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Association of Human Leukocyte Antigen and Esophageal Cancer in North of Iran

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Abstract: Several risk factors including environmental factor, Genetic and nutritional deficiencies have been associated with Esophageal Cancer (EC) in high risk areas. In a case-control association study, the association of HLA class I and esophageal carcinoma has been investigated. A total number of 30 patients and 30 clinical healthy individuals matched for sex and age with the same ethnicity and residence status were enrolled. Five milliliter defibrinated blood taken from each individual and diluted 1:1 with Hanks balanced salt solution buffer. The diluted blood added to 3.0 mL separating medium (ficol hypaqe). Lymphocyte separated and HLA, A, B and C molecules were determined by using the Terrasaki Mirocytotoxicity test. Data were analyzed by Chi square and Fisher exact tests. Thirty patients (20 females and 10 males) with age 61±2.4 enrolled in this study. Only patients with definite diagnosis of Squamous Cell Carcinoma (SCC) included and Patients with other esophageal cancer (adenocarcinoma) excluded. The frequency of many HLA molecules were different in comparison of patients and control groups, but statistical analysis of the data revealed that only difference between frequency of HLA A11, B41 and Cw3 are significant ($p<0.05$). We concluded that HLA-A11, -B41 and -Cw3 molecules may be risk factors for esophageal cancer in northern part of Iran.

Key words: HLA, esophageal carcinoma, North of Iran

INTRODUCTION

Esophageal Cancer (EC) is one of the most common fatal cancers worlds wide. It shows marked geographical variation with exceptionally high rates. North and North Eastern region of Iran and shanaxi province in Northern china are known areas with a high incidence of esophageal cancer (Ghavamzadeh *et al*., 2001). Although, epidemiological studies indicate that tobacco smoking and alcohol consumption are the major risk factors for squamous esophageal cancer in low risk region of Europe and North America, the etiological agent and molecular etiology in high risk regions have yet to be convincingly identified (Su *et al*., 2003). Many studies have been conducted to examine the epidemiological patterns, incidence and etiology of esophageal cancer in high risk regions (Kamangar *et al*., 2007). Several risk factors including nutritional deficiencies and low socio-economics statue have been associated with EC in this area. However it is unlikely that the extraordinary high rate EC, observed in this area are solely due to these factors, as they are also reported in many area of the world with low EC rates (Kamangar *et al*., 2007). It is likely that one or more major risk factors yet undiscovered, exist in high risk areas of the world (Kamangar *et al*., 2007).

Within high risk regions, studies have shown a strong tendency toward familial aggregation, suggesting that genetic susceptibility, in Conjunction with potential environmental exposures, may be involved in the etiology of EC, although the exact mechanism is unclear (Su *et al*., 2003; Hu *et al*., 2003; Biramijamal *et al*., 2001; Watanabe *et al*., 2002).

Several recent studies have indicated that HLA gene complex may mediate susceptibility to a number of malignancies (Bateman and Howell, 1999; Little and Stern, 1999). The HLA System is a kind of genetic marker of human being, the most complicated human genetic polymorphic system with hereditary features of haplotype inheritance, allele polymorphism and linkage disequilibrium. It plays an important role in the event of antigen recognition and presentation, immune response and modulation and in destroying foreign antigen targeted cells. The alleles of HLA system control a variety of immune functions and influence the susceptibility to

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more than 40 diseases; many of which have an autoimmune component. Association of a particular HLA allele with a disease implies that the frequency of the allele is different in the patient population as compared with that of an ethnically matched control population. However, there are few reports regarding the association between HLA alleles and EC (Watanabe *et al*., 2002; Lin *et al*., 2003; Eivazi-Ziaei *et al*., 2006).

The aim of this study was to identify the association of HLA class I molecules and EC in Mazandaran Province, North of Iran, one of the very high risk areas in the world.

PATIENTS AND METHODS

The study consisted of 30 patients (20 females and 10 males, mean age 61±2.4 years) with clinical and pathologically diagnosis of esophageal cancer from areas of Northern Iran, who referred to the Imam Teaching Hospital of Mazandaran University of Medical Sciences during April 2007 and November 2008. Detailed medical records, including demographic data, disease status, clinical presentation and laboratory findings were reviewed. Control subjects included 30 clinically healthy individuals residing in the same geographic area and were age- and sex-matched subjects without inflammatory or autoimmune diseases. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by Mazandaran University of Medical Sciences medical research committee. Informed consent was obtained from all patients and controls enrolled in the study.

Five milliliter blood samples obtained from each patient and control. HLA A, B and C haplotype were determined by using the Terasaki Microcytotoxicity test (Terasaki *et al*., 1978). We use 72 well trays coated anti HLA-A, B and C reagents from Biotest Company (Biotest, Landsteiner Street, Germany).

Statistical analysis were performed using SPSS 10.0 for Windows (SPSS, Chicago, Illinois). Statistical significance of continuous variables was determined by using a Student's T test. Discontinuous variables were analyzed by χ^2 and Fisher Exact tests, appropriately. All tests were two-tailed and $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Clinicodemographic characteristics of patients with esophageal cancer and healthy controls are shown in Table 1. There was no significant age and sex differences between patients and controls ($p>0.05$). Stratification of age in the two groups was also revealed no significant differences. In addition, the majority of patients resided in rural area. Squamous cell carcinoma was more prevalent than adencarcinoma (90% versus 10%) in the patients with esophageal cancer.

To determine any association of HLA class I molecules and esophageal cancer, we typed a haplotype of more common HLA class-I in the histopathologically confirmed patients with esophageal cancer and healthy controls. We typed isolated lymphocyte of patients and controls against 51 alloantisera. As shown in Table 2, among several typed-HLA molecules, the frequency of HLA-A11 (33.3 vs. 10%), -B41 (13.3 vs. 0%) and -CW3 (13.3 vs. 0%) molecules were significantly different in patients compared to controls ($p<0.05$).

There was an association between HLA A11, B41 and CW3 with esophageal cancer in North of Iran. Watanbe *et al*. (2002) identified the association of HLA A24, A26, B54, B61 and DR9 with esophageal cancer in Japanese population. Allele frequency of HLA DR 37*0901 was significantly higher in esophageal carcinoma patients than normal control in Hubei Han Chinese study (Lin *et al*., 2003). HLA B14 and A24 were increased and showed statistically significant correlation with SCC in

Table 1: Clinicodemographic characteristics of patients with esophageal cancer and healthy controls

Characteristics	Esophageal cancer (n = 30)	Healthy control (n = 30)
Age (Mean±SD)	61±2.38	59.3±2.89
Sex (F/M)	20/10	20/10
Residential status		
Urban area	23	22
Rural area	7	8
Pathological pattern		
Well differentiated	13	-
Moderate differentiated	2	-
Poor differentiated	15	-
Type of tumor		
Squamous cell carcinoma	27	-
Adenocarcinoma	3	-

Values are the numbers of patients or controls

Table 2: Frequency of HLA class I molecules in patients with esophageal cancer and healthy controls

	HLA												
Group	A_1	A_2	A_3	A_{11}	A_{32}	B_{13}	B_{41}	B_{49}	CW_1	CW_3	CW_4	CW_6	CW_7
Patients-n (%)	5 (16.6)	13 (43.3)	4 (13.3)	10 (33.3)	6 (20)	4 (13.3)	4 (13.3)	6 (20)	7 (23.3)	4 (13.3)	7 (23.3)	11 (36.6)	4 (13.3)
Controls-n (%)	3 (10)	10 (33.3)	7 (23.3)	3 (10)	3 (10)	1 (3.3)	0 (0)	4 (13.3)	8 (26.6)	0 (0)	10 (33.3)	5 (16.6)	8 (26.6)
p-value	NS	NS	NS	p<0.02	NS	NS	p<0.03	NS	NS	p<0.03	NS	NS	NS

NS: Not significant

North Western region of Iran (Eivazi-Ziaei *et al*., 2006). These discrepancies in the results of the different studies might be due to differences in ethnic background and differing in exposure to environmental risk factors. These differences could reflect either selective advantage of certain alleles under conditions to which the different racial groups have been exposed or may simply reflect the attainment of independent equilibria due to restricted interbreeding between racial groups (David *el al*., 2008).

A growing number of diseases have been shown to occur with a high incidence in individuals who possess particular HLA gene polymorphisms. The majority of these HLA-disease associations, involve none neoplastic conditions including several autoimmune diseases, however, association of many neoplastic diseases, including Hodgkin's lymphoma, cutaneous T cell lymphoma, cervical squamous cell carcinoma and entropathy-associated T cell lymphoma (EATL) with class II and I HLA have been reported (Bateman and Howell, 1999). Even the relative risks except EATL (44.2) are not high (Su *et al*., 2003; Hu *et al*., 2003; Little and Stern, 1999).

In HLA associated diseases, certain HLA alleles usually appear to be necessary for disease development, however, not every individual possessing a disease associated HLA allele, will develop that condition, indicating that other factors are required, in combination with the presence of particular HLA alleles, for the disease to occur. Environmental factors, infective agents, nutritional deficiency and HLA are examples of co-modulated factors for disease susceptibility (Bateman and Howell, 1999).

The immune response involving HLA molecules thought to play an important role in eliminating mutated cells or suppressing carcinoma progression (Little and Stern, 1999). Tumor cells often express new antigens as a result of the multiple genetic alteration that are associated with cell transformation, although such antigens are self in origin, they might not have been presented during thymic education. These antigens can originate from a variety of sources. T cells can potentially monitor genetic changes, including those associated with transformation in the context of novel peptide presentation by HLA molecules. HLA class 1 alleles are required for the presentation of tumor neo antigen to cytotoxic T-lymphocyte but some new peptide might fail to be presented to T cell by particular HLA allele, Therefore, tumor cell proliferates and evades the cytotoxicity of immune system and carcinoma protected themselves from immune responses. Thus, with such operational immune surveillance of tumors, individual's HLA type might increase the risk of developing particular cancer.

HLA alleles associated with esophageal cancer in different ethnic groups i.e., Japanese and Iranian (Watanabe *et al*., 2002; Eivazi-Ziaei *et al*., 2006) are different. Data from the study of the world population groups demonstrated that the frequencies of HLA alleles differ significantly among ethnic population group and HLA alleles that present the same antigen may vary within different ethnic groups.

The associations of HLA alleles within the same ethnic groups but live in different geographical areas i.e. north of Iran and north east of Iran, japans and Chinese are also different (Watanabe *et al*., 2002; Lin *et al*., 2003; Eivazi-Ziaei *et al*., 2006). Thus, we can conclude that in each geographical area, the environmental factors influencing malignancy may be different.

There is a limitation to present study that should be mentioned. It is possible that present results may be false positives due to a modest sample size that affects the power of the study to reliably detect the effect of HLA-A11B41CW3 haplotype on esophageal cancer. However, independent population in our study might be compensated this type of error.

CONCLUSION

Present study suggests that the HLA-A11B41CW3 haplotype may be involved in the susceptibility to esophageal cancer in a population of the northern part of Iran. This association, taken in the context of the role of HLA-I molecules in presenting tumor antigens, suggests that carriage of HLA-A11B41CW3 haplotype should be investigated further as a risk factor for esophageal cancer.

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