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Clinicoimmunological Study of Autoimmune Hepatitis

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Abstract

Auto immune hepatitis is associated with characteristic autoantibodies and hypergammaglobulinemia. The aim of study is to see prevalence of various types of AIH. **Material and Method:** Study included 66 cases of AIH, 16 cases of chronic viral hepatitis (8 hepatitis B, 8 hepatitis C) and 18 healthy control between a period of 1.5 years. Antinuclear antibody (ANA), anti-mitochondrial antibody (AMA), anti smooth muscle antibody (ASMA), anti F-actin antibody, anti parietal cell antibody were done by indirect fluorescent method (IIF) on frozen section of rat liver, stomach, and kidney. It was also done by immunodot method. ANA was repeated by ELISA technique. **Result:** AIH-1 formed maximum cases (89.39%) followed by AIH-2 (9.09%) and AIH-3 (1.51%). In AIH-1 23.7% and in AIH-2 66.66% were children. Male predominance was in AIH-1 (59.3%).Weakness, loss of appetite, weight loss and jaundice were common in more than 86% patients in all types of AIH. Ascitis was more common in AIH-1 (61%) than in AIH-2 (33.3%). ASMA antibody was positive in all cases of AIH-1 while anti LKM was positive in AIH-2. ANA was positive in 16.9% cases of AIH by IFT method and 30.54% by ELISA. APCA was positive in 27% cases. Two cases each of AIH-1 were positive for AMA, endomysium, anticentromere, anti Smith, anti RNP and anti-laminin antibody. All AIH-1 and AIH-2 cases had significantly elevated transaminases, bilirubin, alkaline phosphatase and serum IgG. Thus, our study concludes that AIH1 is more common than AIH2 and ASMA is more common antibody.

Keywords: Autoimmune hepatitis, autoimmune liver disease, chronic hepatitis, anti smooth muscle antibody, anti Factin antibody, anti LKM antibody, anti soluble liver antigen antibody.

Introduction

Autoimmune hepatitis is a chronic disease characterized by interface hepatitis hyper gamma globulinemia and auto antibodies[1,2].Like other autoimmune diseases its pathogenesis is not clear. It is both due to genetic (mostly HLA related) and environmental factor and impaired T cell function. It was first described in 1950 by Waldenstrom in young female who presented with chronic hepatitis, jaundice, elevated serum immunoglobulins and amenorrhoea[3-5]. Kunkel et al in 1951 confirmed Waldenstrom observation and described additional extra hepatic manifestations of fever and arthritis.

In 1954 Italian physician Leoni reported lupus erythematous cells (LE cells) in the asitic fluid of patient with cirrhosis and one year later Josbe and King in 1955 from Melbourne Australia described LE cells in blood of two patients who had chronic hepatitis with hypergammaglobulinemia. In 1956 MacKaye et al reported 6 additional cases of chronic active hepatitis with hypergamma-globulinemia and LE cells. In view of LE cells they proposed the term of lupoid hepatitis[6-8].

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AIH is serologically heterogenous disease. Based on the nature of autoantibodies it is classified into 2 major types – type 1 AIH which shows for positivity for antinuclear antibody (ANA) and anti smooth muscle antibody(SMA) and type 2 AIH which shows positivity for anti-liver kidney microsomal type 1 (anti LKM- 1) or anti liver cytosol type 1 antibody (anti LC-1)[3,9-12] Antibody to soluble liver antigen (SLA) also called liver pancreas antigen(LP) was identified in 1987 by Mann *et al.*,[13]. It is less frequent but specific diagnostic marker of AIH and carries poor prognosis, short survival and 3 fold increased risk of death and more relapse[14-16]. It was kept in AIH 3 by some worker but later was considered in AIH 1. Most of the liver specific autoantibodies are detected by Indirect Immunofluorescent Test (IIF) on combined frozen sections of liver, kidney and stomach of rodent where serum is diluted in more than 1:40 for adult and 1:20 for children[17].

In IIF, anti SMA is detected in smooth muscle of arterial wall of all 3 substrate (SMA-V pattern), in mesangium of kidney (SMA-G pattern), gastric muscularis externa, muscularis mucosa, and smooth muscle fibre that extend from muscularis mucosa to lamina propria. Factin smooth muscle antibody binds to contractile fibres around tubules (SMA-T) giving rise to picket fence appearance which frequently stains mesangial cells of glomeruli. SMA VGT pattern was described by Bottazzo *et al.*, 1976[18]. Out of this SMA-V pattern is not specific because this staining can be due to antibody to vimentin intermediate filaments which can be seen in many viral infections. ELISA for Factin may give rise to false positive result specifically when SMA is present but not with SMAT[17].

Autoantibody to liver microsome antibody is found in type 2 AIH. It is directed against cytocthrome P4502D6 (Cyp2D6)[19,20]. It gives staining to proximal renal tubules and hepatocytes cytoplasm but 10% of patients of Hepatitis C also have anti LKM-1 antibody[11,16].

Antibodies of LC-1 whose target antigens for a immunotransferasecyclodeaminase, 67 kD cytosolic proteins found in 30% with LKM-1 positive AIH 2 and in 10% it is only autoantibody of AIH 2. Anti LC-1 stains hepatocytes cytoplasm but spares hepatocytes around central vein. Its presence is associated with poor clinical course and more rapid progression[21-23].

ANA is another autoantibody found in AIH. It may be directed against ribonucleotide protein complex[24]. Homogenous pattern is more typical[25]. In 55-60% cases both ANA and SMA may coexist together[24]. Besides this antibody to asialoproteins receptors (ASGPR), perinuclear anti neutrophil cytoplasmic antibody (pANCA), antibody to histone, ds DNA, chromatin and anti mitochondrial antibody (anti M_2) may also be found in AIH[26-29].

AIH is very common in our area but very few studies have been done in India. Aim of present study is to find clinical, biochemical, and autoantibody profile of AIH and its comparison with healthy controls and chronic viral hepatitis.

Materials and Methods

Present study was conducted in UGC Advanced Immunodiagnostic Training and Research Centre of Institute of Medical Sciences, Banaras Hindu University. Clinical details and sample were taken from patients between January 2016 to August 2017. This included 66 patients of AIH, 16 patients of chronic viral hepatitis and 18 healthy controls. Diagnosis of AIH was done by International AIH criteria as described by Alvasez *et al.*, 1999[30].

In all patients after consent 3 ml blood was taken in plain vial for serum bilirubin, aminotransferases, alkaline phosphatase, another 4 ml blood was taken in plain vial for autoantibody. Serum after separation was stored in -20^0C deep freezer till test was performed.

Anti smooth muscle antibody (ASMA) anti Factin antibody, anti LKM antibody, ANA, anti mitochondrial antibody, anti parietal cell antibody, anti laminin antibody was detected by indirect fluorescent test on combined frozen section of rat liver, kidney and stomach. In children serum was diluted in 1:20 dilution and in adult it was diluted in 1:40 dilution initially.

In all cases test was repeated by immunodot method. The kit was of D- tek company supplied by Anand Brother. Kit of immunofluorescent of Bio scientifica company of Argentina was used. This was supplied by Pamed services Pvt. Limited.

Results

Study included 66 patients of AIH, out of which 59 cases (84.4%) were of type 1 AIH, 6 cases of type 2 AIH, (9.09%) who had anti LKM antibody, and only one case had type 3 AIH (1.51%) – positive for anti SLA antibody. Besides this 16 cases of chronic viral Hepatitis (8 cases were hepatitis B positive and 8 cases were hepatitis C positive) and 18 cases of healthy controls for comparison, were also taken.

Age wise distribution showed that 1/3rd cases (32.2%) of AIH 1 were between 21 -30 years, 22% were between 31-40 years of age and 22% were above 40 years of age (41-55 years). In AIH type 2 66.66 % were children below 16 years of age, out of this 33.33% children were below 10 years of age. Rest 33.33%

patients were young adults between 21-40 years of age. In type 3 AIH only one patient was 10 years old female child.

Contray to this in chronic viral hepatitis 31.2% were between 21-30 years, 25% were between 31-50 years of age, 31.2% were above 50 years of age, and 12.4% patients were children below 16 years (Table 1)

Table 1: Showing age wise distribution of various patients of chronic hepatitis

Age in years	Type 1 AIH n = 59		Type 2 AIH n = 6		Type 3 AIH n = 1		Chronic viral hepatitis n = 16	
	No.	%	No.	%	No.	%	No.	%
0-10.9	1	1.7	2	33.33	1	100	1	6.2
11-20.9	13	22.0	2	33.33	0	0	1	6.2
21-30	19	32.2	1	16.7	0	0	5	31.2
31-40	13	22.0	1	16.7	0	0	2	12.5
41-50	6	10.2	0	0	0	0	2	12.5
>50	7	11.9	0	0	0	0	5	31.2

Sex wise distribution showed that in AIH males were predominantly affected (59.3%) whereas in AIH 2 both males and females were equally affected while in AIH 3 only one female patient was found (Table 2)

Table 2: Showing sex wise distribution of various types of chronic hepatitis

Sex of the patient	AIH 1		AIH 2		AIH 3		Chronic Viral hepatitis	
	No.	%	No.	%	No.	%	No.	%
Females	24		3	50	1	100	8	50
Males	35		3	50	0	0	8	50
Total Cases	9		6		0	0	16	
Male : Female ratio	1.5:1		1:1				1:1	

Analysis of clinical features showed that weakness (93.2%), loss of appetite (86.4%), pallor (84.7%), weight loss (81.4%), icterus (72.9%) were more common in AIH 1.Ascitis (61.1%) and pain in abdomen (69.5%) were also seen.

One patient of AIH 3 had weakness, weight loss, loss of appetite, pain in abdomen and pallor.

In type 2 AIH weakness (100%), loss of appetite (86.4%), pallor (100%) were more commonly seen. Jaundice was present in 66.66% cases while ascites was present in 33.33% cases.

Contrary to this patients of chronic viral hepatitis were mostly asymptomatic, above described features were present in only 18.7% cases. (Table 3)

Table 3: Showing clinical features of AIH

Clinical Features	AIH 1(59)		AIH 2(6)		AIH 3(1)		Viral Hepatitis(16)	
Diarrhoea	10	16.9	0	0	0	0	0	0
Weakness	55	93.2	6	100	1	100	3	18.7
Weight Loss	48	81.4	2	33.33	1	100	3	18.7
Loss of Appetite	51	86.4	5	83.3	1	100	3	18.7
Pain abdomen	41	69.5	2	33.33	1	100	3	18.7
Pallor	50	84.7	6	100	1	100	3	18.7
Icterus	43	72.9	4	66.66	0	0	3	18.7
Ascitis	36	61.0	2	33.33	0	0	1	6.2

Among the autoantibody profile most common autoantibody detected was anti smooth muscle antibody (ASMA-76.3%) followed by Factin antibody (50.8%), antinuclear antibody (ANA-30.54%) and anti parietal cell antibody (APCA-27.11%). Besides this anti centromere antibody, anti Smith (Sm) antibody, anti ribonucleoprotein antibody (anti RNP), anti nuclear laminin antibody and anti endomysium antibody were detected in small number of cases (3.41% each) (Table4) (Figure 1, 2, 3, 4, 5, 6, 7)

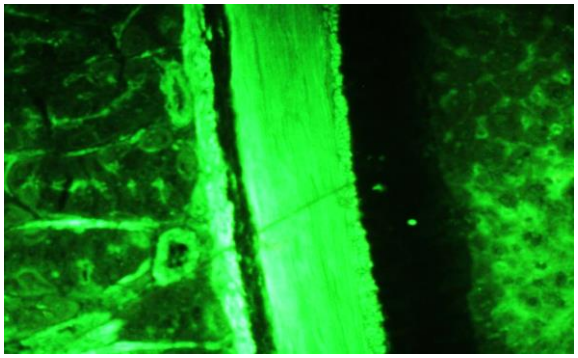

Figure 1: Case of AIH type 1, positive for anti smooth muscle antibody, wall of blood vessel, muscularis propria of stomach wall are showing fluorescence of ASMA. On the left side F-actin fibres in the parietal layer of stomach due to F-actin Ab is also found. (IIF×400).

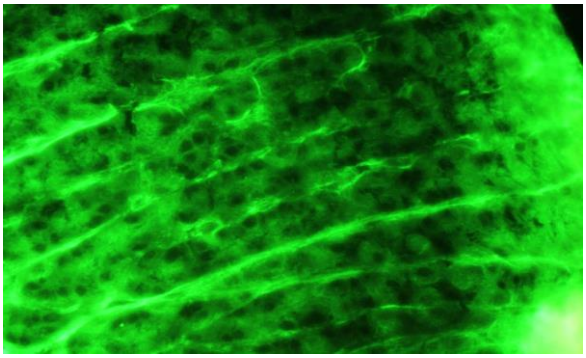

Figure 2: Case of AIH type 1 showing F-actin Ab positivity, thread like F-actin in the parietal layer of stomach showing fluorescence. (IIF×400)

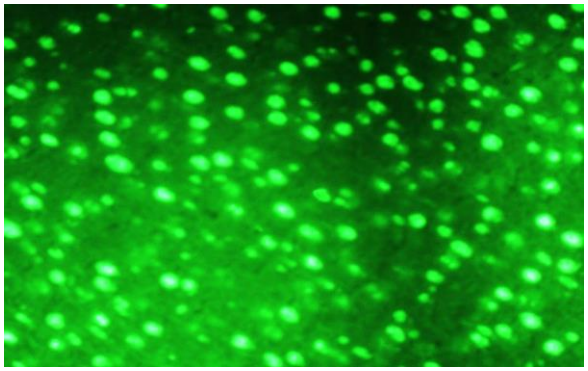

Figure 3: Case of type 1 AIH showing ANA positivity. Nuclei of liver cells show diffuse fluorescence & some nuclei showing nucleolar & speckled pattern. (IIF×400)

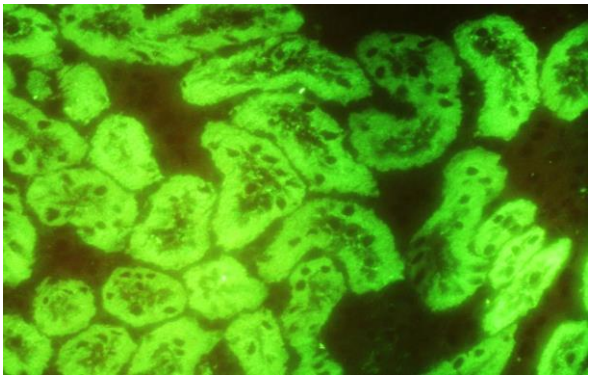

Figure 4: Type 2 AIH showing LKM Ab positivity. Diffuse fluorescence of proximal tubular epithelial cells is seen. (IIF×400)

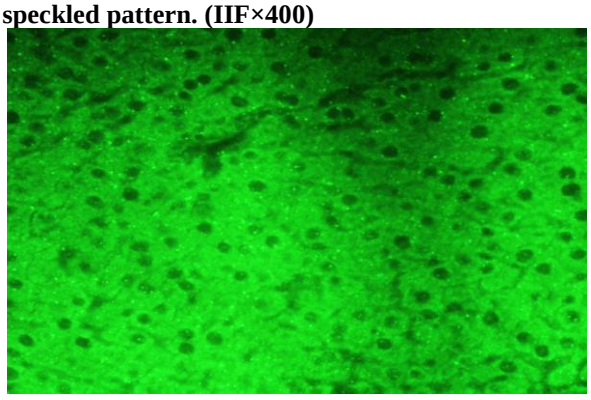

Figure 5: AMA positive case showing diffuse small multiple dot like fluorescence in cytoplasm of hepatocyte. (IIF×400)

Figure 6: Case of AIH type 1 showing anti parietal cell Ab positivity. The parietal cell of stomach showing strong fluorescence. There is no fluorescence on hepatocyte cytoplasm & renal tubular epithelium. (IIF×100)

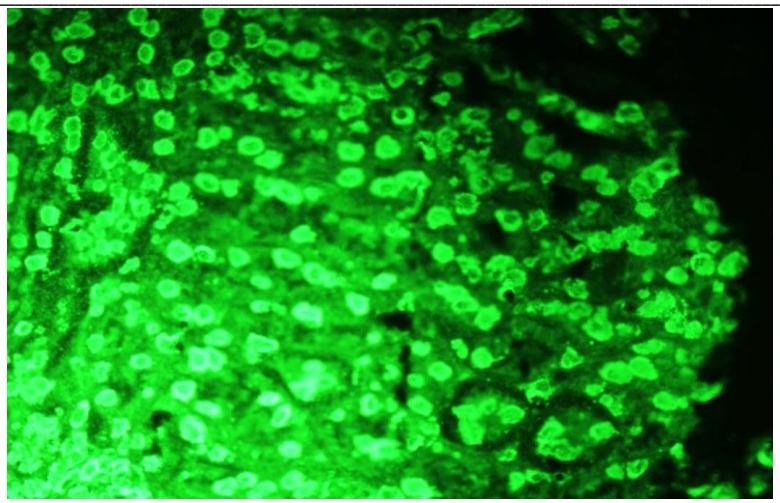

Figure 7: AIH type 1 showing predominantly peripheral staining of nuclei of hepatocyte, some cells show diffuse pattern also. This case was positive of ANA along with anti laminin Ab. This case was anti dsDNA Ab negative. (IIF×400)

Table 4: Frequency of various autoantibodies in different types of hepatitis

Autoantibodies	AIH 1 n=59		AIH 2 n=6		AIH 3 n=1		Viral Hepatitis n=16		Control(12)	
	No	%	No	%	No	%	No	%	No	%
ASMA	45	76.3	2	33.33	0	0	2	125 Weakly positive	2	16.6 Weakly positive
Factin	30	50.8	1	16.6	0	0	1	6.2	0	0
IIF method ANA	10	16.9	1	16.6	0	0	0	0	0	0
ELISA	18	30.54	1	16.6	1	100	0	0	0	0
LKM	0	0	6	100	0	0	0	0	0	0
AMA	2	3.4	0	0	0	0	0	0	0	0
APCA	16	27.1	0	0	0	0	0	0	0	0
EMA	2	3.4	0	0	0	0	0	0	0	0
SLA	0	0	0	0	0	0	0	0	0	0
Anticentromere	2	3.38	0	0	0	0	0	0	0	0
Anti Smith + anti RNP antibody	2	3.38	0	0	0	0	0	0	0	0
Anti Laminin antibody	2	3.38	0	0	0	0	0	0	0	0

Serum SGOT and SGPT were significantly elevated in AIH 1, AIH 3 and hepatitis B and hepatitis C as compared to controls. One case of AIH 3 also had raised SGOT, SGPT. Intra group comparison showed no significant difference between AIH 1 vs AIH 2, AIH 1 vs Hepatitis C. Only SGOT was significantly more in AIH 1 as compared to hepatitis B. (Table 5 and 6)

Total serum bilirubin, direct bilirubin, indirect bilirubin and alkaline phosphatase were significantly elevated in AIH 1, AIH 2 and AIH 3 as compared to control. In hepatitis B patients only alkaline phosphatase was significantly elevated as compared to controls while bilirubin rise was not significant. Contrary to hepatitis B, in hepatitis C all total bilirubin, direct bilirubin, indirect bilirubin and alkaline phosphatase was significantly more as compared to control (Table 6 and 7)

Table 5: Showing serum SGOT, SGPT in different type of hepatitis

Groups	No of Cases	SGOT (U/ml) Mean ± SD	SGPT (U/ml) Mean ± SD
Healthy contacts	18	6.778 ± 4.7719	11.000 ± 7.3324
AIH 1	59	176.522 ± 424.857	156.802 ± 237.03
AIH 2	6	1057.333 ± 2210.67	330.333 ± 426.08
AIH 3	1	269.000	223.000
Hepatitis B	8	40.646.59	85.188115.89
Hepatitis C	8	70.50071.51	73.25036.38

Table 6: Showing statistical analysis at SGOT /SGPT in AIH vs control

Groups	SGOT	SGPT
Healthy control vs AIH 1	Z=6.313 P=0.000	Z=5.313 P=0.000
Control vs AIH 2	Z=3.701 P=0.000	Z=3.546 P=0.000
Control vs Hepatitis B	Z=2.954 P=0.003	Z=2.986 P=0.003
Control vs Hepatitis C	Z=4.060 P=0.000	Z=3.900 P=0.000
AIH 1 vs AIH 2	Z=1.247 P=0.213	Z=1.167 P=0.243
AIH 1 VS Hepatitis B	Z=2.553 P=0.011	Z=1.354 P= 0.176
AIH 1 vs Hepatitis C	Z=1.189 P=0.234	Z=0.184 P= 0.854

Table 7: Showing total bilirubin, direct bilirubin, indirect bilirubin and alkaline phosphatase in different types of hepatitis

Group	No of cases	Total Bilirubin Mean± SD	Direct Bilirubin Mean ± SD	Indirect Bilirubin Mean ± SD	Alkaline Phosphatase Mean ±SD
Controls	18	0.422 ± 0.10033	0.2333 ± 0.09701	0.1889 ± 0.8324	100.44 ± 32.522
AIH 1	59	5.0958 ± 7.4580	3.2268 ± 5.5935	1.8708 ± 2.46741	244.17 ±174.390
AIH 2	6	7.7000 ± 6.78999	4.666 ± 5.0630	3.0333 ± 2.0539	319.17 ± 215.511
AIH 3	1	1.200	.400	0.800	268.00
Hepatitis B	8	0.8500 ± 0.89921	0.5125 ± 0.73764	0.3375 ± 0.25600	170.13 ± 89.788
Hepatitis C	8	4.0500 ± 8.99413	2.4250 ± 5.3293	1.6125 ± 3.67246	245.13 ± 76.48

Table 8: Showing statistical significance of various bilirubin and alkaline phosphatase in various groups

Groups	Direct Bilirubin	Indirect Bilirubin	Total Bilirubin	Alkaline phosphatase
Healthy Controls vs AIH 1	Z=5.312 P=0.000	Z=4.565 P=0.000	Z=5.280 P=0.000	Z=4.267 P=0.000
Controls vs AIH 2	Z=3.700 P=0.000	Z=2.953 P=0.003	Z=3.695 P=0.000	Z=2.236 P=0.025
Healthy Control vs Hepatitis B	Z=1.206 P=0.228	Z=0.406 P=0.685	Z=1.024 P=0.306	Z=2.281 P=0.023
Control vs Hepatitis C	Z=4.033 P=0.000	Z=3.292 P=0.001	Z=3.218 P=0.001	Z=3.669 P=0.000
AIH 1 vs AIH 2	Z=0.975	Z=0.454	Z=1.565	Z=0.805

	P=0.330	P=0.650	P=0.118	P=0.421
AIH 1 vs Hepatitis B	Z=2.960 P=0.003	Z=2.615 P= 0.009	Z=3.166 P=0.002	Z=1.276 P=0.202
AIH 1 vs Hepatitis C	Z=1.789 P=0.074	Z=0.833 P=0.405	Z=2.255 P=0.024	Z=0.793 P=0.428

Table 9: Serum immunoglobulins in various types of hepatitis and healthy contacts

Groups	Number of cases	Serum IgG (mg/dl) Mean±SD	Serum IgA (mg/dl) Mean±SD	Serum IgM (mg/dl) Mean±SD
Healthy Controls	18	1226.00±173.771	253.69±131.81	150.194±90.87
AIH 1	59	2493.24±578.64	412.74±177.32	216.97±94.53
AIH 2	6	1949.67±571.66	262.000±110.55	223.11±178.79
AIH 3	1	3130.00	197.00	99.00
Hepatitis B	8	1937.88±742.21	294.88±160.81	172.37±98.77
Hepatitis C	8	1690.75±533.43	291.91±164.21	195.175±138.088

Table 10 Showing statistical significance of immunoglobulins in various types of chronic hepatitis

Groups	Serum IgG (mg/dl) Mean±SD	Serum IgA (mg/dl) Mean±SD	Serum IgM (mg/dl) Mean ±SD
Healthy Contacts vs AIH 1	Z=6.392 P=0.000	Z=3.491 P=0.000	Z=2.775 P=0.006
Controls vs AIH 2	Z=2.468 P=0.014	Z=0.267 P=0.790	Z=0.867 P=0.386
Controls vs Hepatitis B	Z=2.112 P=0.035	Z==0.722 P=0.470	Z=0.667 P=0.505
Controls vs Hepatitis C	Z=2.723 P=0.006	Z=0.500 P=0.617	Z=0.556 P=0.578
AIH 1 vs AIH 2	Z=1.825 P=0.068	Z=2.41 P=0.041	Z=0.453 P=0.578
AIH 1 vs Hepatitis B	Z=1.779 P=0.075	Z=1.595 P=0.111	Z=1.073 P=0.283
AIH vs Hepatitis C	Z=3.307 P=0.001	Z=1.934 P=0.053	Z=0.938 P=0.348

Table 9 and 10 shows in AIH 1 there was polyclonal hypergammaglobulinemia. All the serum IgG (P.000), IgA (P.000), IgM (P.006) were significantly increased in comparison to controls. In AIH 2 and chronic hepatitis B and C only serum IgG was significantly increased in comparison to controls. Only one case was SLA antibody positive and this case also showed raised IgG. Hepatitis B, Hepatitis C, did not show any significant difference in IgA, IgM. Serum IgA & IgM were raised significantly in AIH 1 as compared to controls. AIH 1 had significantly raise IgG as compared to Hepatitis C (Table 9 and 10)

Discussion

AIH is a chronic necroinflammatory disease of the liver characterized by hypergammaglobulinemia, interface hepatitis, characteristic autoantibodies and association with HLA antigens.

In present study type 1 AIH was found to be more common (89.41%), followed by type 2 AIH (9.11%) and only one patient had type 3 AIH (1.5%). Although now days anti SLA antibody positive cases are also kept in type 1 AIH.

Like us other Indian studies have also found high prevalence of type 1 AIH which is 82% from Delhi, 71.2% from Mumbai and 92% from Lucknow[31-33]. Western literature also found AIH 1 in 80% of cases of total AIH[11].

Sex wise distribution in majority of studies done earlier showed female predominance which varies from 2.5 to 6 times more as compared to males. (Table 11) where as in our study males were slightly more affected than females with male to female ratio of 4:3

Table 11 : Showing male to female ratio in AIH

Group	Region	Year	Male to female ratio
Chowdhuri*et al.,* [33]	Lucknow, India	2005	1:4
Gupta *et al.,* [31]	Delhi, India	2001	1:3
Koay and Chine-Nanlin[34]	Taiwan	2005	1:6
Al-obeidy*et al.,* [35]	Baghdad	2009	1:2.5
Present study	Varanasi India	2017	4:3

Clinical features revealed that jaundice (71.2%), anorexia (86%), weight loss, weakness (93.3%), pallor (8.6%) were more common in present study. Clinical features are highly variable in different studies (Table 12)

Table 12: Showing comparative clinical features of patients with autoimmune hepatitis

Clinical features	Amrapurkar *et al.,* 2015 n= 126		Koay and Ching-Nanlin 2005 n=27		Chaudhuri *et al.,* 2005 Lucknow n= 38		Rajesh *et al.,* 2001 New Delhi 2001 n= 39		Present study n= 66	
	No	%	No	%	No	%	No	%	No	%
Jaundice	75	59.5	14	57.9	21	55.2	32	83	4	71.2
Nausea, vomiting, abdominal pain	0	0	8	29.6	9	23.6	0	0	44	66.66
Anorexia	_	_	11	40.7	_	_	32	83	57	86.4
Weight loss	_	_	_	_	_	_	_	_	51	77.2
Weakness and Fatigue	_	_	_	_	_	_	32	83	62	93.3
Pallor	_	_	_	_	_	_	_	_	57	86.3
Diarrhoea (recurrent)	_	_	_	_	_	_	_	_	10	15.2
Encephalopahy	7.0	5.55	1	37.7	9	23.6	6	15	8	12
Ascitis and edema of feet	41	32.53	6	22.2	13	34.2	17	43	38	57.6

Autoantibody profile showed that anti smooth muscle antibody was the commonest antibody detected in our series in type 1 AIH (71.2%)

Contrary to our study earlier western literature[40] reported ASMA in only 35% cases but some recent Western Literature like us also reported ASMA in 70-80% cases[41,42]. One of the Indian Studies also found ASMA in 60-65% cases[33].

ANA was detected by indirect immunofluorescence as combined frozen section of rat, liver, kidney and stomach in only 16.6% cases but all samples were tested by ELISA method, then ANA was found to be positive in (30.3%) cases.

More or less similar to our observation other workers from Minnesota and USA reported prevalence of ANA in only 13-15% cases by Immunofluorescent Test method which is very much closer to our observation[24,41].

Contrary to our findings some other workers from India reported prevalence of ANA positivity in 65.6% and one study from Taiwan reported ANA positivity in 96.5%[32,34]. Difference between ELISA test and Immunofluorescent Test (IIF) for ANA is well reported.

Many studies have noticed that ANA positivity on rat liver kidney is low as compared to human epithelial line and ELISA ANA positivity is reported to be 12% in chronic viral hepatitis but in our study none of the patients was positive. This could be due to small number of cases[43-46].

Anti parietalcell antibody was positive in 27 % cases of AIH 1. APCA may be detected in 2.5 to 19.5% healthy controls. APCA is marker of autoimmune gastritis and pernicious anaemia where it is detected in 85-90% cases. Besides this it is also detected in autoimmune thyroid diseases, type 1 diabetes mellitus, and vitiligo[47-51].

Two of our ANA positive patients (3.38%) of AIH 1 had anti smith (anti Sm) antibodies and anti RNP antibodies. One patient was 36 years old male and other was 6 years old female. Both these patients presented with chronic recurrent jaundice, hepatosplenomegaly, anaemia and arthritis. This suggests that both patients had SLE. Like us other

workers also reported that 1 to 2.1 patient of AIH 1 have associated SLE[52].

Another 2 patients had anticentromere antibody, one was 18 years old female and another was 26 years old female. No classical feature of scleroderma and primary biliary cirrhosis was present but disease was of severe intensity. Anti centromere antibody was reported in 131 patients of AIH type 1 in Korea[53].

Anti-mitochondrial antibody (AMA) have been reported in 8.20% patients of AIH[29] but in India Chowdhuri *et al.*,[33] reported very low frequency of AMA which is very close to our observation because we found AMA in only 3.4% cases. Type 2 AIH formed only 9% cases where anti LKM antibody was detected. Males and females were equally affected and mostly patients were children. Its incidence is variable .Most of the studies[10,42,54] reported it in low frequency (3 to 4 %). Some of the studies from India[32] and Baghdad[35] reported its higher prevalence in 14% and 16.4% respectively.

Anti SLA antibody is uncommon in our country. We found only one case of SLA antibody in female child. Contrary to our study several studies[2,12,55] reported very high prevalence of this antibody varying from 18.7% to 40%. Anti SLA antibody positivity is associated with more severe disease and poor outcome[12]. Serum SGOT, SGPT, total bilirubin, direct and indirect bilirubin, alkaline phosphatase were significantly elevated in AIH which is in accordance with other studies[33,34].Raised serum gammaglobulin is found in 73% patients of AIH[8]. Now it remains a cornerstone features of the diagnosis of AIH. It is due to increased number of plasma cells in bone marrow and liver. Mean value of Serum IgG was 2493.24 ±578.649 mg/dl . Raised serum IgG was reported in patients of AIH with mean value of 1850 ± 70 mg/dl by Koay and Chin[34]. Alobeidy *et al.*,[33] studied 73 patients at AIH and found elevated IgG with mean of 2447±248 mg/dl but did not found elevated IgG in type 2 AIH.

Thus our study concludes that ASMA and or Factin antibody is positive in all cases of AIH but ANA positivity in AIH is low in our country. Our study also includes that LC -1 antibody and anti SLA antibody are very rare in AIH in our country. Similarly anti LKM antibody and its related AIH 2 is less common. Study also shows that anti parietal cell Ab is frequently found in AIH 1 cases.

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