The correlation between HLA class II and β-thalassemia major in Al-Karama teaching hospital

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Abstract:

Background: Thalassemia is a form of inherited autosomal recessive blood disorder characterized by abnormal formation of hemoglobin.

Objective: Determine frequencies & association of HLA class II alleles (DRB1& DQB1) in Iraqi β-thalassemia major patients.

Patients: seventy unrelated randomly selected β-thalassemia major patients, and one hundred unrelated randomly selected healthy individuals, composed the control group.

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

Results: HLA DQB1*5 give significance importance as an etiological risk factor for β-thalassemia major; HLA DQB1*3 give significance importance as a preventive risk factor for β-thalassemia major.

Conclusion: The positive association of HLA DQB1*5 and DQB1*3 with β-thalassemia major may have the possibility that these antigens, or the genes encoding them, are closely linked with other possible susceptibility genes.

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Introduction:-

The thalassemia is the commonest monogenic syndrome in man & it is a form of inherited autosomal recessive blood disorder characterized by abnormal formation of hemoglobin. (1,2). It was described at first in 1927 as a kind of severe anemia associated with splenomegaly and bone abnormalities (3). Every year there are more than 60000 child born with thalassemia (4). There are two main classes of thalassemia, α and β, in which the α- and β-globin genes are involved, and rarer forms caused by abnormalities of other globin genes (1, 5). Reduction in synthesis of one of the two globin polypeptides leads to deficiency in hemoglobin gathering, resulting in hypochromic and microcytic red cells (6, 7). In beta thalassemia major, there is a large lack of normal beta chain production. This causes a relative excess of alpha chains, the latter are insoluble and tend to precipitate, forming intracellular inclusions that distort the structure of erythrocytes and lead to premature destruction within the bone marrow and spleen (8, 9). 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 (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 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, 12) .

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Subjects and Methods

This study was conducted in period from November 2010 to January 2011 on the following groups; seventy unrelated randomly selected β-thalassemia major patients (aged 11-21y; 43 males, 27 females), who were attending Thalassemia Center at Al-Karama hospital, Baghdad, Iraq, and one hundred unrelated randomly selected healthy individuals (aged 18-35y; 67 males 33 females), composed the control group, collected from donors of kidney and bone marrow transplant, who were attending the Tissue Typing Center at Al-Karama hospital, Baghdad, Iraq. All patients were diagnosed and registered at Thalassemia Center in Al-Karama hospital, Baghdad, Iraq. All laboratory work was undertaken in Al-Karama hospital, Tissue Typing Center. 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-DQB1Multiplex and Update kits, Innogenetics®, Belgium and INNO-LiPA HLA-DRB1Amp 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 Z-test. Level of significance was set to (P-value <0.05). Statistical analysis was done by using Minitab® statistical software.

Results:
The HLA class II (DRB1, DQB1) typing were done for 70 β-thalassemia major patients and compared with 100 healthy control individuals.

Table and figure (1) illustrate the frequencies of HLA DRB1 in control group.

Table 1: frequency of DRB1 in control group

HLA DRB1 Ag	FRQUANCY	%
1	4	3%
2	4	3%
3	34	24%
4	20	14%
5	3	2%
6	5	4%
7	9	6%
8	3	2%
9	3	2%
10	1	1%
11	20	14%
12	4	3%
13	18	13%
14	3	2%
15	4	3%
16	5	4%
TOTAL	140	100%

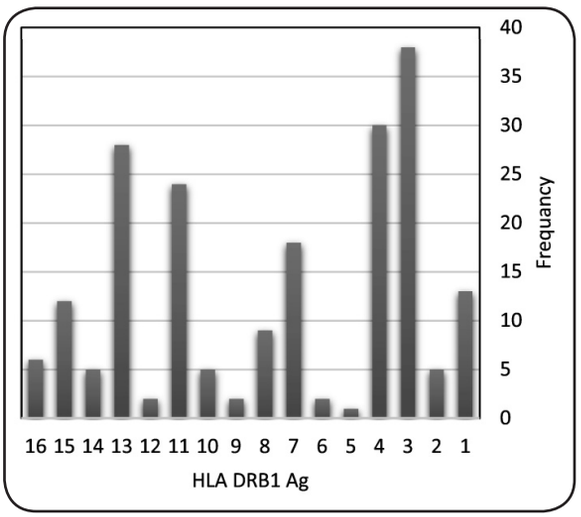

Figure 1: frequencies of HLA DRB1 in control

Table and figure (2) show the frequencies of HLA DQB1 in control group.

Table 2: frequency of DQB1 in control group.

HLA DQB1 Ag	FRQUANCY	%
1	3	1.50%
2	48	24.00%
3	94	47.00%
4	8	4.00%
5	12	6.00%
6	30	15.00%
7	2	1.00%
8	1	0.50%
9	1	0.50%
10	1	0.50%
Total	200	100%

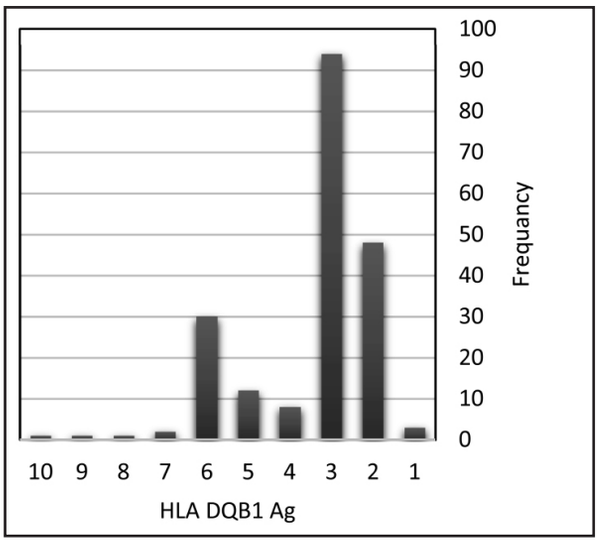

Figure 2: frequencies of HLA DQB1 in control group.

Table and figure (3) demonstrate the frequencies of HLA DRB1 in patients group.

Table 3: frequency of DRB1 in β-thalassemia patients.

HLA DQB1 Ag	FRQUANCY	%
1	8	6%
2	36	26%
3	40	29%
4	6	4%
5	8	6%
6	30	21%
7	3	2%
8	4	3%
9	4	3%
10	1	1%
Total	140	100%

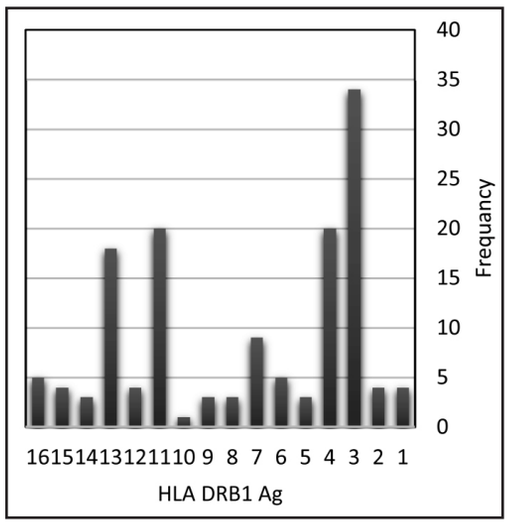

Figure 3: frequencies of HLA DRB1 in patients group.

Table and figure (4) display the frequencies of HLA DQB1 in patients group.

Table 4: frequency of DQB1 in β-thalassemia patients.

DRB1 Ag	FRQUANCY	%
1	13	6.5%
2	5	2.5%
3	38	19%
4	30	15.0%
5	1	0.5%
6	2	1.0%
7	18	9.0%
8	9	4.5%
9	2	1.0%
10	5	2.5%
11	24	12.0%
12	2	1.0%
13	28	14.0%
14	5	2.5%
15	12	6.0%
16	6	3.0%
TOTAL	200	100.0%

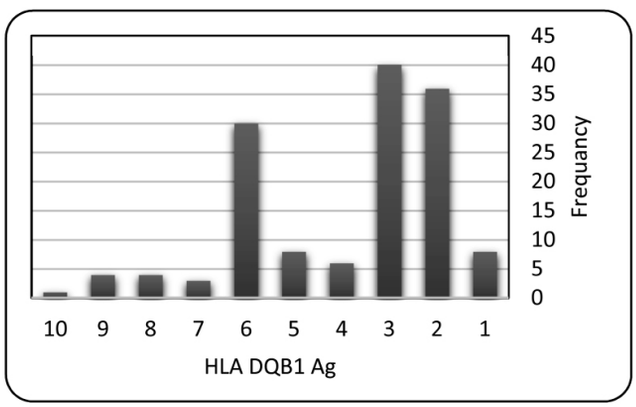

Figure 4: frequencies of HLA DQB1 in patients group.

The frequencies of HLA DRB1 in β-thalassemia major patients when compared with healthy control group, no significant associations were observed between groups. The frequencies of HLA DQB1 in β-thalassemia major patients when compared with healthy control group, HLA DQB1*5 give significance association with a (P-value = 0.03078) and may form an etiological risk factor for β-thalassemia with relative risk (RR) of 3.979 and an etiological fraction (EF) of 0.042. Other finding point out that HLA DQB1*3 give significance association with a (P-value = 0.00062) and may form a preventive risk factor for β-thalassemia with relative risk (RR) of 0.451 and a preventive fraction (PF) of 0.257 as illustrated in table 5.

Table 5: Associations between class II HLA (DRB1, DQB1) in β-thalassemia major patients and in control group.

HLA Ag	P-value	Relative Risk value (RR)	Etiological Fraction (EF)	Preventive Fraction (PF)
DQB1*1	0.03078	3.979	0.042	0
DQB1*3	0.00062	0.451	0	0.257

Discussion

Studying the genes that could be associated with β-thalassemia major may have a lot of importance as the identification of those patients at great risk might contribute to different clinical approach concerning treatment or predication of prognosis. Studies that correlates genetic relationship with HLA in β-thalassemia major would be helpful in clearing up pathogenesis in addition to minimizing symptoms and/ or future treatment planning (13). The significance of HLA association in β-thalassemia major is emphasized by the fact that HLA typing may be involved in the identification of candidate genes, which give the help of resistance and the drawback of disease susceptibility (14). To the best of our knowledge , no previous study in Iraq handled the plink between HLA and β-thalassemia major, only two studies found in china; the first one by Long G1, Mohamed AA in 1998 (15) and they concluded that the susceptibility to beta-thalassemia in the Guangxi Zhuang individuals is associated with HLA-DQB1*0604 allele. The second study by Bao R. et al 2002(16) and their data suggested that HLA-DQB1*06 allele is associated with pathogenesis of the major beta-thalassemia in Guangdong area.In Iraq, studies that linked genetics to thalassemia were concerned mainly with gene mutation. In 2006 Al- Allawi et al (17) provided a base for prenatal genetic testing that will be part of a thalassemia prevention program in the Dohuk region. In 2009 Al Allawi et al (18) handled the molecular level of alpha thalassemia in Iraq. In 2010 the same researcher (19) picked up beta-Thalassemia Mutations among Transfusion-Dependent Thalassemia Major Patients in Northern Iraq. In the same year Jalal S. D. et al (20) discussed the beta-Thalassemia mutations in the Kurdish population of northeastern Iraq. In 2013 Al Allawi et al (21) discussed the spectrum of beta-thalassemia mutations in Baghdad, Central Iraq. In 2013 (22) Abdulwahid et al picked up beta- and alpha thalassemia intermedia in Basra and in 2014 Al Allawi et al studied beta thalassemia intermedia in North of Iraq (23). This study included seventy β-thalassemia major participant and one hundred healthy control participant. Class II HLA DRB1 & DQB1 typing was done and revealed that HLA DQB1*5 was more in frequency in patient and could be an etiological risk factor for β-thalassemia with relative risk (RR) of 3.979 and an etiological fraction (EF) of 0.042. And HLA DQB1*3 was more in frequency in control group which can be a preventive risk factor for β-thalassemia with relative risk (RR) of 0.451 and a preventive fraction (PF) of 0.257. This positive association of DQB1*5 & HLA DQB1*3 might had the probability that these antigens, or the genes encoding them, are closely linked with other possible susceptibility genes. The presence of an association between an HLA allele and a disease should not interpreted to imply that the expression of the allele has caused the disease, the relationship between HLA alleles and development of disease is complex. When the associations between HLA 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 HLA. This finding suggested that multiple genetic and environmental factors have roles in the development of disease, with the HLA playing an important but not exclusive role. An additional difficulty in associating a particular HLA product with disease was the genetic phenomenon of linkage disequilibrium (24) . Further advanced molecular techniques make it promising to analyze the linkage between the HLA and β-thalassemia major more completely and to evaluate the possible influences from other loci.

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