Increased Frequency of Complement C4 "Null" Alleles in Autoimmune Hepatitis

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Summary:

Background: Human leukocyte antigen (HLA) is the most polymorphic genetic system in man. The genes of this region influence susceptibility to certain diseases.

Patients and methods: Immunofixation test is the method used to asses C4 polymorphism of 100 blood samples of 60 AIH patients and 40 healthy normal controls.

Results: An increased frequency of C4A*Q0 was observed for patients group versus control group with P-value (0.003).

Conclusions: This finding demonstrated that C4A*Q0 might play a role in AIH susceptibility.

Key words: autoimmune hepatitis, HLA, C4A*Q0, Immunofixation test.

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Introduction:

Autoimmune hepatitis (AIH) is an unresolving inflammation of the liver of mysterious etiology, characterized by interface hepatitis on histological examination, hypergammaglobulinemia, and autoantibodies, which in most cases, responed to immunosuppressive treatment (1, 2). Accordingly to several observations, attention has been brought to the believe that a predisposition to autoimmunity is inherited, while the liver specificity of this injury is triggered by environmental (chemical or viral) factors (3,4). The MHC region between class I and class II is called class III region contain several different genes coding for complements C2, C4, BF, two cytokines (TNF α and β), 21hydroxylase "an enzyme important in steroid metabolism"and two HSP (hsp 70-1 & hsp 70-2) (5) Concerning C4, it is well known that, they synthesized mainly in the liver, but it is also produced by macrophages. It is 202 kDa in size, synthesized from a 5.5 kb mRNA. There are two isotypes of C4, C4A and C4B and they have a 99% homology on sequence level, but the chemical and serological properties are divergent (6,7). In addition to the isotypic variation, there are roughly 40 different allotypic variants for C4A and C4B (8). They differ from each other by electrophoretic mobility or hemolytic properties. It was found that C4A3 and C4B1is the most frequent alleles in Finnish population since they represent (87%) and (58%) respectively (9). Altogether, six alleles for C4A (A0, A2, A3, A4, A5 and A6) and five for C4B (B0, B1, B2, B3, B5) are represented in Finns. Moreover, the most common C4 combination is C4A3-C4B1, but C4A3-C4BQ0, C4A2-C4BQ0, and C4AQ0-C4B1 also have frequencies over 10% (10). It was reported that increased susceptibility to infections, immune complex diseases and rheumatological disorders are the most common consequences of complement deficiencies (11,12).

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Patients and Methods:

Patients: The present study included 60 Arab, Iraqi AIH patients (42 females and 18 males), attending The Gasteroentestinal and Hepatology Teaching Hospital, Baghdad Teaching Hospital and Al-Yarmook Teaching Hospital in a period between November 2006 and July 2007.Their age raged between 4-62 years, compared with 40 healthy individuals (age and sex matched). Both groups were typed for HLA class-III (C4 complement components).Detection of C4 polymorphism by Imminofixation test : Human blood was collected into EDTA-coated tubes and the separated plasma from each individual was stored at -70C° until required. Samples of plasma (50*Ml)* were treated with 20 *M*l (6.5 units) of carboxypeptidase B and incubated at 25C° for 30 min, after that the sample was treated with neuraminidase; agarose gel electrophoresis was carried out at PH 8.6 and C4 was detected with rabbit anti-human C4 antibodies. Intensity of Coomassie Blue staining of C4-anti-C4 on gel was determined by scanning gels with vitatron densitometer.

Results:

The genetic polymorphisms of the C4 complement components comprising two loci, C4A and C4B, were investigated in AIH patients compared to healthy control groups.

In healthy control groups, for C4A locus seven structural alleles (C4A*1, *11,*2,*3,*4,*51 and *6) and for C4B locus five structural allelels (C4B*1,*11,*2,*3 and *6) were encountered. The most frequent alleles at C4A and C4B loci were C4A*3 (80.0%) and C4B*1 (75.0%), and the least frequent were C4A*11, C4A*4 and C4B*Q0 each with an allele frequency of 2.5%. The second most frequent allele at both these loci were C4A*6=25.0% and C4B*2=17.5%, (table-1), (figure-1). In AIH patients, 7alleles were detected for C4A locus. The C4A*3 was the most frequent and accounted for 81.6%, which was almost identical to that observed in the healthy control group. A significant increased frequency of a deficient (C4A*Q0) was observed in the AIH patients compared to the healthy control (28.3% Vs. 2.5%), P value=0.003. At the C4B locus, it was possible to detect 5 alleles C4B*1, C4B*2, C4B*3, C4B*4 and C4B*Q0. The comparison of the C4B alleles frequencies revealed significantly increased frequencies of C4B*2 (29.6% Vs. 17.5%, P=0.05), and C4B*3 (14.8% Vs 7.5%, P=0.05) alleles among the total AIH patients compared to the controls. With respect to other C4A and C4B alleles, no other significant difference was observed between the patients and healthy control groups (table-1).

So from all what had been mentioned previously it appeared that C4A*Q0 formed a big significant difference between patients and normal persons since its P value 0.003.

Table-1: Observed numbers and percentage frequencies of C4A and C4B alleles in AIH patients and healthy controls

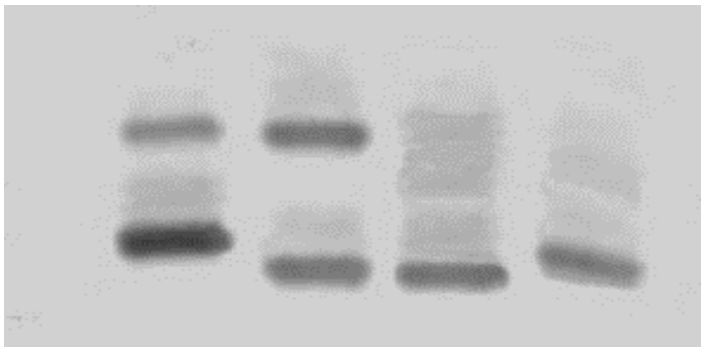
Allele	AIH patients (No.=60)		Healthy control (No.=40)	
	No.	%	No.	%
C4A				
*1	N.D.	N.D.	3	5.0
*11	N.D.	N.D.	1	2.5
*12	N.D	N.D	N.D.	N.D.
*13	1	1.6	N.D.	N.D.
*2	4	6.6	7	17.5
†*3	49	81.6	32	80.0
*4	1	1.6	1	2.5
*5	N.D	N.D	N.D.	N.D.
*51	2	3.3	4	10.0
*6	6	10.0	10	25.0
** C4A*Q0	17	28.3	2	5.0
C4B				
*1	40	66.6	30	75.0
*11	N.D.	N.D.	2	5.0
* *2	15	29.6	7	17.5
* *3	7	14.8	3	7.5
*4	1	1.6	N.D.	N.D.
*5	N.D.	N.D.	N.D.	N.D.
*6	N.D.	N.D.	3	7.5
C4B*Q0	1	1.6	1	2.5

N.D.: Not detected

* P=0.05

**P=0.003

† P =0.4 (Not significant)



1 2 3 4

Figure-1: Gel electrophoresis showing some C4 phenotypes in AIH patients: track 1,A3-B1; track 2, A3-B2; track 3, AQ0-B2; track 4, AQ0-B1.

Table-2: Observed numbers and percentage frequencies of C4A and C4B alleles in different types of AIH types.

Alleles	AIH-1 (No. = 35)		AIH-2 (No. = 15)		AIH-3 (No.=10)	
	No.	%	No.	%	No.	%
C4A						
*1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
*11	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
*12	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
*13	N.D	N.D	N.D	N.D	1	10.0
*2	2	5.7	1	6.6	1	10.0
*3	30	85.7	10	66.6	9	90
*4	1	2.8	N.D.	N.D.	N.D.	N.D.
*5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
*51	1	2.8	N.D.	N.D.	1	10.0
*6	3	8.5	1	6.6	2	20.0
C4A*Q0*	2	5.7	14	93.3	1	10.0
C4B						
*1	28	80.0	7	46.6	5	50.0
*11	N.D	N.D.	N.D.	N.D.	N.D.	N.D.
*2	7	19.9	3	20.0	5	50.0
*3	5	14.2	2	13.3	2	20.0
*4	1	2.8	N.D.	N.D.	N.D.	N.D.
*5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
*6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C4B*Q0	1	2.8	N.D.	N.D.	N.D.	N.D.

N.D.: Not detected

* P= 0.001

Association of HLA-Class III (C4) Ags among different types of AIH:-

Among C4A locus, C4A*Q0 had a major significant effect, they formed the dangerous factor in type-2 of the disease with (P value 0.001). Non of other alleles of C4A locus showed significance between the 3 types of AIH.

Discussion:

The study of C4 polymorphism is limited to few European populations and some mongoloid groups. However, for the first time the genetic polymorphism of HLA-class III (C4) antigens were included as part of the ongoing genetic investigation in our country, these polymorphic studies provide information of genetic variation of complement components (C4A and C4B) on patients with AIH compared to healthy control group.

It has been reported that C4 loci is highly polymorphic (13), these result resemble ours since, this study revealed that in healthy control group at C4A locus seven structural alleles has been detected, whereas five structural alleles were identified at C4B locus which make their polymorphism was slightly less extensive. At present, it is difficult to comment if these alleles are characteristic to Iraqi population or if they have any other anthropological significance. In this study the most common allotypes was C4A*3 (80.0%) and C4B*1 (75.0%) followed by C4A*6 (25.0%) and C4B*2 (17.5%).

A comparison with healthy control group revealed a significant increased frequency of C4A null allele in the AIH patients (P value 0.003). This finding is in agreement with many studies that were done on AIH patients in other countries, for instance European patients (14), Brazilian (15), Germany (16). All these studies qualify the C4A*Q0 to be a predisposing immunogenetic markers for AIH in populations of different ethnic origins.

It has been reported that the association between C4B*Q0 allele and AIH is much less strong than C4A*Q0 allele. This fact was true since our study revealed that, only 1.6% of patients with AIH express this allele. Indeed the majority of studies have found no increase in the C4B*Q0 allele frequency in Caucasoid (16,17), Asian (18), and African-American populations (250). It is therefore necessary to explain why a partial deficiency of C4A and not C4B might predispose to AIH. It is well known that, the C4A isotype was preferentially bind to amino groups and as a result they bind to immune complex (19), whilst C4B is hematologically more active than C4A, so it could be hypothesized that deficiency of C4A null allele result in impaired processing of immune complexes or apoptotic cells which may allow infectious agent or immune complex to persist which may be important in the pathogenesis of AIH.

So, generally the development of AIH would depend upon the expression of the susceptible HLA alleles and absence of the protective alleles, along with the environmental factors that trigger the autoimmune process. This fact was confirmed recently by Czaja(1).

Its generally accepted that, three types of AIH has been proposed based on immunoserological markers, these types differ from each other in many aspects, including the genetic markers. This fact was proved by many investigators (1,8). So, the second important categories in the current study were to find the allelic risk factors that distinguish each type of the disease.

The importance of class III genes, particularly C4 in the pathogenesis of AIH-2 has been confirmed previously by many workers. For instance Czaja revealed a strong association between C4A*Q0 allele and this type in Caucasian patients (7). This fact was emphasized by the current study since C4A*Q0 present in 93.3% of type 2-AIH patients compared to 5.7% and 10.0% of patients with type 1 and 3 respectively.

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