ASSOCIATION OF CLASS I HUMAN LEUKOCYTE ANTIGEN IN IRAQI PATIENTS WITH HODGKIN'S LYMPHOMA

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ABSTRACT

Objective:-

To assess the correlation between human leukocytes antigen class I in Iraqi patients with Hodgkin's lymphoma.

Subject and Methods:-

A study was conducted in period between the June 2003 and November, 2004. Study groups included 80 newly diagnosed Hodgkin's lymphoma patients and two control groups, patient controls which include 50 newly diagnosed by Non Hodgkin's lymphoma and 50 healthy controls. Antibody-Mediated complement dependent cytotoxicity assay was done by treating sample of patient's lymphocytes with a panel of anti- human leukocyte antigen antisera and complement.

Results:-

The frequency expression of human leukocyte antigen- A1, A28, B5, B51 and Cw7 was significantly greater in Hodgkin's lymphoma patients compared to healthy control group with ($p < 0.001$) for human leukocyte antigen- A1, A28 and Cw7 and ($p < 0.01$) for B5 and B51.

Conclusion:-

Human leukocyte antigen- A1, A28, B5, B51 and Cw7 are more related with Hodgkin's lymphoma in Iraqi population that reflects allelic association to Hodgkin's lymphoma.

***Key word*:-** Class I human leukocyte antigen , Hodgkin's lymphoma

INTRODUCTION

Hodgkin's lymphoma (HL) is a form of malignant lymphoma, that is unusual in that the bulk of the tumor is composed of normal cells within which the malignant Reed-Sternberg (RS cells) lie (Derek *et al*., 1995). Such classic binucleated (owls eyes) malignant cells in all histologic subtypes usually express antigens found on resting or activated lymphocytes (Urba *et al*., 1992).

While the specific cause of HL is unknown, clusters of the disease in certain regions have been noted, and both genetic and infectious processes are suspected (Ambindar, 2002 and Jarrett, 2003).

In Iraq, among the common malignancies HL ranks the tenth (Ministry of Health, 1993, 1996 and 1999), while in Gulf countries and south east governorate of Yemen, HL is the fourth common cancer (Rabadi , 1987; Ezzata *et al*., 1996 and Bawazir, 1998).

The human leukocyte antigen classes (HLA) region has been subdivided into class I, class II, and class III regions. Each region contains numerous gene loci, and each locus may encode a large number of polymorphic alleles. Class I and class II antigens are composed of two polypeptide chains, associated together by non-covalent link, usually called alpha and beta chains, which dimerize to form the final molecule (Hyde, 2000).

Strong arguments supporting a genetic linkage between susceptibility to HL and HLA antigens (Hors *et al*.,1983) . Moreover, other studies denote that HL is determined by both an HLA-associated major gene and other non-HLA genetic factors together with environmental effects (Shugart *et al*., 2000).

PATIENTS AND METHODS

Patients: -

Cross sectional study was conducted in the following study groups in period between the Jun 2003, and November, 2004. The patients included in this case - control study were classified into 2 groups, patients and control groups.

Eighty newly diagnosed HL patients (32 females and 48 males) who were either attending the Institute of Radiology and Nuclear Medicine or admitted to AL-Mansour and Baghdad Teaching Hospitals in Baghdad City.

All patients were in their new onset of the disease (not on chemo or radiotherapy) at which the histopathological samples were taken and diagnosed as HL according to the National

Cancer Institute Working Formula. All patients were subjected to personal interview using especially designed questionnaire format.

Control groups were age, sex and ethnic matched with patients group, they consisted of two groups patient control which included 50 patients who were newly diagnosed to be affected by NHL and healthy control which included 50 healthy individuals whom were not complaining of any malignant problem.

METHODS

Ten ml of venous blood were drawn from each subject (patients, patients controls and healthy controls). The venous blood was dispensed into plastic or glass universal tubes containing either lithium heparin (10 Iu/ml blood) as anti- coagulant, or glass beads followed by a gentile mixing for HLA typing.

Typing of HLA -A, B and C antigens was carried out in the tissue typing laboratory, Al - Karama hospital, and in the Teaching Laboratories of Medical City in Baghdad .The test microlymphocytoxicity was established by (Terasaki and MaClelland, 1964) and modified by (Dick *et al*., 1979 and Bender, 1984).

Antibody-Mediated complement dependent cytotoxicity assay was done by treating sample of patient's lymphocytes with a panel of anti-HLA antisera and complement.

Anti- HLA sera react with the corresponding lymphocyte antigens without visible cell alteration .The addition of rabbit complement, leads to a change in the structure of lymphocyte cell membrane which can be made visible by means of an indicator vital dye (eosin). The lysed and vital lymphocytes were assessed using an inverse phase contrast microscope.

The significance of an association between HLA alleles and both patients and control calculated using the Chi-square test with Yates correction as well as Fisher exact test (Dorak *et al*., ,2002). To determine the strength of association between HLA specificities and disease, the relative risk (RR), etiological fraction (EF), preventive fraction (PF) and type of association were estimated.

RESULTS

In this study, the HLA class I (A, B and C) allele frequency were determined by microlymphocytotoxicity assay in the 2 groups of lymphoma patients. The first group included 80 patients with HL, while the second included 50 individuals with NHL considered as patients control. Their allele frequencies were compared with 50 healthy controls. The HLA frequencies were compared by Fisher exact test.

Human leukocyte antigen (HLA) typing results obtained for HLA-Class I antigens are summarized in tables (1, 2, 3, 4, 5 and 6). The frequencies of HLA- A1, A28, B5, B51 and Cw7 were significantly increased in HL patients (37.5, 21.2, 37.5, 28.7 and 43.7% respectively) compared with healthy controls ($p < 0.001$) for HLA- A1, A28 and Cw7 and ($p < 0.01$) for B5 and B51 (Tables 1, 2 and 3). Such positive associations were presented with RR values of 5, 3.7, 4, 5.4 and 6.4, respectively and EF values of 0.299, 0.155, 0.244, 0.234 and, 0.369, respectively with positive association.

In comparison between results of HL patients with NHL patients control as shown in tables (4, 5 and 6), it was found that the HLA-Cw6 frequency is significantly ($p<0.04$) increased in NHL patient control than in HL patient (44% versus 28%) ($p < 0.05$).

Table 1: Frequency of HLA-A antigens in Hodgkin lymphoma patients and healthy control

HLA –A antigens	Healthy control No. 50		HL patients No. 80		RR	P	EF	PF	Type of Association
	N	**%**	**N**	**%**					
1	5	10.0	30	37.5	5	0.0003	0.299	--	PA
2	24	48.0	22	27.5	0.4	NS	--	0.278	NA
3	14	28.0	15	18.7	0.6	NS	--	0.112	NA
9	7	14.0	9	11.2	0.7	NS	--	0.032	NA
10	4	8.0	11	13.7	1.7	NS	0.057	--	PA
11	3	6.0	5	6.2	0.9	NS	--	0.0007	NA
23	3	6.0	4	5.0	0.7	NS	--	0.012	NA
24	3	6.0	4	5.0	0.7	NS	--	0.012	NA

25	1	2.0	2	2.5	1.0	NS	0.001	--	PA
26	2	4.0	1	1.2	0.3	NS	--	0.021	NA
28	3	6.0	17	21.2	3.7	0.0008	0.155	--	PA
29	2	4.0	3	3.7	0.8	NS	--	0.005	NA
30	5	10.0	3	3.7	0.3	NS	--	0.059	NA
31	1	2.0	4	5.0	1.9	NS	0.024	--	PA
32	1	2.0	2	2.5	1.0	NS	0.001	--	PA
33	5	10.0	2	2.5	0.2	NS	--	0.065	NA
34	3	6.0	6	7.5	1.1	NS	0.011	--	PA
36	3	6.0	3	3.7	0.6	NS	--	0.023	NA
Blank	11		17						
Total	100		160						

RR = Relative Risk; EF= Etiologic Fraction; PF=Preventive fraction ; PA=Positive Association; (EF> 0.15); NA=Negative Association(PF>0.15); NS=Non Significant; -- = Nill ; P=Probability

Table 2 : Frequency of HLA-B antigens in Hodgkin lymphoma patients and healthy control

HLA –B antigens	Healthy control No 50		HL patients No 80		RR	P	EF	PF	Type of Association
	No.	**%**	**No.**	**%**					
5	5	10.0	26	37.5	4.0	0.0024	0.244	--	PA
7	2	4.0	8	10.0	2.2	NS	0.056	--	PA
8	4	8.0	14	17.5	2.2	NS	0.097	--	PA
12	3	6.0	1	1.2	0.2	NS	--	0.035	NA
13	1	2.0	2	2.5	1.0	NS	0.001	--	PA
14	3	6.0	1	1.2	0.2	NS	--	0.035	NA
15	1	2.0	2	2.5	1.0	NS	0.001	--	PA
16	3	6.0	4	5.0	0.7	NS	--	0.012	NA
17	2	4.0	2	2.5	0.6	NS	--	0.015	NA
18	3	6.0	1	1.2	0.2	NS	--	0.035	NA
21	5	10.0	4	5.0	0.4	NS	--	0.050	NA
22	1	2.0	5	6.2	2.4	NS	0.036	--	PA
27	1	2.0	1	1.2	0.6	NS	--	0.007	NA
35	5	10.0	4	0.0	0.4	NS	--	0.050	NA
37	0	0.0	0	0.0	0.6	NS	0.0	0.0	ND *
38	2	4.0	2	2.5	0.6	NS	--	0.015	NA
39	2	4.0	1	1.2	0.3	NS	--	0.021	NA
40	3	6.0	1	1.2	0.2	NS	--	0.035	NA
41	3	6.0	1	1.2	0.2	NS	--	0.035	NA
44	4	8.0	2	2.5	0.3	NS	--	0.048	NA
45	2	4.0	3	3.7	0.8	NS	--	0.005	NA
47	1	2.0	3	3.7	1.4	NS	0.012	--	PA
48	0	0.0	1	1.2	1.9	NS	0.005	--	PA
49	2	4.0	4	5.0	1.1	NS	0.006	--	PA
50	2	4.0	1	1.2	0.3	NS	--	0.021	NA
51	4	8.0	23	28.7	5.4	0.0032	0.234	--	PA
52	1	2.0	3	3.7	1.4	NS	0.012	--	PA
53	1	2.0	0	0.0	0.2	NS	0.0	0.0	ND
54	1	2.0	2	2.5	1.0	NS	0.001	--	PA
55	2	4.0	0	0.0	0.1	NS	0.0	0.0	ND

56	2	4.0	2	2.5	0.6	NS	--	0.015	NA
57	2	4.0	2	2.5	0.6	NS	--	0.015	NA
60	2	4.0	2	2.5	0.6	NS	--	0.015	NA
62	1	2.0	1	1.2	0.6	NS	--	0.007	NA
63	1	2.0	2	2.5	1.0	NS	0.001	--	PA
70	2	4.0	3	3.7	0.8	NS	--	0.005	NA
73	1	2.0	5	6.2	2.4	NS	0.036	--	PA
Blank	20		21						
Total	100		160			* ND: Non determined			

Table 3: Frequency of HLA-C antigens in Hodgkin lymphoma patients and healthy control

HLA –C antigens	Healthy control No. 50		Hodgkin lymphoma patients No. 80		RR	P	EF	PF	Type of association
	No.	**%**	**No.**	**%**					
1	4	8.0	5	6.3	0.7	NS	--	0.020	NA
2	8	16.0	9	11.3	0.6	NS	--	0.053	NA
3	7	14.0	6	7.5	0.5	NS	--	0.068	NA
4	13	26.0	16	20.0	0.7	NS	--	0.075	NA
5	2	4.0	3	3.8	0.8	NS	--	0.005	NA
6	11	22.0	22	28.0	1.3	NS	0.066	--	PA
7	5	10.0	35	43.7	6.4	0.00002	0.369	--	PA
8	1	2.0	3	3.8	1.4	NS	0.012	--	PA
Blank	49		61						
Total	100		160						

Table 4: Frequency of HLA-A antigens in Hodgkin lymphoma patients and Non- Hodgkin lymphoma control

HLA –A antigens	NHL control No. 50		HL patients No. 80		P
	N	**%**	**N**	**%**	
1	23	46	30	37.5	NS
2	15	30	22	27.5	NS
3	11	22	15	18.7	NS
9	4	8	9	11.2	NS
10	8	16	11	13.7	NS
11	2	4	5	6.2	NS
23	1	2	4	5.0	NS
24	3	6	4	5.0	NS
25	2	4	2	2.5	NS
26	3	6	1	1.2	NS
28	9	18	17	21.2	NS
29	1	2	3	3.7	NS
30	6	12	3	3.7	NS
31	1	2	4	5.0	NS
32	2	4	2	2.5	NS
33	3	6	2	2.5	NS
34	2	4	6	7.5	NS
36	1	2	3	3.7	NS
Blank	3		17		
Total	100		160		

Table 5: Frequency of HLA-B antigens in Hodgkin lymphoma patients and non-Hodgkin lymphoma control

HLA –B antigens	NHL control No. 50		HL patients No. 80		P
	No.	**%**	**No.**	**%**	
5	19	38.0	26	37.5	NS
7	9	18.0	8	10.0	NS
8	6	12.0	14	17.5	NS
12	1	2.0	1	1.2	NS
13	1	2.0	2	2.5	NS
14	0	0.0	1	1.2	NS
15	1	2.0	2	2.5	NS
16	2	4.0	4	5.0	NS
17	1	2.0	2	2.5	NS
18	0	0.0	1	1.2	NS
21	1	2.0	4	5.0	NS
22	1	2.0	5	6.2	NS
27	0	0.0	1	1.2	NS
35	2	4.0	4	0.0	NS
37	1	2.0	0	0.0	NS
38	1	2.0	2	2.5	NS
39	0	0.0	1	1.2	NS
40	0	0.0	1	1.2	NS
41	2	4.0	1	1.2	NS
44	1	2.0	2	2.5	NS
45	1	2.0	3	3.7	NS
47	1	2.0	3	3.7	NS
48	0	0.0	1	1.2	NS
49	3	6.0	4	5.0	NS
50	0	0.0	1	1.2	NS
51	15	30.0	23	28.7	NS
52	1	2.0	3	3.7	NS
53	1	2.0	0	0.0	NS
54	1	2.0	2	2.5	NS
55	1	2.0	0	0.0	NS
56	1	2.0	2	2.5	NS
57	2	4.0	2	2.5	NS
60	1	2.0	2	2.5	NS
62	0	0.0	1	1.2	NS
63	2	4.0	2	2.5	NS
70	1	2.0	3	3.7	NS
73	2	4.0	5	6.2	NS
Blank	18		21		
Total	100		160		

Table 6: Frequency of HLA-C antigens in Hodgkin lymphoma patients and non- Hodgkin lymphoma control

HLA –C antigens	NHL control No.50		HL patients No.80		P
	N	**%**	**N**	**%**	
1	2	4.0	5	6.3	NS
2	8	16.0	9	11.3	NS
3	4	8.0	6	7.5	NS
4	12	24.0	16	20.0	NS
5	1	2.0	3	3.8	NS
6	22	44.0	22	28.0	0.041
7	17	34.0	35	43.7	NS
8	3	6.0	3	3.8	NS
Blank	31		61		
Total	100		160		

DISCUSSION

Hodgkin's lymphoma is relatively rare lymphoma that affects younger as well as elder persons (Mueller, 1996).The disease aggregation in families and persons with specific HLA types indicates genetic susceptibility (Glaser *et al*., 1996).

Human Leukocyte Antigen is located on the short arm of chromosome 6, due to its extreme polymorphism it is considered as one of the best genetic markers and the most voluble prognostic factors used nowadays (Fogdell *et al*., 1998).

A number of reports have studied the associations between HL and HLA, some of them established correlation between several antigens and HL while other found no correlations. The present study is the first to determine the frequencies of HLA-class I using lymphocytotoxicity test in Iraqi patients with HL. Examination has been done for 80 HL patients, 50 NHL patients control and 50 healthy controls. The present study revealed that in patients with HL there were significantly increase in frequency for certain alleles,A1, A28, B5, B51 and Cw7 in HLA-class I , as compared to healthy control, which may confirm the existence of antigenic linkage between such alleles and the susceptibility to the HL in certain individuals.

The frequencies of class I HLA-A1, 28, B5, 51 and Cw7 were increased significantly in HL patients compared to healthy control, however, non of these alleles showed significant positive or negative association with NHL patient control excluded Cw6 allele that showed significant negative association in NHL patients compared to patients with HL.

Different from our results, Amiel, (1967); Green *et al*., (1979) and Hors *et al*., (1983) found significant differences in the HLA- B8 allele while they found B5 allele were found to be similar to that of our study. Racial differences and methods of analysis may be the key factors for the controversy of these results from ours. In addition, 60% to 70% of cases in familial HL may be linked to this region (Chakravorti *et al*., 1986). In 1993, Conte *et al*., in their work on familial cases of NS subtype of HL, found that B18 allele is related to the development of familial HL, particularly the familial NS subtype, however the incidence of HLA-B locus antigens especially that involved B5, B15, B18 and B35 was more associated with HL patients(Tiwari *et al*., 1980). Xixiong *et al*., (1996) reported that HLA28, B18, B51, Cw4 and DQ4 alleles significantly increased as compared NHL patients with healthy control.

In addition, the association of HL with different alleles as evident in this work and other published studies may be explained by more than one possibility. One implies that genes determining these conditions are located close to the MHC loci, and are thus transmitted along with whatever HLA haplotypes in the family (Hafez *et al*., 1985). On the other word heritable factors contribute to development of HL (Jox *et al*., 2002).A second possibility is the cross-tolerance to a self competent (Woda *et al*.,

1981). A third possibility is that the immunogenic responsiveness to oncogenic viruses may be linked to genes coding for HLA antigens, if the concerned coding of the key antigens is missing or present and active, the person will become susceptible to the virus.

In conclusion, The susceptibility to HL in Iraqi population is supposed to be related to certain specific alleles(HLA- A1, A28, B5, B51 and Cw7) which suggests that certain HLA alleles may be associated with predisposition to HD.

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پەیوەندی نێوان پۆلی یەکەمی ئەنتیجینی لیوکوسایتی مرۆڤی بە نەخۆشە عیراقییە تووش بووەکان بە نەخۆشی هۆچکنی لیمفاوی

پوختە

ئامانج: بۆ زانینی پەیوەندی نێوان پۆلی یەکەمی ئەنتیجینی لیوکوسایتی مرۆڤی بە نەخۆشە عیراقییە تووش بووەکان بە نەخۆشی هۆچکنی لیمفاوی هەیە

ڕێگا و کەرەسە بەکارهێنراوەکان: ئەم لێکۆڵینەوەیە ئەنجام درا لە ماوەی بێوان مانگی شەشی ٢٠٠٣ بۆ مانگی یانزەی ٢٠٠٤. گروپی لێکۆڵینەوەکەمان پێکهاتبوو لە٨٠ نەخۆشی تازە دەست نیشانکراو بە نەخۆشی هۆچکنی لیمفاوی. لەگەل دوو کۆمەڵەی کۆنترۆل کە پێکهاتبوو ٥٠ نەخۆشی تازە دەست نیشان نەکراو بە نەخۆشی هۆچکنی لیمفاوی و ٥٠ کەسانی تەندروست. بە دەست هێنانی (Antibody-Mediated complement dependent cytotoxicity assay) **لە لیمفی نەخۆشەکان کرا بەبەکارهێنانی** panel of anti human leukocyte antigen antisera and complement

ئەنجام: ئەنجامی ئەم لێکۆڵینەوەیە دەریخست کەوا دووبارەبوونەوەی ئەنتیجینی لیوکوسایتی مرۆڤی پۆلی A1, A28, B5, B51,Cw7 **لە نەخۆشی هۆچکنی لیمفاوی زیاترە بە بەراوورد کردن لەگەڵ هەردوو کۆنترۆلەکە و جیاوازي دیارو بەلگەداری ئامارکاری تۆمارکرا بە ڕادەی** ($p < 0.001$) **لەگەڵ پۆلی** A1, A28, Cw7 **. وە بە ڕادەی** ($p < 0.01$) **لە لەگەڵ پۆلی** B5, B51 **دەردانی ئەم دوو ماددەیە لەلایەن میزەڵدان ڕۆلێکی گرنگ دەبینن لە نەخۆشی هۆچکنی لیمفاوی**

دەرئەنجام: ئەنتیجینی لیوکوسایتی مرۆڤی پۆلی A1, A28, B5, B51,Cw7 **زۆرتر پەیوەندیدار بوون بە نەخۆشی هۆچکنی لیمفاوی لە خەڵکانی عیراق، ئەمەش ڕۆلی ئەلیلی ئەبەستێت بە نەخۆشییەکەوە.**

ووشە سەرەکیەکان: ئەنتیجینی لیوکوسایتی مرۆڤی پۆلی یەکەم، لە نەخۆشی هۆچکنی لیمفاوی

Cancer Institute Working Formula. All patients were subjected to personal interview using especially designed questionnaire format.

Control groups were age, sex and ethnic matched with patients group, they consisted of two groups patient control which included 50 patients who were newly diagnosed to be affected by NHL and healthy control which included 50 healthy individuals whom were not complaining of any malignant problem.

METHODS

Ten ml of venous blood were drawn from each subject (patients, patients controls and healthy controls). The venous blood was dispensed into plastic or glass universal tubes containing either lithium heparin (10 Iu/ml blood) as anti- coagulant, or glass beads followed by a gentile mixing for HLA typing.

Typing of HLA -A, B and C antigens was carried out in the tissue typing laboratory, Al - Karama hospital, and in the Teaching Laboratories of Medical City in Baghdad .The test microlymphocytoxicity was established by (Terasaki and MaClelland, 1964) and modified by (Dick *et al*., 1979 and Bender, 1984).

Antibody-Mediated complement dependent cytotoxicity assay was done by treating sample of patient's lymphocytes with a panel of anti-HLA antisera and complement.

Anti- HLA sera react with the corresponding lymphocyte antigens without visible cell alteration .The addition of rabbit complement, leads to a change in the structure of lymphocyte cell membrane which can be made visible by means of an indicator vital dye (eosin). The lysed and vital lymphocytes were assessed using an inverse phase contrast microscope.

The significance of an association between HLA alleles and both patients and control calculated using the Chi-square test with Yates correction as well as Fisher exact test (Dorak *et al*., ,2002). To determine the strength of association between HLA specificities and disease, the relative risk (RR), etiological fraction (EF), preventive fraction (PF) and type of association were estimated.

RESULTS

In this study, the HLA class I (A, B and C) allele frequency were determined by microlymphocytotoxicity assay in the 2 groups of lymphoma patients. The first group included 80 patients with HL, while the second included 50 individuals with NHL considered as patients control. Their allele frequencies were compared with 50 healthy controls. The HLA frequencies were compared by Fisher exact test.

Human leukocyte antigen (HLA) typing results obtained for HLA-Class I antigens are summarized in tables (1, 2, 3, 4, 5 and 6). The frequencies of HLA- A1, A28, B5, B51 and Cw7 were significantly increased in HL patients (37.5, 21.2, 37.5, 28.7 and 43.7% respectively) compared with healthy controls ($p < 0.001$) for HLA- A1, A28 and Cw7 and ($p < 0.01$) for B5 and B51 (Tables 1, 2 and 3). Such positive associations were presented with RR values of 5, 3.7, 4, 5.4 and 6.4, respectively and EF values of 0.299, 0.155, 0.244, 0.234 and, 0.369, respectively with positive association.

In comparison between results of HL patients with NHL patients control as shown in tables (4, 5 and 6), it was found that the HLA-Cw6 frequency is significantly ($p<0.04$) increased in NHL patient control than in HL patient (44% versus 28%) ($p < 0.05$).

Table 1: Frequency of HLA-A antigens in Hodgkin lymphoma patients and healthy control

HLA –A antigens	Healthy control No. 50		HL patients No. 80		RR	P	EF	PF	Type of Association
	N	**%**	**N**	**%**					
1	5	10.0	30	37.5	5	0.0003	0.299	--	PA
2	24	48.0	22	27.5	0.4	NS	--	0.278	NA
3	14	28.0	15	18.7	0.6	NS	--	0.112	NA
9	7	14.0	9	11.2	0.7	NS	--	0.032	NA
10	4	8.0	11	13.7	1.7	NS	0.057	--	PA
11	3	6.0	5	6.2	0.9	NS	--	0.0007	NA
23	3	6.0	4	5.0	0.7	NS	--	0.012	NA
24	3	6.0	4	5.0	0.7	NS	--	0.012	NA

25	1	2.0	2	2.5	1.0	NS	0.001	--	PA
26	2	4.0	1	1.2	0.3	NS	--	0.021	NA
28	3	6.0	17	21.2	3.7	0.0008	0.155	--	PA
29	2	4.0	3	3.7	0.8	NS	--	0.005	NA
30	5	10.0	3	3.7	0.3	NS	--	0.059	NA
31	1	2.0	4	5.0	1.9	NS	0.024	--	PA
32	1	2.0	2	2.5	1.0	NS	0.001	--	PA
33	5	10.0	2	2.5	0.2	NS	--	0.065	NA
34	3	6.0	6	7.5	1.1	NS	0.011	--	PA
36	3	6.0	3	3.7	0.6	NS	--	0.023	NA
Blank	11		17						
Total	100		160						

RR = Relative Risk; EF= Etiologic Fraction; PF=Preventive fraction ; PA=Positive Association; (EF> 0.15); NA=Negative Association(PF>0.15); NS=Non Significant; -- = Nill ; P=Probability

Table 2 : Frequency of HLA-B antigens in Hodgkin lymphoma patients and healthy control

HLA –B antigens	Healthy control No 50		HL patients No 80		RR	P	EF	PF	Type of Association
	No.	**%**	**No.**	**%**					
5	5	10.0	26	37.5	4.0	0.0024	0.244	--	PA
7	2	4.0	8	10.0	2.2	NS	0.056	--	PA
8	4	8.0	14	17.5	2.2	NS	0.097	--	PA
12	3	6.0	1	1.2	0.2	NS	--	0.035	NA
13	1	2.0	2	2.5	1.0	NS	0.001	--	PA
14	3	6.0	1	1.2	0.2	NS	--	0.035	NA
15	1	2.0	2	2.5	1.0	NS	0.001	--	PA
16	3	6.0	4	5.0	0.7	NS	--	0.012	NA
17	2	4.0	2	2.5	0.6	NS	--	0.015	NA
18	3	6.0	1	1.2	0.2	NS	--	0.035	NA
21	5	10.0	4	5.0	0.4	NS	--	0.050	NA
22	1	2.0	5	6.2	2.4	NS	0.036	--	PA
27	1	2.0	1	1.2	0.6	NS	--	0.007	NA
35	5	10.0	4	0.0	0.4	NS	--	0.050	NA
37	0	0.0	0	0.0	0.6	NS	0.0	0.0	ND *
38	2	4.0	2	2.5	0.6	NS	--	0.015	NA
39	2	4.0	1	1.2	0.3	NS	--	0.021	NA
40	3	6.0	1	1.2	0.2	NS	--	0.035	NA
41	3	6.0	1	1.2	0.2	NS	--	0.035	NA
44	4	8.0	2	2.5	0.3	NS	--	0.048	NA
45	2	4.0	3	3.7	0.8	NS	--	0.005	NA
47	1	2.0	3	3.7	1.4	NS	0.012	--	PA
48	0	0.0	1	1.2	1.9	NS	0.005	--	PA
49	2	4.0	4	5.0	1.1	NS	0.006	--	PA
50	2	4.0	1	1.2	0.3	NS	--	0.021	NA
51	4	8.0	23	28.7	5.4	0.0032	0.234	--	PA
52	1	2.0	3	3.7	1.4	NS	0.012	--	PA
53	1	2.0	0	0.0	0.2	NS	0.0	0.0	ND
54	1	2.0	2	2.5	1.0	NS	0.001	--	PA
55	2	4.0	0	0.0	0.1	NS	0.0	0.0	ND

56	2	4.0	2	2.5	0.6	NS	--	0.015	NA
57	2	4.0	2	2.5	0.6	NS	--	0.015	NA
60	2	4.0	2	2.5	0.6	NS	--	0.015	NA
62	1	2.0	1	1.2	0.6	NS	--	0.007	NA
63	1	2.0	2	2.5	1.0	NS	0.001	--	PA
70	2	4.0	3	3.7	0.8	NS	--	0.005	NA
73	1	2.0	5	6.2	2.4	NS	0.036	--	PA
Blank	20		21						
Total	100		160			* ND: Non determined			

Table 3: Frequency of HLA-C antigens in Hodgkin lymphoma patients and healthy control

HLA –C antigens	Healthy control No. 50		Hodgkin lymphoma patients No. 80		RR	P	EF	PF	Type of association
	No.	**%**	**No.**	**%**					
1	4	8.0	5	6.3	0.7	NS	--	0.020	NA
2	8	16.0	9	11.3	0.6	NS	--	0.053	NA
3	7	14.0	6	7.5	0.5	NS	--	0.068	NA
4	13	26.0	16	20.0	0.7	NS	--	0.075	NA
5	2	4.0	3	3.8	0.8	NS	--	0.005	NA
6	11	22.0	22	28.0	1.3	NS	0.066	--	PA
7	5	10.0	35	43.7	6.4	0.00002	0.369	--	PA
8	1	2.0	3	3.8	1.4	NS	0.012	--	PA
Blank	49		61						
Total	100		160						

Table 4: Frequency of HLA-A antigens in Hodgkin lymphoma patients and Non- Hodgkin lymphoma control

HLA –A antigens	NHL control No. 50		HL patients No. 80		P
	N	**%**	**N**	**%**	
1	23	46	30	37.5	NS
2	15	30	22	27.5	NS
3	11	22	15	18.7	NS
9	4	8	9	11.2	NS
10	8	16	11	13.7	NS
11	2	4	5	6.2	NS
23	1	2	4	5.0	NS
24	3	6	4	5.0	NS
25	2	4	2	2.5	NS
26	3	6	1	1.2	NS
28	9	18	17	21.2	NS
29	1	2	3	3.7	NS
30	6	12	3	3.7	NS
31	1	2	4	5.0	NS
32	2	4	2	2.5	NS
33	3	6	2	2.5	NS
34	2	4	6	7.5	NS
36	1	2	3	3.7	NS
Blank	3		17		
Total	100		160		

Table 5: Frequency of HLA-B antigens in Hodgkin lymphoma patients and non-Hodgkin lymphoma control

HLA –B antigens	NHL control No. 50		HL patients No. 80		P
	No.	**%**	**No.**	**%**	
5	19	38.0	26	37.5	NS
7	9	18.0	8	10.0	NS
8	6	12.0	14	17.5	NS
12	1	2.0	1	1.2	NS
13	1	2.0	2	2.5	NS
14	0	0.0	1	1.2	NS
15	1	2.0	2	2.5	NS
16	2	4.0	4	5.0	NS
17	1	2.0	2	2.5	NS
18	0	0.0	1	1.2	NS
21	1	2.0	4	5.0	NS
22	1	2.0	5	6.2	NS
27	0	0.0	1	1.2	NS
35	2	4.0	4	0.0	NS
37	1	2.0	0	0.0	NS
38	1	2.0	2	2.5	NS
39	0	0.0	1	1.2	NS
40	0	0.0	1	1.2	NS
41	2	4.0	1	1.2	NS
44	1	2.0	2	2.5	NS
45	1	2.0	3	3.7	NS
47	1	2.0	3	3.7	NS
48	0	0.0	1	1.2	NS
49	3	6.0	4	5.0	NS
50	0	0.0	1	1.2	NS
51	15	30.0	23	28.7	NS
52	1	2.0	3	3.7	NS
53	1	2.0	0	0.0	NS
54	1	2.0	2	2.5	NS
55	1	2.0	0	0.0	NS
56	1	2.0	2	2.5	NS
57	2	4.0	2	2.5	NS
60	1	2.0	2	2.5	NS
62	0	0.0	1	1.2	NS
63	2	4.0	2	2.5	NS
70	1	2.0	3	3.7	NS
73	2	4.0	5	6.2	NS
Blank	18		21		
Total	100		160		

Table 6: Frequency of HLA-C antigens in Hodgkin lymphoma patients and non- Hodgkin lymphoma control

HLA –C antigens	NHL control No.50		HL patients No.80		P
	N	**%**	**N**	**%**	
1	2	4.0	5	6.3	NS
2	8	16.0	9	11.3	NS
3	4	8.0	6	7.5	NS
4	12	24.0	16	20.0	NS
5	1	2.0	3	3.8	NS
6	22	44.0	22	28.0	0.041
7	17	34.0	35	43.7	NS
8	3	6.0	3	3.8	NS
Blank	31		61		
Total	100		160		

DISCUSSION

Hodgkin's lymphoma is relatively rare lymphoma that affects younger as well as elder persons (Mueller, 1996).The disease aggregation in families and persons with specific HLA types indicates genetic susceptibility (Glaser *et al*., 1996).

Human Leukocyte Antigen is located on the short arm of chromosome 6, due to its extreme polymorphism it is considered as one of the best genetic markers and the most voluble prognostic factors used nowadays (Fogdell *et al*., 1998).

A number of reports have studied the associations between HL and HLA, some of them established correlation between several antigens and HL while other found no correlations. The present study is the first to determine the frequencies of HLA-class I using lymphocytotoxicity test in Iraqi patients with HL. Examination has been done for 80 HL patients, 50 NHL patients control and 50 healthy controls. The present study revealed that in patients with HL there were significantly increase in frequency for certain alleles,A1, A28, B5, B51 and Cw7 in HLA-class I , as compared to healthy control, which may confirm the existence of antigenic linkage between such alleles and the susceptibility to the HL in certain individuals.

The frequencies of class I HLA-A1, 28, B5, 51 and Cw7 were increased significantly in HL patients compared to healthy control, however, non of these alleles showed significant positive or negative association with NHL patient control excluded Cw6 allele that showed significant negative association in NHL patients compared to patients with HL.

Different from our results, Amiel, (1967); Green *et al*., (1979) and Hors *et al*., (1983) found significant differences in the HLA- B8 allele while they found B5 allele were found to be similar to that of our study. Racial differences and methods of analysis may be the key factors for the controversy of these results from ours. In addition, 60% to 70% of cases in familial HL may be linked to this region (Chakravorti *et al*., 1986). In 1993, Conte *et al*., in their work on familial cases of NS subtype of HL, found that B18 allele is related to the development of familial HL, particularly the familial NS subtype, however the incidence of HLA-B locus antigens especially that involved B5, B15, B18 and B35 was more associated with HL patients(Tiwari *et al*., 1980). Xixiong *et al*., (1996) reported that HLA28, B18, B51, Cw4 and DQ4 alleles significantly increased as compared NHL patients with healthy control.

In addition, the association of HL with different alleles as evident in this work and other published studies may be explained by more than one possibility. One implies that genes determining these conditions are located close to the MHC loci, and are thus transmitted along with whatever HLA haplotypes in the family (Hafez *et al*., 1985). On the other word heritable factors contribute to development of HL (Jox *et al*., 2002).A second possibility is the cross-tolerance to a self competent (Woda *et al*.,

1981). A third possibility is that the immunogenic responsiveness to oncogenic viruses may be linked to genes coding for HLA antigens, if the concerned coding of the key antigens is missing or present and active, the person will become susceptible to the virus.

In conclusion, The susceptibility to HL in Iraqi population is supposed to be related to certain specific alleles(HLA- A1, A28, B5, B51 and Cw7) which suggests that certain HLA alleles may be associated with predisposition to HD.

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پەیوەندی نێوان پۆلی یەکەمی ئەنتیجینی لیوکوسایتی مرۆڤی بە نەخۆشە عیراقییە تووش بووەکان بە نەخۆشی هۆچکنی لیمفاوی

پوختە

ئامانج: بۆ زانینی پەیوەندی نێوان پۆلی یەکەمی ئەنتیجینی لیوکوسایتی مرۆڤی بە نەخۆشە عیراقییە تووش بووەکان بە نەخۆشی هۆچکنی لیمفاوی هەیە

ڕێگا و کەرەسە بەکارهێنراوەکان: ئەم لێکۆڵینەوەیە ئەنجام درا لە ماوەی بێوان مانگی شەشی 2003 بۆ مانگی یانزەی 2004. گروپی لێکۆڵینەوەکەمان پێکهاتبوو لە80 نەخۆشی تازە دەست نیشانکراو بە نەخۆشی هۆچکنی لیمفاوی. لەگەل دوو کۆمەڵەی کۆنترۆل کە پێکهاتبوو 50 نەخۆشی تازە دەست نیشان نەکراو بە نەخۆشی هۆچکنی لیمفاوی و 50 کەسانی تەندروست. بە دەست هێنانی (Antibody-Mediated complement dependent cytotoxicity assay) لە لیمفی نەخۆشەکان کرا بەبەکارهێنانی panel of anti human leukocyte antigen antisera and complement

ئەنجام: ئەنجامی ئەم لێکۆڵینەوەیە دەریخست کەوا دووبارەبوونەوەی ئەنتیجینی لیوکوسایتی مرۆڤی پۆلی A1, A28, B5, B51,Cw7 لە نەخۆشی هۆچکنی لیمفاوی زیاترە بە بەراوورد کردن لەگەڵ هەردوو کۆنترۆلەکە و جیاوازي دیارو بەلگەداری ئامارکاری تۆمارکرا بە ڕادەی ($p < 0.001$) لەگەڵ پۆلی A1, A28, Cw7, . وە بە ڕادەی ($p < 0.01$) لە لەگەڵ پۆلی B5, B51 دەردانی ئەم دوو ماددەیە لەلایەن میزەڵدان ڕۆلێکی گرنگ دەبینن لە نەخۆشی هۆچکنی لیمفاوی

دەرئەنجام: ئەنتیجینی لیوکوسایتی مرۆڤی پۆلی A1, A28, B5, B51,Cw7 زۆرتر پەیوەندیدار بوون بە نەخۆشی هۆچکنی لیمفاوی لە خەڵکانی عیراق، ئەمەش ڕۆلی ئەلیلی ئەبەستێت بە نەخۆشییەکەوە.

ووشە سەرەکیەکان: ئەنتیجینی لیوکوسایتی مرۆڤی پۆلی یەکەم، لە نەخۆشی هۆچکنی لیمفاوی