

The Association between HLA class II and Iraqi Leukemic Patients

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Summary:

Background: Leukemia is a type of cancer of the blood or bone marrow that is characterized by an abnormal increase of white blood cells.

Objective: Determine frequencies of HLA class II alleles (DRB1 & DQB1) in Iraqi leukemic patients and roll out the association between HLA class II and leukemia types.

Patients: Ninety patients with leukemia were included in this study, fifty three cases were males and thirty seven were females. Patients were attending the National Center of Hematology / Almustansiriya University in Baghdad. 120 healthy individuals represented the control group collected from donors of kidney and bone marrow transplant.

Methods: low resolution PCR-SSO (Sequence Specific Oligonucleotide) technique was used for HLA typing.

Results: Males were predominant in all 4 types of leukemia with a ratio of 1.4:1 and with an age mean of 39.9 (± 16) years. The most frequent HLA DRB1 was DRB1*3 in all leukemia patients but showed no statistical significance when compared with control group. HLA DRB1*5, HLA DRB1*5 and DRB1*6 were found significantly in association with CLL, CML and ALL respectively with a P-value ≤ 0.05 . Regarding to HLA DQB1 typing, only HLA DQB1*3 was significantly associated with CML patients.

Conclusion: The positive association of DRB1*5, DRB1*6 and DQB1*3 with leukemia may have the possibility that these antigens, or the genes encoding them, are closely linked with other possible susceptibility genes that could initiate oncogenesis process in leukemia patients.

Keywords: Acute leukemia, PCR-SSO, HLA class II, chronic leukemia.

Introduction:

Leukemia is a type of cancer of the blood or bone marrow that is characterized by an abnormal increase of white blood cells. Leukemia is a broad term covering a spectrum of diseases. In turn, it is part of the even broader group of diseases called hematological neoplasm (1). Leukemia was first observed by a French physician, Alfred Donné, in 1844 (2). (Donné, 1844 #19; Vinay Kumar, 2007 #24)

Leukemia is clinically and pathologically subdivided into a variety of large groups. There are 4 main types, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) (3).

The causes of the leukemia are unknown in the majority of patients. Several factors, however, are associated with the development of leukemia such as exposure to a variety of environmental agents like ionizing radiation and alkylating agents (4). Ionizing radiation used in the treatment of malignancies such as Hodgkin's disease also has been linked to the (5). Oncovirus such as Human T-cell lymphotropic virus type 1 (HTLV-1) have been isolated from cells of

patients with adult T-cell leukemia which is considered as an endemic disease in certain parts of world (6). Familial leukemia may occur in the context of a clinical syndrome in which it is one component of the overall disease, or as an isolated leukemia predisposition trait that is not specifically associated with co morbid conditions (7).

The term HLA refers to the Human Leukocyte Antigen System, which is controlled by genes on the short arm of chromosome six. The HLA loci are part of the genetic region known as the Major Histocompatibility Complex (MHC). The MHC has genes (including HLA) which are integral to normal function of the immune response (8).

The first HLA study in human leukemia showed an increase of HLA-A2 allele frequency in acute lymphocytic leukemia (ALL) in 1967 (9). A slight but definite increase of A2 has been demonstrated after that in numerous worldwide studies (10).

Based on the structure of the antigens produced and their function, there are two classes of HLA antigens, termed accordingly, HLA Class I and Class II. The overall size of the MHC is approximately 3.5 million base pairs. Within this the HLA Class I genes and the HLA Class II genes each spread over approximately one third of this length. The

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remaining section, sometimes known as Class III, contains loci responsible for complement, hormones, intracellular peptide processing and other developmental characteristics. Thus the Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes (11).

HLA System had Clinical Relevance with Leukemia, Seremetis et al in 1985 have reported the strongest association between AML and HLA alleles using a monoclonal antibody specific for the third hyper variable region (HVR3) epitope of HLA-DR53(12). In another study by Duncan Gowans in 150 AML patients from UK no significant association with HLA-DRB1 alleles was found (13). However in CML, P210 fusion protein as a novel protein to the immune system is presented to T-cells in association with HLA molecules. In this case HLA molecules play an important role in immune response to the tumoral cells (14). In Chinese CML patients, HLADPB1* 1301 and DPB1*20011 frequencies were higher in patients compared with control group(15). HLA-DRw6 frequency was significantly lower in Sicilian CML patients (16).

Study done in the Arabian Gulf Peninsula (AGP), suggested that the presence of HLA A*01, B*35 and DRB1*07 phenotypes in the AGP population provides increased susceptibility to Leukemias. These results also imply that susceptibility/ resistance to leukemias is associated with HLA phenotypes specific to each population (17).

Subjects and Methods:

This study was conducted in period from October 2010 to February 2011 on the following groups, ninety male and female patients (aged 11-75y) with leukemia who were attending the National Center of Hematology / Almustansiriya University, Baghdad, Iraq, and one hundred twenty unrelated healthy male and female individuals (aged 12-63y) composed the control group, collected from donors of kidney and bone marrow transplant which were age and sex matched with the patients group, who were attending the Tissue Typing Center at Al-Karama hospital, Baghdad, Iraq.

All patients were diagnosed by hematologist according to bone marrow aspiration and biopsy at National Center of Hematology / Almustansiriya University.

All laboratory work was undertaken in Al-Karama hospital "Tissue Typing Center". And a low resolution PCR-SSO technique was used for HLA typing. Blood sample of 2.5ml were collected from patients and control groups and stabilized with EDTA in sterile plastic test tubes (AFCO*, Jordan). The samples were stored at -70C°. Materials used in this work are Invisorb* Spin Blood Mini Kit, Invitek*, Germany ; INNO-LiPA HLA-DQB1 Multiplex and Update

kits, Innogenetics*, Belgium and INNO-LiPA HLA-DRB1 Amp Plus 100 and Update kits, Innogenetics*, Belgium. Procedures and standardization were followed according to protocols delivered with each kit. The strength of association between disease and genetic marker is generally expressed in term of relative risk value (RR), which indicates how many times more frequently a disease develops in individuals carrying the marker and in individuals lacking it. The RR is defined by the following formula:

$$RR = \frac{a \times d}{b \times c}$$

a: number of patients positive for the marker.

b: number of patients negative for the marker.

c: number of control positive for the marker.

d: number of control negative for the marker.

The (RR) value can range from less than (negative association) to more than one (positive association). In the latter case an etiological fraction (EF) was given, which indicates how much of a disease is "due to" the disease associated factor.

The EF is defined by the following formula:

$$EF = \left(\frac{RR - 1}{RR} \right) \times \left(\frac{a}{a + b} \right)$$

In the former case, a preventive fraction (PF) was given, which indicates how much of a disease is prevented by the disease associated marker. The PF is defined by the following formula:

$$PF = \frac{(1 - RR) \times \left(\frac{a}{a + b} \right)}{RR \left(1 - \frac{a}{a + b} \right) + \left(\frac{a}{a + b} \right)}$$

Both the EF and PF value can vary between zero (no association) and one (maximum association).

Associations between each allele in patients and controls were compared by means of Chi-square or a two-tailed Fisher's exact test. Level of significance was set to (P-value <0.05). Statistical analysis was done by using Minitab* statistical software.

Results:

It was worthwhile to demonstrate the age, sex in relation to study groups as shown in table 1, in which 53 of leukemia patients were male and 37 were female with ratio of 1.4:1, age mean was 39.9 (±16y) with a range of (11-75) years of age. Regarding control groups, 65 were male, 55 were female with a ratio of 1.18:1, age mean was 33.57 (±12) with a range of 12-63 years of age.

Table 1: Age & sex distribution for study groups

Types of leukemia	SEX		M:F	TOTAL NO. (%)	AGE/YEARS	
	male	female			MEAN ($\pm$ SD)	RANGE
ALL	15	13	1.15:1	28 (31.2)	27.7($\pm$ 11.79)	11-65y
CML	12	10	1.2:1	22 (24.4)	51.7 ($\pm$ 4.76)	25-63y
CLL	12	8	1.5:1	20 (22.2)	47.45 ($\pm$ 16.89)	40-75y
AML	14	6	2.3:1	20 (22.2)	36.45 ($\pm$ 9.5)	12-75y
Total patients	53	37	1.4:1	90	39.9 ($\pm$ 16)	11-75y
Control	65	55	1.18:1	120	33.57 ($\pm$ 12)	12-63y

In healthy control group, figure 1 & 2 depict the frequencies of HLA DRB1 and HLA DQB1 respectively

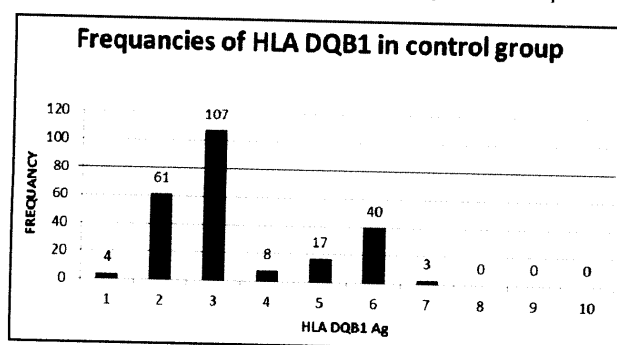


Figure 1: Frequencies of HLA DRB1 in Control Group

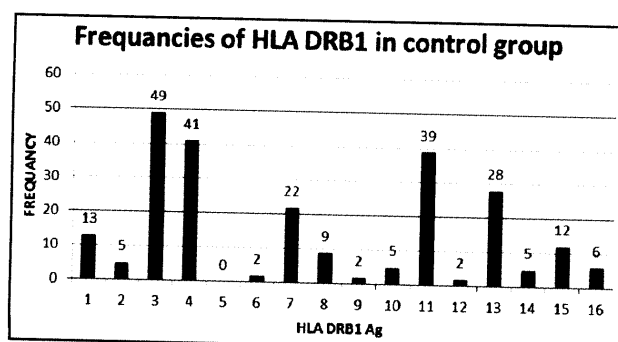


Figure 2: Frequencies of HLA DQB1 in Control Group.

Frequencies of HLA DRB1 in (ALL) patients when compared with healthy control group, only HLA DRB1*6 provide significance association with a (P-value = 0.0129) and may form an etiological risk factor for (ALL) with relative risk (RR) 9.153 and etiological fraction (EF) of 0.59 as illustrated in table 2.

The frequencies of HLA DQB1 in (ALL) patients when compared with healthy control group, no significant associations were observed between groups.

Frequencies of HLA DRB1 in (CML) patients when compared with healthy control group, only HLA DRB1*5 give significance association with a (P-value = 0.0035) and may form an etiological risk factor for (CML) with relative risk (RR) 40.566 and etiological fraction (EF) of 0.85 as

illustrated in table 2.

Frequencies of HLA DQB1 in (CML) patients, these when compared with healthy control group, only HLA DQB1*3 give significance association with a (P-value = 0.00259) and may form a preventive risk factor for (CML) with relative risk (RR) 0.31 and preventive fraction (PF) of 0.147 as illustrated in table 2.

Frequencies of HLA DRB1 in (CLL) patients when compared with healthy control group, only HLA DRB1*5 give significance association with a (P-value = 0.01966) and may form an etiological risk factor for (CLL) with relative risk (RR) 31.234 and etiological fraction (EF) of 0.81 as illustrated in table 2.

While the frequencies of HLA DQB1 in (CLL) patients when compared with healthy control group, no significant associations were observed between groups.

Regarding frequencies of HLA DRB1 & HLA DQB1 in (AML) patients, these when compared with healthy control group, no significant associations were observed between groups.

Table 2: Significant Associations between class II HLA (DRB1, DQB1) in leukemia patients and in control group.

HLA Ag	leukemia type	P-value	RR	Etiological fraction	preventive fraction
DRB1*5	CLL	0.01966	31.234	0.81	0
DRB1*6	ALL	0.01293	9.153	0.59	0
DRB1*5	CML	0.0035	40.566	0.85	0
DQB1*3	CML	0.00259	0.31	0	0.147

Discussion:

The identification of genes associated with leukemia may have a great importance because the identification of those patients at great risk might contribute to different clinical approach concerning treatment or predication of prognosis. Several associations between HLA and human leukemia have been quoted; however, the HLA-DR53 molecule has been one of the most consistently observed in several populations (12, 18,19). Genetic association studies in leukemia disorders are helpful in clarification of pathogenesis as well as reduction or treatment of leukemia disorders of poor prognosis. The biological importance of HLA association in leukemia is emphasized by the fact that HLA may be involved in the identification of candidate genes, which give the advantage of resistance and the disadvantage of disease susceptibility (20). These associations are found in more than one locus, which suggest haplotype association or the existence of several susceptibility loci (18,21). According to sex distributions, the present study had revealed that male is more susceptible to both acute and chronic leukemia than females and this is agree with many studies (22,23). This remains unexplained but might be due to fact that males exposed more to environmental hazards during their life. Doraket al declared that gender associations might be a reflections of a male-specific increase in homozygosity of certain HLA locus (21). Main age group for acute myeloid leukemia patients were between 51-60 years of age, and for acute lymphocytic leukemia were between 21-30 years of age, this was in accordance with other studies (24, 25). Main age group for chronic myeloid & chronic lymphocytic leukemia were 31-40, 41-50 years of age respectively which were documented and approved in plenty of studies (26, 27). There are numerous differences between various ethnic groups concerning HLA & leukemia association (28-31); this may be due to differences in method used in study, racial type, and size of involved groups, in which inadequate group sizes may account for many of the conflicting reports in the literature describing the presence or absence of HLA associations with leukemia. A hypothesis might arise, in which, the positive association of DRB1*5, DRB1*6 and DQB1*3 with leukemia might had the possibility that these antigens, or the genes encoding them, are closely linked with other possible susceptibility genes. Improved genomic mapping techniques make it possible to analyze the linkage between the MHC and leukemia more fully and to assess the contributions from other loci (32). The existence of an association between an MHC allele and a disease should not interpreted to imply that the expression of the allele has caused the disease, the relationship between MHC alleles and development of disease is complex. When the associations between MHC alleles and disease are weak, reflected by low relative risk values, it is likely that

multiple genes influence susceptibility, of which only one is in the MHC. This finding suggested that multiple genetic and environmental factors have roles in the development of disease, with the MHC playing an important but not exclusive role. An additional difficulty in associating a particular MHC product with disease was the genetic phenomenon of linkage disequilibrium (32).

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