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Viral Markers and Autoantibodies in Autoimmune Liver Disease Patients

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ABSTRACT

Autoimmune liver disease is a chronic condition caused by immune-mediated auto-aggressive inflammatory reactions in genetically susceptible individuals. The diagnosis of the disease is multipronged and detection of autoantibodies in Autoimmune liver disease is an important diagnostic tool and it also helps in the classification of the disease. This study evaluated the autoantibodies and analyzed viral makers for Hepatitis B and Hepatitis C in Autoimmune liver disease patients. Autoimmune Liver disease was evaluated by various immuno-serological assays which include Anti-nuclear antibodies, Anti-double stranded DNA, Anti-liver kidney microsomal antibody and Anti-mitochondrial antibody. Association of viruses with Type I and Type II Autoimmune hepatitis were found. Viral infection can act as a trigger for Autoimmune hepatitis by initiating a self-perpetuating immune-mediated liver inflammation and can present acutely after the resolution of viral Hepatitis.

Keywords: Autoimmune liver disease, Autoimmune hepatitis, Hepatitis B Virus, Hepatitis C Virus

1. INTRODUCTION

Autoimmune liver disease (AILD) occurs when the body's immune system attacks the liver, causing inflammation. Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) are liver diseases having a likely autoimmune origin. Hepatocytes are the target of AIH, while biliary epithelial cells are the target of the autoimmune onslaught in PBC and PSC. After non-alcoholic fatty liver disease, AILD is the second most frequent cause of chronic liver disease in the second decade of life. AIH can be asymptomatic or present in various forms from subclinical disease to acute liver failure and end-stage liver disease. AIH can be divided into two subtypes. The presence of Anti – nuclear antibodies (ANA), anti-smooth muscle antibodies (SMA) and perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) may indicate AIH type 1 (AIH-1), and anti-liver kidney microsomal anti-body type one (LKM1) and anti-LKM3 and/or anti-liver cytosol type one antibody (LC1) are disease markers for AIH 2. Antimitochondrial antibodies are the diagnostic markers of PBC and they are directed against members of the 2-oxo-acid dehydrogenase complex. Antineutrophil

cytoplasmic antibodies (ANCA) are autoantibodies directed against various neutrophilic components and they are common in PSC, chronic ulcerative colitis, and autoimmune hepatitis. Viruses in general, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in particular have long been associated with the induction of autoimmune diseases. Agarwal, *et al*. (2018) study reports India has around 40 million HBV carriers accounting 10%–15% of the entire pool of HBV carriers of the world. HCV affects 3% of the world population and India has a major share of this HCV burden with an estimated prevalence between 0.5% and 1.5% [1]. The aim of this study is to evaluate the association of autoimmune liver disease triggered by the Hepatitis B and Hepatitis C virus.

2. METHODOLOGY

2.1 Study Design: The study design was Descriptive case series.

2.2 Study Population

The study was carried out in King Institute of Preventive Medicine and Research, Chennai. 90 patients with suspected autoimmune liver disease referred from various government hospitals in around Chennai from April 2022 to August 2022.

2.3 Clinical assessment

In all patients, a detailed history was taken. History of onset of illness, acute or precipitating events, blood transfusion, surgery, menstrual abnormalities, and presence of extra-hepatic manifestations of autoimmune diseases were specifically noted in detailed proforma with informed consent. Family history of autoimmune diseases was also noted.

2.4 Immunoserologic assessment

Serological tests for autoantibodies antinuclear antibody (ANA), anti-liver kidney microsomal antibodies (anti-LKM-1) were done using the ELISA method. Anti-mitochondrial antibody (AMA) by Indirect Immunofluorescence assay (IIFA), sp 100 and gp210 by line immuno assay.

2.5 Laboratory tests and virological assessment

All patients underwent biochemical evaluation using standard automated techniques. Liver function test was done in all patients. HBsAg, HBcAb, HBeAg and Anti-HCV were measured by Enzyme linked immunosorbent assay (ELISA). HBV DNA were confirmed by Real Time-PCR.

3. RESULT

Table 3.1 Detection of Autoantibodies in AILD patients

n=90	Anti-LKM		ANA		Anti-ds DNA		AMA	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
	%	%	%	%	%	%	%	%
Male (n=25)	0%	27.77%	2.22%	25.55%	0%	27.77%	1.11%	26.66%
Female(n=65)	3.33%	68.89%	11.11%	61.11%	3.33%	68.89%	2.22%	70%
Total	3.33%	96.66%	13.33%	86.66%	3.33	96.66%	3.33%	96.66%

Table 3.1 Depicts the detection of Autoantibodies in AILD patients which shows 3.33% of Anti-LKM positives, 13.33% of Anti-Nuclear Antibody (ANA), 3.33% of Anti-ds DNA positives and 3.33% of Anti-mitochondrial (AMA).

Table 3.2 Classification of AILD patients based on Autoantibodies

n=90	AIH – I		AIH -II		PBC		PSC	
	No.	%	No.	%	No.	%	No.	%
Male	2	2.22%	-	-	1	1.11%	-	-
Female	13	14.44%	3	3.33%	2	2.22%	-	-
Total	15	16.66%	3	3.33%	3	3.33%	-	-

Table 3.2 Depicts the classification of AILD patients based on liver autoantibodies.

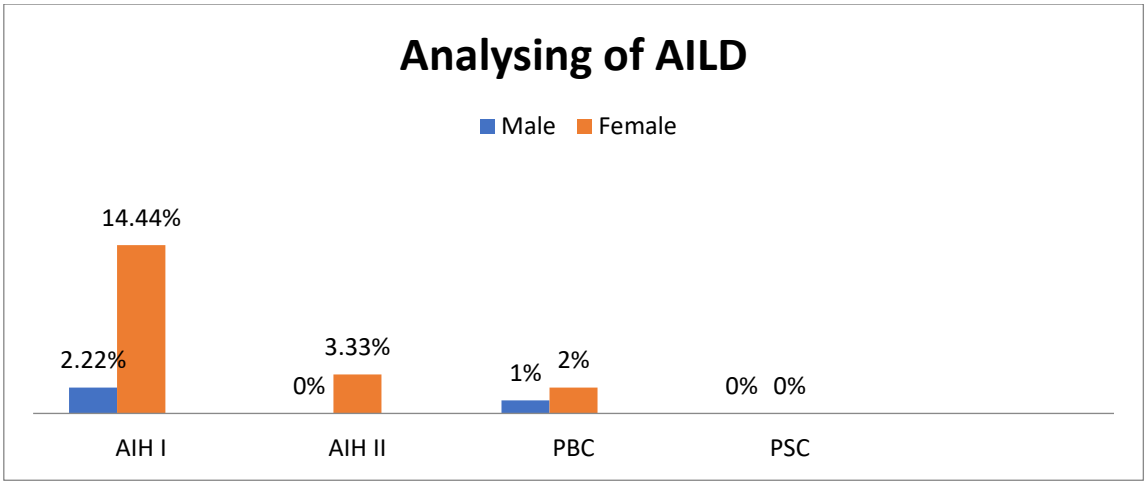

Fig 3.1: Analysis of AILD

Figure 3.1 Demonstrates that out of ninety patients who were suspected to autoimmune liver disease, of these eighteen (20%) were confirmed to have autoimmune hepatitis. Among patients with autoimmune hepatitis, 15(16.66%) were of type 1, 3(3.33%). Primary Biliary Cirrhosis were found in three (3.33%) and none of them were found to be Primary sclerosing cholangitis as they lacked of Perinuclear anti-neutrophil cytoplasmic antibodies.

Table 3.3 HBV MARKERS

HBV MARKERS No.=90	POSITIVE		NEGATIVE	
	No.	%	No.	%
HbsAg	23	25.55%	67	74.44%
HbeAg	10	11.11%	80	88.88%
Anti-Hbe	13	14.44%	77	85.55%
Anti-Hbc	31	34.44%	59	65.55%

Table .3 Summarizes that, HBV markers were evaluated, out of 90 patients 31(34.44%) patients were positive for HBV markers. HBsAg with 25.55% (23), HBeAg with 11.11% (10), Anti-Hbe with 14.44% 14.44% (13), Anti-Hbc with 34.44% (31).

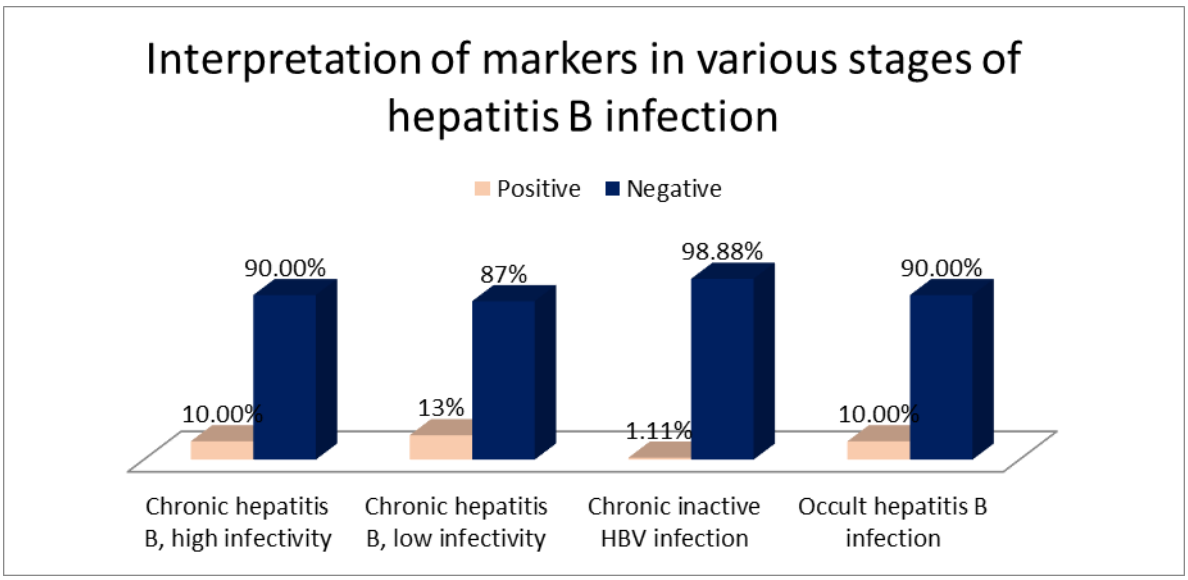

Fig 3.2: Interpretation of markers in various stage of HBV infection

Figure 3.2 depicts interpretation of markers in various stage of HBV infection, in which 10% (9) were having Chronic hepatitis B infectivity with high infectivity, 13.33% (12) was infected with Chronic hepatitis B infectivity with low infectivity, 1.11% (1) was in chronic inactive HBV infection stage and 10% (9) was infected with Occult hepatitis B infection.

Association of HBV markers with AIH was found in which 5(5.55%) was associated with Type I AIH. Anti-HCV was evaluated and 5(6.25%) patients were positive for Anti-HCV out which One patient (1.11%) with Anti-HCV was associated with type -2 AIH. Co-infection of HBV and HCV were also observed in this study.

4. DISCUSSION

This study assessed the HBV and HCV markers in the Autoimmune Liver Disease Patients. Study population included 90 patients suspected of autoimmune liver disease with 28 % (25) male and 72 % (65) with female predominance. Elisabeth *et al*. (2019) studies suggest that the female to male ratio is 4: 1 [2]. Fallatah *et al*. (2012) studies report AIH typically affects younger individuals [3].

Type 1 AIH was present in 16.66% (15) with female predominance of 14.44% (13) and male was 2.2% (2). In which 13.33 % (12) was positive for ANA with 2.2% (2) male and 11.11(10) female and 3.33% (3) was positive for anti-ds DNA. Both show the female predominance. The mean age for type 1 AIH was 30-45. This indicates the type 1 AIH which is mainly associated with middle aged women. Indian studies of Rajesh gupta *et al.* (2016) reported mean ages have been 31and 40 years with a female predominance [4].

In this study the presence of Anti-LKM autoantibodies was 3.33% (3) which includes only pediatric patients with majority of female child. This indicates the type 2 AIH which is mostly associated with children. Elisabeth et al studies demonstrate Type 2 AIH only accounts for 5%to10% of cases [2]. Giorgina et al (2014) studies study shows that type 2 AIH is mainly described in children [5]. Fallatah *et al*. study shows that the AIH type 2 predominantly affects children younger than 18 years of age with a female-to-male ratio of 9:1 [3].

Anti- mitochondrial antibodies were detected in three (3.33%) patients by IIFA. Out of the three patients one (1.11%) was male and the rest two (2.22%) were female. AMA M2 is a marker for Primary Biliary Cirrhosis. These samples were subjected Liver Imnunoassay which showed positivity for gp210 and sp100 antigens. This confirms Primary Biliary Cirrhosis. The mean age group was 30 to 45. Albert *et al*. study suggest that Antimitochondrial antibodies are present in 95% of patients who satisfy clinical, laboratory, and histological criteria for PBC. They are not essential for the diagnosis, and 5% of individuals are designated AMA negative PBC [6]. An Indian study of Sarin *et al*. reported 15 female positive patients for AMA and the mean age group was 40 to 60 [4].

In this study population HBV markers were evaluated, out of 90 patients 31 patients were positive for HBV markers. In earlier study from Tamil Nadu Kurien *et al*. [7,8] reported a HBV prevalence rate of 5.7% from Vellore and Mahalakshmi *et al*. [7,9] reported a 3.5% rate in Chennai. Studies from Western India Chowdhury *et al*. reported lower HBV prevalence rates Maharashtra – 0.92%, Ahmedabad – 1.42% [7,10,11]. Bangalore 4.4%, Gulbarga –7.2%, Northern (Jodhpur – 3.5%, Chandigarh 2.9% and Central (Uttar Pradesh – 2.1%) and Eastern India West Bengal – 2.9% [7,12]. The varied HBV prevalence reported across India could be owing to population heterogeneity in the testing strata, varying exposure to risk factors, different sample sizes, etc. Studies from different parts of India found Occult HBV Infection ranging from 3.9% in Kolkata (eastern India) and 0.78% in New Delhi (northern India) to 0.05% in Chandigarh (north-western India.) and 0.15% in Vellore (southern India) [13]. The prevalence of Occult HBV among the anti-HBc

seropositives was found to be 2.2% in Madhavan *et al*. study [13].

Type 1 AIH were also associated with HBV markers. Two (2.22%) patients with Anti-HBc were associated ANA positive, two (2.22%) patients with HBeAg were associated with ANA Positive and anti-ds DNA positive respect3ively, one (1.11%) patient with Anti-HBe was associated with ANA positive. Nobili *et al*. study reported the presence of HBV markers in AIH patient [14].

Anti-HCV antibody was only positive in 5 (5.55%) patients out of 90 patients included in the study. In which only one (1.11%) patient with AIH 2 was associated Anti – HCV antibody positive. A study from Japan, Toda *et al*. reported anti-HCV positivity in 12.5% of patients of autoimmune hepatitis [15,16]. Puri *et al.* reported the prevalence of HCV in India 0.5% [17]. Chowdhury *et.al* reported HCV prevalence rate was much higher in most community-based reports from North, South and East India [18].

Co-infection of HBV and HCV were also observed in this study. One patient (1.11%) was found to HBV/HCV coinfection. Prabha *et al.* study shows that the pooled prevalence of HBV/HCV co-infection in India was found to be 1.89 % [19,20].

CONCLUSION

In this study Autoantibodies and Viral markers in Autoimmune Liver Disease were assessed. Primary biliary cirrhosis was found only in few patients compared to Autoimmune Hepatitis. Overlap Autoimmune Hepatitis and Primary biliary cirrhosis syndrome was not observed in this study. Viral infection can act as a trigger for Autoimmune Hepatitis by initiating a self-perpetuating immune-mediated liver inflammation and can present acutely after the resolution of viral Hepatitis. Liver biopsy is considered as a critical element in the differential diagnosis of liver disease. Liver histology has to be done for long term follow-up for disease staging and assessment for inflammation and fibrosis.

The identification Autoimmune Hepatitis as the aetiology of acute hepatitis or fulminant hepatic failure is important because a delay in the diagnosis would lead to delay in initiation of therapy ultimately resulting in poor prognosis of Autoimmune Hepatitis. A more extensive and sensitive autoimmune liver serology testing could be helpful in classification, early diagnosis and treatment of autoimmune liver disease.

Declaration by Authors

Ethical Approval: Ethical clearance was obtained from the institutional Ethics Committee at King Institute of Preventive Medicine and Research, Guindy.Ref No-001/KIPMR/2022/Date:12.05.2022.

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Conflict of Interest: The authors declare no conflict of interes

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