A1* rs4646903 polymorphism was classified into homozygous wild type and variant allele containing genotypes. Wild type was used as reference group to assess the effects of the different alleles. Analyses were performed by SPSS (Version 20.0).

RESULTS

Subject characteristics of lymphoma patients

Demographic and other subject characteristics of NHL patients are shown in table 1. Mean age of healthy subjects (controls) and cancer patients were 56.02±10.83 and 49.14±15.91years, respectively. Almost equal distribution was found in the 4th, 5th and 6th decades of life with twenty-six percent of the patients in the age range of 20-40 years; similar percentage in the age ranges of 41-50 and 60+ years each. All the patients were male and no female patient during study period was observed. Highest equal incidence (17.48%) of lymphoma was observed in Dir & Chitral, Mardan, Peshawar districts and tribal belt. Lowest incidence (4%) was observed in Bannu DI Khan, Nowshera and Swabi districts; while no patient (0%) was observed from district Kohat. Highest incidence (cumulative 56.82%) was observed in patients having laborious jobs followed by patients having farming as profession (30.60%).Ninety-six percent of the patients were naswar (A local smokeless tobacco product) addicts while 39.34% were smokers. Majority (48.08%) started tobacco at the age of 10-15 years, with mean age of 14 years. Similarly, majority (91.25%) were mild users with interestingly no patient (0%) having moderate use.

Genetic susceptibility to NHL due to CYP1A1 gene

The allele frequencies and genotype of CYP1A1 rs4646903 polymorphism among controls and cases are given in table 2 and represented by figure 1. In control group they were not departure from Hardy-Weinberg equilibrium. The distributions of genotypes of *CYP1A1* rs4646903 were significantly different between the cases and controls groups (Pearson chi Square $\chi 2_{0.05,\ 2}$=0.000, P>0.05). The prevalence of CC genotype was almost equal, while that of TC genotypes was more in cases (3% and 47.5% respectively) when compared to controls (3.57% and 25%). Interestingly, homozygous variant type (C/C) had an almost no association with NHL compared with wild genotype (T/T), both in the absence and presence of other confounding factors (OR: 1.212 (.395-3.716), p-value= .736); while heterozygous variant (T/C) had a significant association with NHL, compared with wild genotype (T/T)(OR: 2.742 (1.783-4.216), p-value= .000) at 95% CI; which further increased when the data were adjusted for age, gender, place of residence, tobacco type used, amount of tobacco used per day and age at first exposure (Adjusted OR=2.885 (1.796-4.634), p-value= .000). Overall the C allele confer no susceptibility to NHL as compared to T allele (OR=.835 (.381-1.829), *p*-value=.652). Allele carriage rate showed no association with the susceptibility to NHL.

Genetic susceptibility to NHL due to GSTT1 and GSTM1 gene

The allele frequencies and genotype of GSTs of both control and NHL cancer cases are given in table 3 and represented by figure 2 and 3. The distribution of *GSTM1* and *GSTT1* genotypes were significantly different between the cases and controls groups (Pearson chi Square $\chi 2_{0.05,\ 2}$=0.000, P>0.05). When analyzed for individual genes the prevalence of null genotypes of *GSTM1* was more in cases (69.39%) when compared to controls (56.95%), whereas null genotype of *GSTT1* was less prevalent in cases as compared to controls. Null genotypes of *GSTM1* gene were having weak association with the increased risk of NHL cancer compared with wild type, which became non-significant when OR were adjusted for confounding factors such as tobacco use habits, age and area of residence etc. Whereas, null genotype of *GSTT1* gene was having non-significant association, with little/no effect of confounding factors. Similarly, when analyzed for two GST gene combinations the association of GSTM1 Null/GSTT1 positive combination was more and significant than GSTM1/GSTT1 Null, which was having non-significant association. Interestingly, GSTM/GSTT both null genotype was having non-significant association.

Combined effect of all three genes on NHL

Combined effect of *GST* and *CYP1A1* gene variants on susceptibility to NHL was analyzed as given in table 4. Presence of all three gene variants showed strong and significant association with NHL as compared to control. Presence and absence of both GSTs along with variant type *CYP1A1* gene also showed significant association with NHL, with slight effects of confounding factors. Whereas, presence of both null GSTs or GSTT1 genotypes alone, along with wild type *CYP1A1* showed weak and non-significant association.

DISCUSSION

Socio-demographic characteristics of cancer patients

In order to improve our understanding of the Etiology of NHL in Pakistani Pashtun population, we have accounted for genetic, ethnic and demographic factors associated with the incidence of the disease. As previously mentioned, well known risk factors for NHL include family history, dietary habits (ingestion of charbroiled meat and fish etc.) immune dysfunction, immune stimulation, and infections. Similarly, identified environmental exposures (e.g., tobacco, alcohol etc) among others are also risk factors for NHL, although the results are variable and inconsistent (Chiu *et al*., 1996; De Stefani *et al*., 1998; Purdue *et al*., 2004; Morton *et al*., 2005; Besson *et al*., 2006; Nieters *et al*., 2006; Lim *et al*., 2007; Monnereau *et al*., 2008).

In this study populationmean age of NHL cancer was 49.14±15.91years. Highest incidence age group was 30-50 years. This incidence pattern has also been reported in many countries throughout the world (Ferlay*et al*., 2010). Generally, ≥ 45 years of age should be considered as risk factor for cancer, as has been reported in previous studies (Llewellyn *et al*., 2004). However, in case of NHL our study reveals that the 4th decade of life is important as much as 5th and 6th decades. As far as gender is concerned we found only male patients fulfilling our criteria during hole of our study period. The reason may be that in Pashtun population tobacco consumption is socially an unacceptable practice for females and secondly the female patients may have provided us inaccurate information, as our main stay regarding tobacco habits were through questionnaire.

Similarly address of the patients revealed that NHL was prevalent equally in many districts like Dir & Chitral, Mardan, Peshawar and Tribal belt. Differential distribution of NHL cases among different districts was observed. For example, Bannu DI Khan and Swabi districts were having no NHL cases; while Dir and Chitral districts were having highest equal incidence of NHL. This distribution points towards certain specific etiologic factors that need to be explored.

Regarding occupation (that reflects socioeconomic status) it was observed that generally the patients were very poor having laborious jobs. Under-nutrition associated with low socioeconomic status has been an established risk

factor for various types of cancers including that of NHL, and our data showed that majority were having laborious low paid jobs. Highest incidence of NHL cancer cases were observed in patients having laborious jobs.

Several tobacco use habits were analyzed to assess exposure to tobacco carcinogens. Almost hundred percent of all cancer cases were naswar addicts with majority having started it in teen age. Majority were mild users of tobacco products alone or in combination. This shows that majority of the patients were exposed to tobacco carcinogens for almost life long period.

Genetic susceptibility

Present data shows that genetic polymorphisms in *GSTM1, GSTT1* and *CYP1A1* genes have complextype of association with the occurrence of NHL in Pashtun population. These observations are partially consistent with the previous studies that have shown controversial results regarding the associations between polymorphisms of drug-metabolizing genes and NHL risk. In four separate studies, no association was observed between *CYP1A1* in California (De Roos *et al*., 2006), Connecticut (Kilfoy *et al.,* 2009), Saudi Arabia (Al-Dayel *et al*., 2008) and Australia (Kerridge *et al*., 2002). In this study, *CYP1A1* variant allele has shown partial association with homozygous C/C having no association, while heterozygous T/C showing significant association. This might point towards certain complex aspect of xenobiotic metabolism that is yet to be explored. One of the reasons may be the effect of gene induction, not the original enzyme activity, as previously reported for yet another site (CYP1A14889 GG genotype) that is not addressed in this study (Crofts *et al*., 1994). This hypothesis is strengthened by the effect of confounding factors such as tobacco use (that induce *CYP1A1*). Our analysis of these polymorphisms suggests that *CYP1A1* gene inducibilty may be more important than does CYP1A1 enzyme activity in modifying the risk of NHL.

Findings of this study regarding GST association with NHL are also interesting. It points towards certain indirect type of association with NHL. *GSTM1* null genotype seems to be more important than *GSTT1* null. Independently weak or no association was observed for both isoforms, that is in partial agreement with the previous studies (Kerridge *et al*., 2002, Chiu *et al*., 2005, De Roos *et al*., 2006, Al-Dayel *et al*., 2008), as previously two reports have shown the association of *GSTT1* null genotype (Kerridge *et al.,* 2002, Al-Dayel *et al*., 2008). Reverse finding of our study may points towards differences in environmental exposure and/or other socio-demographic factors in the study population. The effect of all three gene polymorphisms shows strong association that point towards the importance of these enzymes/genes although the picture continues to be blurred and further explorable. In conclusion, our study shows that the *CYP1A1, GSTM1* and *GSTT1* genes play role in the pathogenesis of NHL, especially in the presence of local confounding factor like tobacco use.

In summary, these complex observations may be due to different lymphoma subtypes, different genotypes frequencies with respect to ethnicity (Pashtun population), sample size variation, differential environmental exposure and/or dietary and life style habits.

CONCLUSION

Despite high prevalence in Khyber Pakhtunkhwa the precise etiologic factors for NHL have yet to be elucidated. Further studies with larger sample size in local ethnicities taking account of various lifestyle factors such as chemical exposures such as to tobacco, alcohol and HCAs and consumption of fresh fruits and vegetables should be conducted to further envisage the associations between genetic polymorphisms of drug-metabolizing enzyme genes and lymphoma genesis. Future large epidemiologic studies are necessary to come to a definite conclusion regarding interaction between these polymorphisms and NHL risk.

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