Preliminary Study of HLA-ABCDR Antigens in CML, ANLL, Thalassemia and Severe Aplastic Anemia in Thais

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Abstract

The objective of this study was to analyse human leukocyte antigen (HLA) and disease association in common blood diseases [chronic myelogenous leukemia (CML), acute non-lymphocytic leukemia (ANLL), thalassemia and severe aplastic anemia] in Thais. The subjects were patients from the Hematological Clinic, Departments of Medicine and Pediatrics, Ramathibodi Hospital who were referred for HLA typing for bone marrow transplantation (BMT) at the Histocompatibility Laboratory from March 1988 to September 1997. A total of 129 patients had complete HLA-ABC typing. The patients included 45 CML, 40 ANLL, 26 thalassemia (*Thal*) and 18 severe aplastic anemia (SAA). Of these, 88 patients were typed for HLA class II. The HLA class I (ABC) and II (DR, DQ) typings were performed by microlymphocytotoxicity test. It was found that HLA class I was associated with CML, ANLL and *Thal*, whereas, HLA class II was associated with SAA. HLA-B8 and HLA-B18 were increased in CML with R.R. values of 12.2 and 3.9, respectively, whereas, HLA-B18 was increased in ANLL with R.R. value of 4.5. In addition, HLA-DR2 and DR3 were increased in SAA with R.R. values of 3.8 and 4.8, respectively. For *Thal*, HLA-A2 and B46 were increased in *Thal* in Central Thais with R.R. values of 3.3 and 6.1, respectively, whereas, HLA-B13 was increased in *Thal* in Northern Thais with R.R. value of 8.5. On the other hand, HLA-B7 was absent in CML. HLA-Cw7 was decreased in CML and SAA, whereas, HLA-DR6 was decreased in ANLL and SAA. Furthermore, HLA-Cw6 was also decreased in CML, whereas, HLA-A33 and Bw4 were decreased in SAA. Although the sample size of each disease was small, the increase of HLA-DR2 was observed in SAA in Thais which was similar to other studies in different ethnic groups. These preliminary data may be useful for further study in HLA and blood disease association.

Key word : HLA, CML, ANLL, Thalassemia, Severe Aplastic Anemia

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HLA antigens play an important role in clinical medicine such as organ or bone marrow transplantation, disease association, platelet transfusion, and paternity test or criminal disputes. For disease association, the first study in human was reported by Amiel in 1967 in patients with Hodgkin's disease[1]. Weak association between 5CREG and Hodgkin's disease was observed. A strong association of HLA-B27 and ankylosing spondylitis was independently reported by Schlosstein et al and Brewerton et al in 1973[2,3]. Since then a large number of reports concerning the possible HLA antigens and various disease associations have been continuously delivered that include autoimmune diseases e.g. IDDM, rheumatoid arthritis, etc; malignancies e.g. nasopharyngeal carcinoma, Kaposi's sarcoma, etc; infections e.g. HIV, Leprosy, etc[4]. The knowledge of HLA and disease association has been applied for disease diagnosis especially in ankylosing spondylitis. Other applications such as prediction of the likelihood for an individual to develop a disease, prognosis of the disease, and the response to medical treatment have been under observation[5,11]. This study aimed to present the association of HLA antigen in various blood diseases.

MATERIAL AND METHOD

A total of 129 patients from the Hematological Clinic, Departments of Medicine and Pediatrics, Ramathibodi Hospital who were referred for HLA typing at Histocompatibility Laboratory for bone marrow transplantation from March 1988 to September 1997 were retrospectively studied for HLA and disease association. The patients included 45 CML, 40 ANLL, 26 thalassemia (*Thal* : 13 ß thalassemia major and 13 ß thalassemia/HbE) and 18 severe aplastic anemia (SAA). The ages of patients with CML, ANLL and SAA ranged from 11-40 years, whereas, the age of patients with *Thal* was less than 10 years. There were more male patients than female patients in CML (2:1) and SAA (3:1), whereas, the sex ratio was not so different in ANLL and *Thal*.

The HLA class I (ABC) and II (DR, DQ) were performed by microlymphocytotoxicity test[6] using local typing trays prepared from local and exchanged sera and trays from One Lambda (USA). All of the patients were typed for HLA-ABC, whereas 88 patients (patients who had a possible HLA identical sibling), were also typed for HLA-DR, DQ. The HLA typing data of 106 normal controls were from our previous study in 11IHW[7]. The disease association was expressed as relative risk (R.R.) calculated from 2 X 2 table, R.R. = ad / bc ; or R.R. = ((2a+1) X (2d+1)) / ((2b+1) X (2c+1)) when b or c = 0. The X^2 test was used for indicating the statistical significance, $X^2 = (ad-bc)^2N / (a+b)(c+d)(a+c)(b+d)$. The X^2 of greater than 3.8415 or p value of less than 0.05 was considered as statistically significant. R.R. > 1 and $p < 0.05$ was interpreted as susceptible to the disease, whereas, R.R. < 1 and $p < 0.05$ was interpreted as resistant to the disease. One third of the thalassemic patients in this study had home towns in the North (7 out of 8 patients were from Nan Province). Therefore, the HLA typings of thalassemic patients were also compared with the control group from the North using data from Fongsatikul L. et al[8].

RESULTS

HLA-ABC phenotype frequencies of 45 CML, 40 ANLL, 26 *Thal*, 18 SAA and 106 normal controls are shown in Table 1. In addition, HLA-DR, DQ phenotype frequencies of 34 CML, 30 ANLL, 13 *Thal*, 11 SAA and 100 normal controls are shown in Table 2. Only one split for HLA-A9 (HLA-A24) and HLA-B12 (HLA-B44) commonly found in Thais is shown in Table 1. Splits within the same broad HLA (class I and II) specificity that had no significant difference in frequency between patient and control groups are not separately shown. The R.R. values of HLA-ABC (Table 1) and HLA-DR, DQ (Table 2) in disease association were calculated. Only R.R. values with $p < 0.05$ are expressed in Table 3. It was found that HLA class I was associated with CML, ANLL and *Thal*, whereas, HLA class II was associated with SAA. HLA-B8 and B18 were increased in CML with R.R. values of 12.2 and 3.9, respectively ($p < 0.05$), whereas, HLA-Cw6 and Cw7 were decreased with R.R. values of 0.1 and 0.5, respectively ($p < 0.05$). HLA-B7 was absent in all of 45 tested CML patients. For ANLL, HLA-B18 was increased with an R.R. value of 4.5 ($p < 0.05$), whereas, HLA-DR6 was decreased with an R.R. value of 0.3 ($p < 0.05$). For *Thal*, HLA-A2, B13 and B46 were increased when using the control group of Central Thais. However, when patients from the North were taken out and reanalysed with the control group from the North using

Table 1. HLA Class I (ABC) phenotype frequencies in patients with CML, ANLL, thalassemia, and severe aplastic anemia.

HLA Specificity	Control		CML		ANLL		Thalassemia		Aplastic anemia	
	n = 106	(%)	n = 45	(%)	n = 40	(%)	n = 26	(%)	n = 18	(%)
A1	6	5.7	0	0.0	3	7.5	2	7.7	1	5.6
A2	42	39.6	24	53.3	15	37.5	18	69.2	9	50.0
A3	6	5.7	0	0.0	0	0.0	0	0.0	0	0.0
A24(9)	35	33.0	11	24.4	11	27.5	5	19.2	7	38.9
A10	8	7.5	5	11.1	1	2.5	1	3.8	1	5.6
A11	47	44.3	22	48.9	23	57.5	15	57.7	11	61.1
A29(19)	2	1.9	0	0.0	1	2.5	0	0.0	0	0.0
A30(19)	2	1.9	0	0.0	0	0.0	0	0.0	0	0.0
A31(19)	1	0.9	1	2.2	2	5.0	1	3.8	0	0.0
A33(19)	34	32.1	17	37.8	12	30.0	5	19.2	1	5.6
A28	1	0.9	2	4.4	0	0.0	0	0.0	0	0.0
Cw1	22	20.8	14	31.1	11	27.5	9	34.6	7	38.9
Cw2	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0
Cw3	44	41.5	19	42.2	13	32.5	16	61.5	8	44.4
Cw4	12	11.3	4	8.9	3	7.5	0	0.0	2	11.1
Cw5	0	0.0	1	2.2	0	0.0	0	0.0	0	0.0
Cw6	14	13.2	1	2.2	2	5.0	2	7.7	0	0.0
Cw7	54	50.9	15	33.3	18	45.0	9	34.6	4	22.2
Cw8*	14	13.2	2	5.0	5	14.7	2	10.0	1	12.5
B5	16	15.1	8	17.8	6	15.0	2	7.7	2	11.1
B7	12	11.3	0	0.0	3	7.5	1	3.8	1	5.6
B8	0	0.0	2	4.4	0	0.0	0	0.0	0	0.0
B44(12)	11	10.4	3	6.7	6	15.0	2	7.7	0	0.0
B13	16	15.1	9	20.0	7	17.5	9	34.6	5	27.8
B15	30	28.3	10	22.2	9	22.5	8	30.8	3	16.7
B16	9	8.5	2	4.4	5	12.5	0	0.0	1	5.6
B17	24	22.6	9	20.0	7	17.5	4	15.4	1	5.6
B18	4	3.8	6	13.3	6	15.0	3	11.5	2	11.1
B22	8	7.5	6	13.3	0	0.0	2	7.7	3	16.7
B27	14	13.2	3	6.7	2	5.0	0	0.0	1	5.6
B35	6	5.7	2	4.4	4	10.0	0	0.0	0	0.0
B37	4	3.8	0	0.0	0	0.0	0	0.0	1	5.6
B40	26	24.5	9	20.0	7	17.5	3	11.5	6	33.3
B46	18	17.0	14	31.1	9	22.5	12	46.2	6	33.3
Bw4	73	68.9	31	68.9	27	67.5	16	61.5	7	38.9
Bw6	78	73.6	39	86.7	31	77.5	22	84.6	14	77.8

* Only 40 CML, 34 ANLL, 20 thalassemia, and 8 SAA were typed with anti-HLA-Cw8.

Table. 2. HLA Class II (DR, DQ) phenotype frequencies in patients with CML, ANLL, thalassemia, and severe aplastic anemia.

HLA	Control		CML		ANLL		Thalassemia		Aplastic anemia	
Specificity	n = 106	(%)	n = 34	(%)	n = 30	(%)	n = 13	(%)	n = 11	(%)
DR1	0	0.0	0	0.0	0	0.0	1	7.7	0	0.0
DR2	34	32.1	13	38.2	11	36.7	4	30.8	7	63.6
DR3	8	7.5	5	14.7	4	13.3	2	15.4	3	27.3
DR4	23	21.7	6	17.6	5	16.7	1	7.7	1	9.1
DR5	48	45.3	11	32.4	12	40.0	5	38.5	3	27.3
DR6	29	27.4	4	11.8	3	10.0	3	23.1	0	0.0
DR7	18	17.0	3	8.8	6	20.0	0	0.0	0	0.0
DR8	7	6.6	5	14.7	3	10.0	2	15.4	0	0.0
DR9	19	17.9	9	26.5	6	20.0	2	15.4	1	9.1
DR10	8	7.5	1	2.9	2	6.7	1	7.7	0	0.0
DR52	76	71.7	24	70.6	22	73.3	8	61.5	7	63.6
DR53	55	51.9	15	44.1	14	46.7	5	38.5	3	27.3
DQ1	70	66.0	19	55.9	16	53.3	8	61.5	8	72.7
DQ2	20	18.9	7	20.6	5	16.7	1	7.7	1	9.1
DQ3	75	70.8	20	58.8	17	56.7	8	61.5	7	63.6
DQ4	9	8.5	1	2.9	0	0.0	0	0.0	0	0.0

Table 3. Relative risk of HLA Class I (ABC) and II (DR, DQ) in patients susceptible and resistant to CML, ANLL, thalassemia and severe aplastic anemia.

HLA	CML		ANLL		Thalassemia		Aplastic anemia	
	R.R.	P	R.R.	P	R.R.	P	R.R.	P
Susceptible								
A2					3.3*	< 0.05		
B8	12.2	< 0.05						
B13					8.5**	< 0.005		
B18	3.9	< 0.05	4.5	< 0.05				
B46					6.1*	< 0.001		
DR2							3.8	< 0.05
DR3							4.8	< 0.05
Resistance								
A33							0.1	< 0.05
Cw6	0.1	< 0.05						
Cw7	0.5	< 0.05					0.3	< 0.05
B7	0.0	< 0.05						
Bw4							0.3	< 0.05
DR6			0.3	< 0.05			0.1	< 0.05

* Comparison with control group from Central Thais

** Comparison with control group from the North (data from Fongsatikul L, et al.).

data from Fongsatikul L, et al[14], it was found that HLA-B13 was increased in the patients. The frequency of HLA-B13 in *Thal* in Northern Thais was 62.5 per cent and the R.R. value was 8.5 ($p < 0.005$). The remaining *Thal* were then reanalysed using the control group of Central Thais. The increase of HLA-A2 and B46 was observed with frequencies of 66.7 per cent and 55.6 per cent, respectively. The R.R. values were 3.3 and 6.1 for HLA-A2 and B46 in *Thal* in Central Thais, respectively ($p < 0.05$ and 0.001, respectively). In addition, HLA-DR2 and DR3 were increased in SAA with R.R. values of 3.8 and 4.8, respectively ($p < 0.05$), whereas, HLA-A33, Cw7, Bw4 and DR6 were decreased with R.R. values of 0.1, 0.3, 0.3 and 0.1, respectively ($p < 0.05$).

DISCUSSION

The information from previous studies of HLA and disease associations in different ethnic groups may suggest that HLA genes and their products contribute to the development or resistance to certain diseases[4,5]. Although HLA itself is not the primary cause of a disease, its linkage disequilibrium to an immune response gene (Ir) will result in susceptibility or resistance to a disease. In addition, one or more genetic determinants may contribute to the development of a disease. In some instances, environmental factors and geographic distribution alter HLA antigen association, and difference in the incidence of the disease in different ethnic groups may be observed[9]. An increase of HLA-DR2 frequency was observed in SAA in both Caucasians[10] and Thais (Table 2, 3). However, HLA-Cw1, DR52 and DR53 were suggested to be protective markers against the development of SAA, AML and ALL in Brazilians[11]. In addition, HLA-Cw3 and Cw4 were suggested to be markers for leukemia susceptibility genes (ALL, AML, CML), whereas, Aw19 may be a marker for leukemic resistance in North American and European Caucasoid patients[12]. The different HLA association in CML and ANLL in Thais may suggest that the etiology of these diseases may be different among various ethnic groups. An increase of HLA-A2 and B46 in *Thal* in Central Thais was different from the increase of HLA-B13 in *Thal* in Northern Thais (Table 3). This finding may indicate that at least two different types of ß thalassemia are found in Thailand. From the report of P. Wasi, the genetic nature of α thalassemia is rather homogeneous while that of ß thalassemia is very heterogeneous in Southeast Asia[13]. More than 60 genotypes of thalassemia syndromes with different combinations of α thalassemia 1, α thalassemia 2, ß thalassemia, HbE and Hb Constant Spring genes will lead to varying degrees of severity from completely asymptomatic to the total lethality of the Hb Bart's hydrops fetalis. Unlike CML, ANLL and SAA diseases, the defect on synthesis of ß globin chain of hemoglobin in thalassemia disease can be inherited by the offspring. The cause of mutation of genes for ß thalassemia in several previous generations is still unknown. The significant associations of HLA and thalassemia were observed in different areas in this study which is worth noting for further study.

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การศึกษาเบื้องต้นของหมู่เลือดเม็ดเลือดขาว HLA ในผู้ป่วย CML, ANLL, thalassemia, และ severe aplastic anemia ในคนไทย

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การศึกษาหมู่เลือดเม็ดเลือดขาว HLA ในผู้ป่วย CML, ANLL, thalassemia (*Thal*) และ severe aplastic anemia (SAA) จากคลินิกโรคเลือด ภาควิชาอายุรศาสตร์ และกุมารเวชศาสตร์ โรงพยาบาลรามาธิบดี ที่ตรวจเนื้อเยื่อสำหรับปลูกถ่ายไขกระดูก ระหว่างมีนาคม พ.ศ. 2531 ถึง กันยายน พ.ศ. 2540 จำนวน 129 ราย เป็นผู้ป่วยโรค CML 45 ราย, ANLL 40 ราย, *Thal* 26 ราย และ SAA 18 ราย ผู้ป่วยทุกรายได้รับการตรวจ complete HLA-ABC typing ในจำนวนนี้ 88 ราย ได้รับการตรวจ HLA-DR, -DQ typing ด้วย การตรวจ HLA typing ใช้วิธี microlymphocytotoxicity test พบว่า HLA class I มีความสัมพันธ์กับโรค CML, ANLL และ *Thal* ส่วน HLA class II มีความสัมพันธ์กับโรค SAA อย่างมีนัยสำคัญ คือพบ HLA-B8 และ B18 สูงในผู้ป่วย CML (R.R. = 12.2 และ 3.9 ตามลำดับ) ขณะที่ HLA-B18 สูงในผู้ป่วย ANLL (R.R. = 4.5) นอกจากนี้ยังพบว่า HLA-DR2 และ DR3 สูงในผู้ป่วย SAA (R.R. = 3.8 และ 4.8 ตามลำดับ) สำหรับผู้ป่วย *Thal* พบความแตกต่างของ HLA ระหว่างภาคกลางและภาคเหนือ กล่าวคือพบ HLA-A2 และ B46 สูงในผู้ป่วยภาคกลาง (R.R. = 3.3 และ 6.1 ตามลำดับ) ขณะที่พบ HLA-B13 สูงในผู้ป่วยภาคเหนือ (R.R. = 8.5) ในทางตรงกันข้าม ไม่พบ HLA-B7 ในผู้ป่วย CML, พบ HLA-Cw6 และ Cw7 ในผู้ป่วย CML ในอัตราที่ต่ำกว่าคนปกติ, และพบ HLA-A33, Bw4, Cw7 และ DR6 ในผู้ป่วย SAA ในอัตราที่ต่ำกว่าคนปกติ ส่วน HLA-DR6 พบน้อยในผู้ป่วย ANLL ถึงแม้ผู้ป่วยที่นำมาศึกษาในแต่ละโรคมีจำนวนน้อย สิ่งที่ตรวจพบเหมือนผู้ป่วยในชนชาติอื่นคือ HLA-DR2 สูงในผู้ป่วย SAA การศึกษาเบื้องต้นนี้จะเป็นประโยชน์สำหรับศึกษาความสัมพันธ์ของหมู่เลือด HLA กับโรคเลือดชนิดต่าง ๆ ต่อไป

คำสำคัญ : HLA, CML, ANLL, Thalassemia, Severe Aplastic Anemia

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