Original Article

Viruses as a Trigger for autoimmune Hepatitis in susceptible Individual

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Summary:

Background: Autoimmune hepatitis (AIH) is a rare chronic liver disease of known etiology, characterized by hypergammaglobulinemia, characteristic auto antibodies, and a favorable response to immunosuppressive treatment.Strong circumstantial evidences denoted that there is quite long list of environmental factors such as (food additives and drugs), viruses and toxins which play an important role in precipitating this disease.

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Patients and Methods: the study was performed on 13 Iraqi patients with acute viral hepatitis, attending the Teaching Hospital for Gastroenterology and Liver Disease and Al-Khadymia Teaching Hospital in the period between November 2003 and July 2005. ANA, SMA and anti-LKM 1 were detected by immunoflurescent technique whereas SLA/LP Ab was detected by Euroline method.

Results: the thirteen patients with acute viral hepatitis were developing AIH after 3 months

Conclusion: acute viral hepatitis is a trigger of AIH.

Keywords: Viral hepatitis, AIH

Introduction

Autoimmune hepatitis (AIH) is a chronic, progressive, and sometimes fluctuating necroinflammatory liver disorder of unknown origin (1). It is characterized by immunologic and autoimmune features, including the presence of circulating autoantibodies and elevated serum globulin levels, a heterogenous clinical picture, and a response to therapeutic immunosuppression (2). In addition, there is a significant predisposition of the female sex and a significant association with the presence of HLA DR3 and DR4 alleles among affected patients (3). Three types of AIH has been proposed on the basis of immunoserologic markers; type- 1 characterized by the presence of ANA and\or anti-smooth muscle antibody (SMA); whereas type -2 is characterized by presence of anti-liver-kidney microsomal antibodies (LKM-1) while, soluble liver antigen/ liver pancreas (SLA/LP) is a characteristic autoantibodies of type -3 of the disease(4,5,6) There is no doubt that a loss of self- tolerance is the pathophysiologic process driving AIH, and this leads to the observed sequelae. However, the precise cause of this disease remains elusive and a number of concepts have been pursued to elucidate the causative agents or mechanisms leading to AIH. A large body of evidence has pointed to a viral etiology of the disease, potentially resulting in either a virus- triggered, sustained autoaggressive immunologic reaction, or an autoimmune reaction directly induced by hepatotropic viruses (7).However, the association of viral infection and AIH was firstly reported in 1985 (8), since then, so many studies were done to explain the nature of such association. All these studies denoted that, the infection with viruses may trigger preexisting autoimmune processes either by the mechanism of molecular mimicry or by induction of inflammatory cytokines (4,9). HCV is on the top of the list which shown to play an important toxic and immune role in the development of AIH in susceptible patients (10). Additional support to such relationship emerged from recent studies by Kerkar, *et ah,* (11) who showed the similarity and cross-reactivity between the immunodominant epitope 193-212 of CYP2D6 and homologues of HCV. Several other series of studies pointed towards HAV, HBV, HEV, EBV, HSV-1 and CMV role followed the detection of virus Abs in AIH patients (12, 13, 14, 15, 16,17). The interested finding was supported by a case report study in a pair of identical twins . Who showed a healthy one while the other one had ICP4 protein of HSV-infected cells serum positive was suffering from AIH-2(18).Epstein-Barr virus (EBV) are not excluded, they found to induce AIH via similarity between EBV lycoprotein 110 and HLA class II which may explain the increased presence of Ab to this virus in AIH (19) .

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Patients and Methods:

This study was conducted in a period between November 2002 and July 2005 in Teaching Laboratories, Medical City. Among one hundred Iraqi patients with AIH, ninety three patients were attending the

Gastroenterology and Hepatology Teaching Hospital, seven patients from Al-Khadymia Teaching Hospital. All of whom met the revised criteria for AIH as defined by the International Autoimmune Hepatitis Group 1999, we describe thirteen patients (10 females and 3 males),who was diagnosed with acute viral hepatitis of different causes by serological and clinical means three months ago, they was admitted to hospital deterioration and elevated liver enzymes.

Laboratory investigation**:** Anti-smooth musc (SMA), and anti-liver/ kidney microsom type -1 (anti-LKM 1) Abs were detecte dilutions of sera by indirect immun technique (IIF) on rat liver-kidney-stom substrate) cryostat section; for anti-nucle (ANA), slides of mouse liver were used. titer for ANA, SMA, and anti-LKM 1 Abs w >1: 40, > and >1: 100 respectively. Posi were recognized by presence of specific flurescence. Euro line methods for presenc liver antigen/liver pancreas antibody (SL anti-LKM 1 Abs (as confirmatory test) wer positive results were recognized by presenc line. Positive and negative controls were included at all stages according to the manufacturers' instructions and to confirm the validity of the test. Euro immune has supplied the above kits company, Germany, virological screen which include IgM anti HEV antibodies by enzyme-linked immunosorbent assay (ELISA)kits ,IgM anti-HAV for hepatitis A virus (HAV), HBS Ag (hepatitis B surface antigen), IgM anti-HBc against core antigen for hepatitis B virus (HBV), and anti HCV antibodies for hepatitis C virus(HCV). Patients with positive results for HCV were retested using a more specific test, a RIBA-based test that allows for the detection of antibodies against specific HCV antigens, and further tested for the presence of HCV RNA by PCR-based test.Biochemical test (AST, ALT, TSB and Alk. Phosphates) were performed using commercially available kits (Randox-UK).

Results:

In this study, three patients had positive serological markers for HAV;one had HBV, four HCV, one HEV, two EBV and two CMV as in table-(1)

Table (1): The distribution of types of viruses among patients

Virus type	Total	
	No	%
HAV	3	23.0
HBV	1	7.6
HCV	4	30.7
HEV	1	7.6
EBV	2	15.3
CMV	2	15.3
Total	13	100

Interestingly, all the 13 patients who presented with viral hepatitis before 3 months ago, they develop AIH after that since they found to be positive for immunoserologic markers of AIH as shown in figure-1. Titer of this auto-Abs in our patients ranging from 1/320-1/640 for ANA and SMA and 1/200-1/400 for anti-LKM 1 Abs and therefore they fulfilling standard diagnostic criteria for AIH.

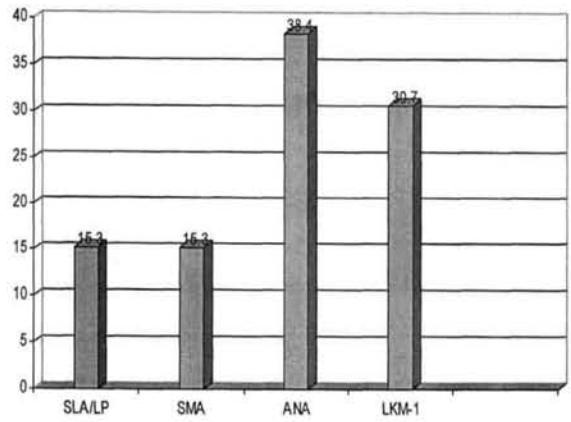


Figure -1: Frequency distribution of positive auto antibodies among patients

The age distribution of the 13 patients were presented in table-2, the mean age was 34.82+10.7 with a range 8-62.

Table 2: The age distribution of viral hepatitis patients

Age (years)	AIH (n=13)	
	No	%
<20	1	7.6
20—29	5	38.4
30—39	3	23.0
40—49	2	15.3
50—59	1	7.6
>60	r	7.6
Total	13	100
Mean+SD	34.82+10.7	
Range	8-62	

The biochemical test were measured at the beginning (during acute viral hepatitis) shown in table-3

Table (3): The biochemical parameters findings in the beginning

Biochemical parameters	AIH(n=12)	Healthy control (n=50)	P value
S.GOT	35.32+17.35 13-80	14.0+2.5 12-20	<0.001
S.GPT	41.5+13.9 12-55	13.5+3.5 10-19	<0.001
S. Alkaline phosphates	158.5+35.4 77-240	71.1+5.8 62-82	>0.05
TSB	3.67+1.77 1.5-7.7	0.4+0.3 0.1-0.9	<0.001

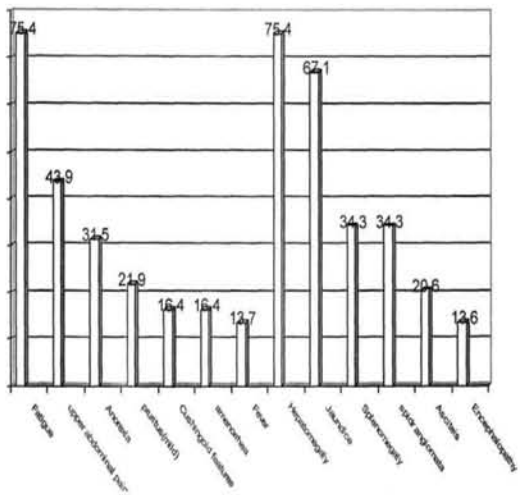
Then after 3 month all these patients developed elevated

liver enzyme, as shown in table-4

Table (4) biochemical parameters increased after resolution of viral infection

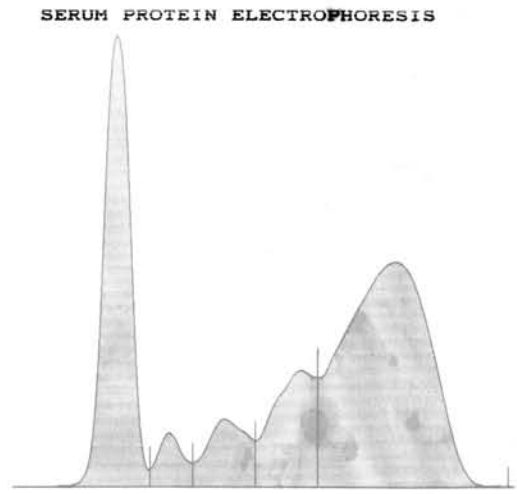
S.GOT	79.63+32.9 45-142
S.GPT	79.7+31.62 24-160
S. alkaline phosphates	137+25.1 80-195
TSB	9.5+4.6 0.7-18.0

Regarding the different clinical sign and symptoms, fatigue is the most common one in our patients ,since they occurs in 75.4% followed by upper abdominal pain (43.9%) and anorexia (31.5%), mild pruritus was found in 21.9% but none had intense pruritus. Fever in absence of explainable cause was found in 13.7%. The most common physical finding was hepatomegaly (75.4%), jaundice (67.1%) and splenomegaly (34.3%) whereas ascites and encephalopathy in *20.6%* and 13.6%) respectively as in figure-2



In addition, polyclonal gammapathy was detected on protein electrophoresis in all patients, Figure-3.

Figure-2: The frequency distribution of clinical features of AIH patients



HELENA Agarose Gel

FRACTION	%	G/dL	REF. RANGES %	REF. RANGES G/dL
ALBUMIN	**29.3-**	**2.5-**	**46.0 - 58.0**	**3.5 - 5.0**
ALPHA 1	3.3	0.3	2.0 - 5.0	0.1 - 0.4
ALPHA 2	6.8-	0.6	11.0 - 16.0	0.6 - 1.0
BETA	**12.4**	**1.1**	**8.0 - 15.0**	**0.6 - 1.3**
GAMMA	48.2+	4.1+	11.0 - 21.0	0.7 - 1.5
TOTAL		**8.6-**		**60.0 - 80.0**

A/G 0.41

COMMENTS.
- reduced albumin
- markedly increased gamma globulin

Figure 3: serum protein electrophoresis

Prednisolon were administered. A liver function tests returned to normal after a month , they still taking maintenance treatment of steroid.

Discussion:

It is generally accepted that, autoantibodies that characterize autoimmune hepatitis may occur in conjunction with antibodies to hepatitis A, B, and C viruses (17, 20, 21).

Despite intensive efforts to elucidate the pathogenesis of AIH, the exact etiology is still vague, however a variety of studies suggest that a blend of environmental and genetic factors could be the cause; a contribution of either one is necessary but not sufficient for full expression of the c sease.Strong circumstantial evidence point towards a viral etiology and this has been investigated in numerous studies. However, the concept that viral infection is the eti ogy of AIH remains a matter of controversy.In anecdotal repots, hepatitis A virus, HBV, EBV, and

herpes simplex virus (HSV have all been implicated in triggering AIH (16,17,19). Taking together, the above studies suggest that multiple exposures to viruses mimicking self may represent an important pathway to the development of autoimmunity (9). In this respect, it was shown that the B-cell epitope of CYP2D6 (drug metabolizing enzyme), which is the targeted by LKM-1 autoantibodies, displays homology with the amino acid sequence of HCV (15).

In addition, Manns and associate reported that EBV is capable of elaborating a viral IL-10, which is 70% homologous with human IL-10. This may enact B-cell stimulation without prior cascade activation (13).

In this study, we report 13 cases of AIH followed viral hepatitis.The thirteen cases were diagnosed according to the AIH scoring system probably triggered Qy viral infection. However, Clinical and biochemical response to steroid therapy is achieved. In spite of the limited facilities available, yet it was possible to deeply search for the role of viruses in the pathogenesis of AIH.

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