Serological analysis of Human Leukocyte Antigens-A and -B Antigens in Thai patients with Nasopharyngeal Carcinoma

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Abstract

Objective : To study the distribution of human leukocyte antigens (HLA) -A and -B antigens by standard microlymphocytotoxicity assay in Thai nasopharyngeal carcinoma (NPC) patients compared to normal controls in order to identify the alleles associated with NPC in Thailand

Design : Retrospective-Analytical study

Subjects : Fifty-three unrelated Thai patients with histologically confirmed NPC diagnosed at King Chulalongkorn Memorial Hospital and 70 healthy unrelated Thai individuals served as controls

Method : Lymphocyte separation and HLA typing were performed from freshly drawn blood by standard microlymphocytotoxicity assay. The significance of differences between the two groups was analyzed by the chi-square test

Results : HLA-A2 was observed at a greater frequency in patients being found in 31/53 (58%) NPC patients compared to 27/70 (38%) controls ($p = 0.02$). An increase in HLA-B46 was also demonstrated. HLA-B46 was present in 16/53 (30%) NPC patients but was observed in 10/70 (14%) in controls ($p = 0.03$).

Conclusions : This study reported two susceptible, HLA-A2 and HLA-B46 antigens, for NPC in a Thai population.

Key word : Nasopharyngeal Carcinoma, HLA-A, HLA-B

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Nasopharyngeal carcinoma (NPC) is one of the most common cancers in Asia, with the highest incidence rate in South China and an intermediate incidence rate in Southeast Asia[1]. This tumor is quite rare in Western populations. Multiple factors have been reported to play a role in the pathogenesis of this disease including Epstein-Barr virus (EBV) infection, environmental carcinogens (e.g., cigarette smoke, certain foods), and genetic factors[2]. HLA is one of the genetic factors reported as having a significant association with NPC [1]. Certain HLA antigens have been reported as associated with either increased or decreased risk in various studies. For example, HLA-A2 and B46 antigens were consistently reported to be positively associated with NPC in Chinese populations living in different countries (e.g., Singapore, China, Hong Kong, Malaysia, California USA)[1,3-6]. HLA-B58 is another NPC-associated antigen observed at a higher frequency in Chinese and Malay patients[7] while HLA-A11 was observed at a lower frequency among Chinese NPC patients[6,7]. Studies of HLA in other low incident populations gave more variable results[8-12].

In the present study, the authors investigated the distribution of HLA-A and -B antigens by standard microlymphocytotoxicity assay in Thai NPC patients compared to normal controls to identify the HLA antigens associated with NPC in Thailand.

Table 1. HLA-A frequencies in patients with NPC and healthy controls from Thailand.

HLA antigen	NPC Patients (n =53)		Controls (n = 70)	
	N	AgF %	N	AgF %
A1	3	5.6	3	4.2
A2	31	58.5	27	38.5
A3	2	3.7	3	4.2
A9 (A23)	0	0	0	0
A9 (A24)	14	26.4	30	42.8
A10 (A25)	0	0	0	0
A10 (A26)	4	7.5	1	1.4
A10 (34)	0	0	0	0
A11	23	43.3	34	48.6
A19 (A29)	0	0	1	1.4
A19 (A30)	1	1.8	4	5.7
A19 (A31)	0	0	2	2.8
A19 (A32)	0	0	0	0
A19 (A33)	17	32	24	34.3
A19 (A74)	2	3.7	0	0
A28	1	1.8	0	0
A36	0	0	0	0

n = the total number of individuals studied in either the patient or control group.
N = the number of individuals positive for each antigen.

MATERIAL AND METHOD

Study population

The study population included 53 unrelated Thai patients with histologically confirmed NPC diagnosed at King Chulalongkorn Memorial Hospital in Bangkok. There were 40 men and 13 women, with a median age of 48 years (range, 16-81). Seventy healthy unrelated Thai individuals served as ethnically and geographically matched controls. There were

Table 2. HLA-B frequencies in patients with NPC and healthy controls from Thailand.

HLA antigen	NPC Patients (n =53)		Controls (n = 70)	
	N	AgF %	N	AgF %
B5 (B51)	0	0	0	0
B5 (B52)	4	7.5	5	7.1
B7	2	3.8	5	7.1
B8	0	0	1	1.4
B12 (B44)	5	9.4	14	20
B12 (B45)	0	0	0	0
B13	10	18.8	16	22.8
B14	0	0	1	1.4
B15 (B62)	5	9.4	7	10
B15 (B75)	6	11.3	5	7.1
B15 (B76)	0	0	0	0
B15 (B77)	0	0	2	2.8
B16 (B38)	4	7.5	4	5.7
B16 (B39)	1	1.8	0	0
B17*	1	1.8	0	0
B17 (B57)	2	3.8	7	10
B17 (B58)	13	24.5	8	11.4
B18	6	11.3	4	5.7
B22 (B54)	1	1.8	2	2.8
B22 (B55)	1	1.8	1	1.4
B22 (B56)	1	1.8	3	4.3
B27	1	1.8	5	7.1
B35	4	7.5	9	12.8
B37	1	1.8	0	0
B40 (B60)	10	18.8	16	22.8
B40 (B61)	4	7.5	3	4.3
B42	0	0	1	1.4
B46	16	30.2	10	14.2
B47	0	0	0	0
B48	0	0	1	1.4
B70	1	1.8	0	0

n = the total number of individuals studied in either the patient or control group
N = the number of individuals positive for each antigen
* = Subtype of B17 could not be interpreted in one individual, who had both B15 and B17, because the key sera that differentiate B17 subtype is duo-specific for B15 and B17 subtype (B57).

Table 3. HLA-A and B antigens that demonstrated significant associations with NPC.

HLA	NPC (n = 53)		Controls (n = 70)		Odd ratio (95% CI)	P-value
	N	%	N	%		
HLA-A2	31	58	27	38	2.24 (1.02-4.97)	0.02
HLA-B46	16	30	10	14	2.59 (0.98-6.95)	0.03

N = the total number of individuals studied in either the patient or control group
n = the number of individuals positive for each antigen

50 men and 20 women, with a median age of 40 years (range, 21-61).

HLA-A, -B typing

Lymphocyte separation and HLA typing were performed from freshly drawn blood by standard microlymphocytotoxicity assay. The panel of 70 antisera were used to define the 17 HLA-A antigens and 30 HLA-B antigens. The antigen frequencies were determined by direct counting.

Statistical analysis

The significance of differences between the two groups was analyzed by the chi-square test. Fisher's exact tests were applied if the expected frequency was less than 5.

RESULTS

The distribution of HLA-A and -B antigens between the two groups is shown in Table 1 and 2. A total of 10 HLA-A antigens and 23 HLA-B antigens were observed in the Thai control group. The common HLA antigens with antigen frequencies (AgF) of more than 20 per cent in Thai controls were A2 (38.5%), A9 (A24) (42.8%), A11 (48.6%), A19 (A33) (34.3%), B12 (B44) (20%), B13 (22.8%), and B40 (B60) (22.8%). Ten HLA-A antigens and 21 HLA-B antigens were detected in NPC patients with the same 4 common HLA-A antigens observed at high frequencies (26.4-58.5%). The pattern of HLA-A and B antigens frequencies was similar to previous independent-studies in healthy Thai individuals indicating normal distribution in this control group[13, 14]. However, the common HLA-B antigens in NPC patients were different from the control group. B17 (B58) and B46 antigens were present in the patient group with the high AgF of 24.5 per cent and 30.2 per cent, respectively. When the frequency of HLA-A and B antigens in NPC patients and normal individuals was compared, significant associations between NPC and 2 HLA antigens were observed, as summarized in Table 3. Specifically, the frequencies of A2 and B46, were significantly increased in NPC patients (58 and 30% *vs* 38 and 14%, $p = 0.02$ and $p = 0.03$, respectively).

DISCUSSION

The present study has confirmed results suggesting that genetic susceptibility of NPC in the Thai population is likely similar to the Chinese population. This observation might be the result of being genetically related since the Thai population in this study were mostly Central Thai or present-day Thai who have a high-degree of Thai-Chinese admixture. As mentioned above, the association with A2 and B46 has been consistently observed in Chinese populations[1,3-6]. The authors' recent report analyzing molecular HLA-B types in Thai NPC patients also demonstrated a positive association of NPC with HLA-B*4601 ($p = 0.005$)[15]. The restricted-antigen binding properties of the B46 molecule[16] might be one explanation for its link to susceptibility to NPC. Interestingly, no EBV epitopes restricted by B46 have been reported so far and further studies are required to prove this hypothesis. Although there are some relationships between HLA types and NPC, the exact nature of this association is not yet clear. It is more likely that HLA antigens are not involved in the causation of the disease but are very closely linked to the "disease susceptibility genes". In fact, many studies supported this latter theory[17,18]. However, no susceptibility genes have been identified so far.

In conclusion, the present study reported 2 susceptible, A2 and B46 antigens, for NPC in Thai populations.

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การวิเคราะห์ความสัมพันธ์ระหว่างแอนติเจน HLA-A และ HLA-B ที่ได้จากการตรวจทางซีโรโลยีในผู้ป่วยมะเร็งโพรงหลังจมูกชาวไทย

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วัตถุประสงค์ : เพื่อศึกษาความสัมพันธ์ระหว่างแอนติเจน HLA-A และ HLA-B ที่ได้จากการตรวจทางซีโรโลยีในผู้ป่วยมะเร็งโพรงหลังจมูกชาวไทย

รูปแบบการวิจัย : การวิจัยเชิงวิเคราะห์ แบบย้อนหลัง

ประชากรที่ทำการศึกษา : ผู้ป่วยมะเร็งโพรงหลังจมูกที่ได้รับการยืนยันจากการตรวจชิ้นเนื้อที่มารักษาที่โรงพยาบาลจุฬาลงกรณ์จำนวน 53 คน และกลุ่มควบคุมที่เป็นอาสาสมัครที่มีสุขภาพดีจำนวน 70 คน

วิธีการ : ทำการแยกเม็ดเลือดขาวจากเลือดและตรวจชนิดของ HLA ด้วยการตรวจทางซีโรโลยีโดยวิธี microlympho-cytotoxicity แล้ววิเคราะห์ความแตกต่างระหว่างสองกลุ่มด้วยวิธีทางสถิติ (Chi-square)

ผลการศึกษา : พบแอนติเจนชนิด HLA-A2 เพิ่มมากขึ้นอย่างมีนัยสำคัญในกลุ่มผู้ป่วยโดยพบถึง 31/53 (58%) เปรียบเทียบกับกลุ่มควบคุมที่พบ A2 เพียง 27/70 (38%) (p = 0.02) และพบแอนติเจนชนิด HLA-B46 เพิ่มมากขึ้นอย่างมีนัยสำคัญในกลุ่มผู้ป่วยโดยพบ 16/53 (30%) เปรียบเทียบกับกลุ่มควบคุมที่พบ B46 เพียง 10/70 (14%) (p = 0.03)

สรุป : การศึกษานี้รายงานแอนติเจน 2 ชนิด นั่นคือ HLA-A2 และ HLA-B46 ที่มีความสัมพันธ์กับการเกิดโรคมะเร็งโพรงหลังจมูกในประชากรไทย

คำสำคัญ : มะเร็งโพรงหลังจมูก, เอ็ชแอลเอ-เอ, เอ็ชแอลเอ-บี

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