HL A ASSOCIATION WITH CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Dr. BATOOL M. MAHDI *M.B.Ch.B., M.Sc.
SALAWA M. SHAREEF **(B.S.)

Summary :

J Fac Med Baghdad
2005; vol.47 No. 2
Received: Oct. 2002
Accepted: Oct. 2003

OBJECTIVE *Many associations have been found between specific HLA antigens and increased susceptibility to various diseases . So we tried to associate class I and class II antigens with acute lymphoblastic leukemia . We also demonstrate the presence of antibodies in serum of acute lymphoblastic leukemic patients against HLA class I.*
DESIGN *: Prospective study.*
SETTING: *Tissue typing and histocompatibility center at Al- Karamah Teaching Hospital.*
PATIENTS AND METHOD*: 70 acute lymphoblastic leukemia patients from pediatric hospitals. HLA (human leukocyte antigens) typing done for them by serological method and cross matching and blood grouping were also done for them.*
RESULTS: *there was significant difference between patients and control groups regarding HLA -C6,DR1, DR4, DR7, DQ1,DQ2 ,DQ3, DQ4. There was 14.2 % (10/70) of patients had antibodies against HLA class I. There was no significant association between blood group and acute lymphoblastic leukemia .*
CONCLUSION: *Genetic factor increased susceptibility with acute lymphoblastic leukemia (HLA- DR1 DQ1, HLA - DR4 DQ4, HLA- DR4 DQ3, HLA- DR7 DQ2. This HLA typing increased susceptibility to be affected with leukemia after infection.*
RECOMMENDATION: *HLA typing was done to acute lymphoblastic leukemic patients by molecular -DNA based method (PCR-SSP, RSCA) in addition to serological method.*
KEY WARD *: ALL, HLA typing, antibodies, blood group.*

Intodution

Leukemias are a group of malignant disorders of the haematopoitic tissues characteristically associated with increased numbers of primitive white cells (blasts) in the bone marrow. The cause of the leukemia is unknown in the majority of patients. Several factors are associated with development of leukemia like: ionizing radiation, cytotoxic drugs, retroviruses, immunological and genetic factors (1). Certain human diseases occur more frequently among individuals who carry particular MHC alleles. The main categories of diseases studied having a positive association with HLA antigens are those with a known or suspected hereditary factor (2) and those with a possible immunological basis (3). So we tried to investigate HLA class I and class II in children with acute lymphoblastic leukemia to detect the presence of antibodies directed against HLA class I and II antigens.

*AL- KINDI COLLEGE OF MEDICEN DEPARTMENT OF MICROBIOLOG
** TISSUE TYPING CENTER AL-KARAMAH TEACHING HOSPITAL

PATIENTS AND METHODS

- PATIENTS GROUP: consist of seventy Iraqi Arab Muslims children patients complaining from acute lymphoblastic leukemia from pediatrics hospitals in first remission after treatment with traditional therapeutic regime. Lastly they were indicated for allogeneic bone marrow transplantation . Their age ranged from 5-11 years with median age (8 years). Fifty of them were male and the rest were female.
- CONTROL GROUP: Consist of 500 healthy control group, their age ranged from 3-45 years, median was 25 years . 355 of them were male and the rest was female.

HLA typing for class I and II , cross matches for detection antibodies in their serum were done by complement dependent lymphocytotoxicity test (4). Blood group were also done for them . Statistical analysis was done by using Chi- square test.

RESULTS:

Acute lymphoblastic leukemia were more common in male children. Their phenotype and gene frequency in those patients were shown in table -1- . there was no significant difference between patients group and control group in the following HLA antigens : A2,A3,A11, A24, A26, A33, B7, B8, B18, B41, B44, B35, B38,B37, B39, B53, B60, B63, C3, C4, C7, DR52, DR53, DR2, DR3, DR5, DR6, DR10). In addition, there was significant difference in the following antigens : (Cw6, DR4, DR1, DR7, DQ1,DQ2,DQ3,DQ4) table -1- . Relative risks were equal one or more or less than one in different antigens table-1A- . The percentage of acute lymphoblastic leukemic patients who had antibodies in their serum against HLA antigens class I (positive cross- matches) was (14.2%) table-2-. Their was no significant difference between patients and control regarding blood groups as shown in the table -3-.

DISCUSSION:

It had been suggested that childhood leukemia may be the abnormal outcome of a common infection. Rare events caused by a common environmental events such as infection are likely to be influenced by host genetic susceptibility (5). We have therefore investigate whether immunogenetic (HLA typing) susceptibility contribute to the risk of childhood acute lymphoblastic leukemia . In this preliminary study we report that children with acute lymphoblastic leukemia carry the following HLA locus alleles: HLA-Cw6, DR1, DR4, DR7, DQ1, DQ2, DQ3, DQ4 with significant difference than the control group , their relative risks were 3 , 2 ,1.7, 7.1, 3.2, 9.2, 10.1, 3.5 and 0.2 respectively. Moreover, there is a linkage disequlibrium between (DR1 DQ1, DR7 DQ2, DR4 DQ3 and DR4 DQ4) which is in agreement with other results (6). These results suggest that HLA -C , HLA- DR and HLA-DQ either alone or with other alleles contribute to the risk of childhood acute lymphoblastic leukemia possibly by increasing susceptibility to an infectious agent (5).Other studies showed that HLA - DPB1* 0201/ *0301/*0401 and / * 0402 were more frequent in patients with acute lymphoblastic leukemia (ALL)(5).

A molecular analysis was carried in ALL patients to investigate the hetrozygosity for HLA-DR53 and were not different between patients and control (7). It is in agreement with our results regarding HLA- DR 53 and DR52. Molecular mimicry of an HLA -DR 53 epitope by oncogenic (retro) viruses or susceptibility genes in linkage disequilibrium with HLA -DR 53 may be responsible for this association (7).

It had been found that DR4 and DQ2 were significantly correlated with acute myeloid leukemia with favorable remission rates and survival (8). While in our study we showed that HLA-DR4 and DQ2 are significantly correlate with ALL. Possible mechanisms for this association include the linkage or co- inheritance of an oncogenen , facilitate the binding of a transforming virus , toxin, cytokine and impaired immunerecognition of an emerging neoplasm (8).

Other HLA antigen that were detected included HLA - B38 which is present in 10-20 % of the Jewish population and is associated with T- cell leukemia (9) and HLA-B35 increased in Ashkenazi Jews of European origin with chronic lymphoblastic leukemia (10). while in our study HLA- DR35 and B38 , there were no significant differences between patients and control probably due to racial factors. We only studied Arab Muslims.

Family studies showed that Cw3 and Cw4 may be markers for leukemia susceptibility genes (17). This observations imply that in leukemia families unknown MHC - linked recessive factors linked to Cw3 and Cw4 alleles may be due to susceptibility genes which also cause segregation distortion of HLA genes and probably development al errors (17). In our study , we only analyzed ALL and we only studied patients and not whole family because of shortage of materials and cost. We found that only Cw6 had significant difference between patients and control. In the future we will do family study.

The frequencies of HLA-DRB1*0403, *0802, *1403 and *1405 were significantly higher in Japanese patients with chronic myelogenous leukemia (11). Our results showed that HLA -DR4 had significant difference in patients with ALL.

DNA typing of HLA - alleles in CLL patients showed that increased frequencies of HLA -DRB4 * 0103, DRB1* 0401, DQB1 * 0302 and HLA - DPB1* 0301 in patients with CLL (12). Our data showed that DR4 and DQ3 had significant association with ALL. This suggest that factors within or close to the human MHC class II regions confer susceptibility to CLL (12).

Other results found that male patients had a higher frequencies of DQA * 0101 / *0104 and DQB1 * 0501 than control group in ALL . this results suggest a male associated susceptibility haplotype in ALL and supports an infectious etiology (13).This is in agreement with our results that there was significant association and increase in HLA -DQ1 in male patients with ALL.

Our data showed no significant increase in antibodies in serum patients .Other studies showed decrease in these antibodies due to using leukocyte depleted blood (14).

Lastly there was no association between blood group and ALL.

This difference in results may be due to racial factors , religion, method we used (serological) while all studies used molecular DNA

based method (PCR-SSP, RSCA) which is more accurate and sensitive (15,16).

REFRENCES:

1. Mackie, M.J., Ludlan,C.A., and Haynes, A.P.. Diseases of the blood. In "Davidson's Principles and Practice of Medicine". Editors: Haslett, C., Chilvers, E.,R., Hunter, J.A.A. and Boon, N.A.. 18th edition. 1999. pp: 737-800. Churchill Livngstone.
2. Brewerton, D.A., Caffrey, M., Hart, F.D., Jame, D.C.O., Nicholls,A. and Sturrock, R.D.. Ankylosing spondylitis and HLA -B27 .Lancet. 1973.I:904-907.
3. Stokes, P.L., Asquith, P., Holmes, G.K.T., Mackintosh, P. and Cooke, W.J.. Histocompatibility antigens associated with adult coeliac diseases. Lancet.1972.I:162-164.
4. Terasaki,P.I. and McClelland, J.D.. Microdroplet assay of human serum cytotoxin. Nature.1964.204:998-1000.
5. Taylor, G.M., Robinson, M.D., Binchy, A.,Birch, J.M., Stevens, R.F., Jones, P.M., Carr, T., Dearden, S., Gokhale, D.A.. Preliminaryevidence of an association between HLA-DPB1* 0201 and childhood common acute lymphoblastic leukemia supports an infectious aetiology. Leukemia. 1999. Mar; 9(3):440-3.
6. Schreuder,I. And Aiyer,K.HLA -DR,DQ common and rare association.Pel-Freez,clinical system.2001.Wisconsen.USA.
7. Dorak, M.T., Owen, G., Galbraith, I., Henderson, N., Webb, D., Mills, K.I., Darke, C. and Burnett,A.K..Nature of HLA associated predisposition to childhood acute lymphoblastic leukemia.Leukemia 1995.May;9(5):875-8.
8. Joventino,L.P.,Stock,W.,Lane,N.J.,Daly,K. M.,Mick,R.,Le-Beau,M.M.,Larson,R.A..Certain HLA antigens are associated with specific morphologic and cytogentic subsets of acute myeloid leukemia.Leukemia .1995.Mar:9(3):433-9.
9. Colovai, A.I. ,Suciu-Foca, N., Baiulescu, G.E., Harris, P.E. HLA class I self peptides isolated from a T - cell leukemia reveal the allele specific motif of HLA- B 38. Tissue Antigens.1994.Aug.44(2):65-72.
10. Cuttner, J., Skerrett, D., Rosina, O., Troy, K.M., Wallenstein, S., Spivack,M..Increased incidence of HLA antigens B35 in patients with chronic lymphocytic leukemia. Leuk. Res. 1994. Aug; 18(8):565-7.
11. Yasukawa, M., Ohminami, H., Kojima, K., Inokuchi, K., Nishimura, Y.,Fujita, S.. Analysis of HLA-DRB1 alleles in Japanese patients with chronic myelocytic leukemia. Am. J. Hematol. 2000. Feb;63(2):99-101.
12. Machulla,H.K.,Muller,L.P.,Schaaf,A.,Kujat ,G.,Schonermarck,U,Langer,J..Association of chronic lymphoblastic leukemia with specific alleles of the HLA-DR4 : DR53 :DQ8 haplotype in German patients.Int.J. Cancer. 2001. Apr. 15; 92(2):203-7.
13. Tylor, G.M., Dearden, S., Payne, N., Ayres, M., gokhale, D.A., birch, J.M., Blair, V., Stevens, R.F., Will, A.M., Eden, O.B.. Evidence that an HLA-DQA1-DQB1 haplotype influences susceptibility to childhood common acute lymphoblastic leukemia in boys provides further support for an infection- related aetiology.Br.J.Cancer.1998.Sep;78(5):561-5.
14. Adamzik, I.K., Jin, J., Sachs, V., Thommsen, H..Prevention of HLA antibody formation in patients with hemato-oncologic diseases by using leukocyte depleted blood preparations. Infusion. There. Transfusioned. 1995. Feb.22;(1):9-13.
15. Tiercy, J.M., Roosnek, E., Speiser, D., Cros, P., Allibert, P., Mach, B., Jeannet, M.. Replacement of HLA class II serology by the HLA-DR microtiter plate oligotyping assay one year experience in unrelated bone marrow donor selection.Br.J.Haematol.1993.Oct;85(2):417-8.
16. Lorentzen, D.F., Iwanaga, K.K., Meuer, K.J., Moritz, T.L. and Watkins, D.I. A 25% error rate in serologic typing of HLA -B homozygotes.Tissue antigens .1997.50.359-365.
17. Dorak, M.T., Chalmers, E.A.. HLA leukemia: is it a simple allelic association? Turk. J.Pediatr.1992.Jan-Mar;34(1):55-9.

HLA antigens	Patient No.	Patient phenotype	Patient gene frequency	Control No.	Control phenotype	Control Gene frequency	Chi square	P-value	(Odd ratio) Relative risk
A2	22	0.3	0.1	200	0.4	0.2	1.2	N.S.	0.7
A3	12	0.1	0.09	89	0.1	0.09	0.005	N.S.	1
A11	8	0.1	0.1	57	o.1	0.1	**0.0004**	N.S.	1
A24	19	0.2	0.1	108	0.2	0.1	1.8	N.S.	1
A26	9	0.1	0.07	40	0.08	0.04	2	N.S.	1.6
A33	18	0.2	0.1	110	0.2	0.1	0.4	N.S.	1.2
B7	7	0.1	0.05	30	0.06	0.03	1.9	N.S.	1.6
B44	9	0.1	0.07	58	0.1	0.06	0.1	N.S.	1
B35	12	0.1	0.09	71	0.1	0.07	0.6	N.S.	1.2
B38	1	0.01	0.05	3	0.006	0.05	0.6	N.S.	2.4
B60	3	0.04	0.05	13	0.02	0.05	0.6	N.S.	1.6
B41	8	0.1	0.1	55	0.1	0.1	0.01	N.S.	1
B8	4	0.05	0.05	29	0.05	0.05	**0.0008**	N.S.	0.9
B63	2	0.02	0.05	16	0.03	0.05	0.2	N.S.	0.8
B39	1	0.01	0.05	1	0.002	0.05	2.6	N.S.	7.2
B18	3	0.04	0.05	11	0.02	0.05	1.1	N.S.	1.9
B37	1	0.01	0.05	2	0.004	0.05	1.2	N.S.	3.6
B53	2	0.02	0.05	6	0.01	0.05	1.2	N.S.	2
C6	6	0.08	0.05	14	0.02	0.05	6.04	**P<0.01**	3.2
C7	20	0.2	0.1	120	0.2	0.1	0.6	N.S.	1.2
C3	2	0.02	0.05	6	0.01	0.05	1.2	N.S.	2.4
C4	16	0.2	0.1	122	0.2	0.1	0.008	N.S.	0.8
DR53	35	0.5	0.2	60	0.1	0.1	0.1	N.S.	7.3
DR52	23	0.3	0.2	160	0.3	0.2	0.02	N.S.	1
DR3	15	o.1	0.1	110	0.2	0.1	0.01	N.S.	0.9
DR4	22	0.3	0.2	30	0.06	0.05	47.8	**P>0.005**	7.1
DR2	22	0.3	0.2	110	0.2	0.1	3	N.S.	1.6
DR5	10	0.1	0.1	40	0.08	0.05	3	N.S.	1.9
DR1	13	0.1	0.1	90	0.1	0.1	6.7	**P<0.01**	1.7
DR7	12	0.1	0.1	30	0.06	0.05	11.1	**P>0.005**	3.2
DR10	3	0.04	0.05	40	0.08	0.05	1.2	N.S.	0.5
DR6	3	0.04	0.05	10	0.02	0.05	1.4	N.S.	2.1
DQ1	26	0.3	0.2	30	0.06	0.05	67.1	**P>0.005**	9.2
DQ2	12	0.1	0.2	10	0.02	0.1	49.9	**P>0.005**	10.1
DQ3	23	0.3	0.2	60	0.1	0.1	28.2	**P>0.005**	3.5
DQ4	4	0.05	0.05	90	0.1	0.1	6.8	**P<0.005**	0.2

Table -1- HLA phenotype and gene frequency in patients with acute lymphoblastic leukemia and controls showing Chi - square, P- values and odd ratios. (N.S.= not significant)

Control		Patients with positive cross matches		Patients with negative cross matches	
No.	%	No.	%	No.	%
-	---	10	14.2	60	85.7

Table-2- the number and percentages of acute lymphoblastic leukemia patients with positive antibodies against HLA class I antigens.

Blood groups	Patients		Control		Chi square	P - values
	No.	%	No.	%		
O	28	40	195	39	0.001	N.S.
A	19	27.1	145	29	0.1	N.S.
B	17	24.2	135	27	0.07	N.S.
AB	6	8.5	25	5	1.7	N.S.

N.S. = not significant

Table -3- the number and percentages of acute lymphoblastic leukemia with different blood groups compared with healthy normal control group.