Studies on Cryptogenic Chronic Liver Disease in Indians - A Retrospective Analysis

A dissertation submitted in partial fulfillment of DM (Gastroenterology) course requirements of the Tamil Nadu Dr. MGR Medical University, Chennai.

Certificate

This is to certify that this dissertation entitled 'Studies on Cryptogenic Chronic Liver Disease in Indians-retrospective analysis'is a bonafide work done by Dr. Kanhu Charan Das in partial fulfillment of the rules and regulation for DM (Gastroenterology) examination of the Tamil Nadu Dr. MGR Medical University, to be held in August 2009.

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Acknowledgement

I thank Dr. George Kurian for his guidance and support in undertaking and completing this study work.

I am indebted to Dr. Banumathi Ramakrishna for her support in the histopathological works.

I am always obliged to Dr. Gagandeep for her valuable opinions and providing Lab facilities needed to carry out this work.

I thank to Mrs. Sheila for helping out in the lab works.

Special thanks to Miss. Nithya for helping me with statistical analysis and Ms. Sumitha for the secretarial support.

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Introduction

Cryptogenic chronic liver disease is defined as chronic liver disease the aetiology of which is unknown or not discernable after exhaustive investigations. The etiology in 5-10 % of cases with cirrhosis, despite reasonably extensive investigations, remains unresolved(1) and these would qualify for the label.

Ever since new causes of parenchymal liver damage were discovered the proportion of patients with Cryptogenic cirrhosis has diminished. The newer causes include Hepatitis C (1999) and more recently Non Alcoholic Steatohepatitis or NASH (2001) Even more recently there has been evidence that Non Cirrhotic portal hypertension (NCPF) ends in chronic liver failure and hepatocellular carcinomas (2). It may turn out that this too might be another cause of cryptogenic Cirrhosis.

Looking for the aetiology of Chronic Liver Disease or Cirrhosis opens up the possibility of the successful use of therapeutic agents with improvement (3), stabilization or the delay in progression. Oral antivirals such as Lamivudine are good examples of such “rescue” therapy (4). Once the stage of Cirrhosis is reached many patients are not intensively studied, except for viral serology, because Cirrhosis is seen as the end game and cursory treatment other than liver transplantation is seen as worthless.

This scenario would change if new agents are discovered that could halt or reverse ongoing liver damage in decompensated Hepatitis C infection, NASH or NCPF as they have in Hepatitis B. This has special relevance to India where transplantation is beyond the scope of most sufferers.

With these as the undergirding considerations, this study was undertaken to investigate covert causes of Cryptogenic Chronic Liver disease. This was a retrospective study and as the issue is complex, we concentrated only on two specific areas – 1) Steatohepatitis as a cause of “cryptogenic chronic liver disease” and 2) Cryptogenic chronic liver disease with elevated alkaline phosphatase levels with special reference to Primary Biliary cirrhosis and the anti mitochondrial antibody.

The first issue has not been examined in previous reports from India as biopsies are rarely done in those with near end stage disease. The second issue has also not received much attention in India. There are, of course, many other causes of “cryptogenic Liver disease” which have not been investigated in this study. Others in our group have studied NCPF(5) as mentioned before. We were constrained by other factors from studying causes such as occult HBV infection which may have been another treatable cause of liver disease (6).Rarer causes such as Iron storage disorders and auto immune liver diseasewere also not covered in the current study.

In steatohepatitis, we attempted to answer these two questions 1) Would laboratory tests and clinical features aid in arriving at an aetiological diagnosis of NASH?(6, 7) Would the severity of the histological changes be reflected and therefore be predictable from scores such as the MELD score.. The implications of these are as follows.

The diagnosis of the condition may be made easier if there are specific clinical signs as biopsies of the liver are not widely conducted or possible in patients with decompensation. A concordant scoring system where histology matches a MELD score would make therapeutic decision making easier.

The second part related to the possibility of those patients with chronic Liver disease with high serum levels of alkaline phosphatase, actually having Primary Biliary Cirrhosis. The test used for the diagnosis of this condition is the antimitochondrial antibody (AMA). The AMA is reported to be more than 90-95% sensitive and more than 90-95 % specific(6). There are about 5% of patients who are negative for AMA but have the other features of PBC(7).

The first question we asked is how frequently in this study population of chronic liver disease with elevated alkaline phosphatase was this test positive. If the test was positive in the majority it would mean that testing need not be done as the pre-test possibility would be high. If on the other hand the test was rarely positive biopsy would need to be done more often.

The second query was to find out if the population that tested positive was in any way different from those who tested negative. If similar then the implications are that the test is falselynegative in the majority of cases. If different then the pristine disease is probably very rare in this country. The former conclusion can be arrived at only after more intense investigation.

These two issues may appear to be unrelated but as they are both in the realm of chronic liver disease of unknown aetiology they will be presented together.

Review of Literature

There have been some studies on Cryptogenic liver disease, Cirrhosis is usually accepted as ‘‘cryptogenic’’ only after an extensive evaluation has excluded recognizable etiologies. The prevalence of cryptogenic cirrhosis ranges from 5% to30% of cirrhotic patients in past series.(5) Several explanations may be offered as possible underlying etiologies include occult alcohol abuse, occult viral (non-B, non-C hepatitis, silent autoimmune hepatitis, or progression of nonalcoholic steatohepatitis (8). The prevalence of clinically silent autoimmune hepatitis is not known; however, asymptomatic patients with autoimmune hepatitis and previously unrecognized cirrhosis have been described (9). Non-B, non-C hepatitis is thought to account for about 15% of post transfusion hepatitis and may exist in a silent form for years.Obesity and non–insulindependent diabetes mellitus are the two most common conditions associated with NASH,(10) which is frequently asymptomatic10 and which can progress silently to cirrhosis with loss of definitive histological features(11).

NONALCOHOLIC STEATOHEPATITIS was first proposed in Nonalcoholic steatohepatitis (NASH) is the term used to describe the distinct clinical entity in which patients lack a history of significant alcohol consumption (≤40gm/d) but have liver biopsy findings indistinguishable from alcoholic steatohepatitis(12). Other terms that have infrequently been used to describe this condition include pseudoalcoholic hepatitis, alcohol-like hepatitis, fatty liver hepatitis, steatonecrosis, and diabetic hepatitis.

NASH is also considered to be a subset of nonalcoholic fatty liver disease (NAFLD). Diagnosis of NASH was based on the following criteria: (i) intake of less than 20 g of ethanol per day, (ii) biopsy proven steatohepatitis; steatosis, inflammatory infiltrates, and ballooning degeneration with or without Mallory bodies or pericellular/perivenular fibrosis, (iii) appropriate exclusion of other liver diseases.

The detection of NASH is usually delayed, since there are no serum Surrogate markers for NASH, and a definitive diagnosis requires a liver biopsy(13). The detection of NASH is usually delayed, since there are no serum surrogate markers for NASH, and a definitive diagnosis requires a liver biopsy(14). Today, it is considered a nonspecific term encompassing several clinicopathologic entities (steatosis alone, steatonecrosis, steatohepatitis and histologic alcoholiclike hepatitis) that are similar to alcoholic liver disease. Studies of nonalcoholic fatty liver disease have come to conflicting conclusions about the course of the disease. It can be argued that the disparate results are largely the result of nonuniform definitions. When histologic features such as hepatocyte ballooning, necrosis, and Mallory hyaline are seen, nonalcoholic fatty liver disease has been shown to be associated with an aggressive outcome. Steatosis alone, in contrast, appears to be benign. The current understanding of nonalcoholic fatty liver disease, the limited treatments available(15).

EPIDEMIOLOGY: The prevalence of NASH in the general population is incompletely understood. The major risk factors for nonalcoholic fatty liver disease (NAFLD), central obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome are common in western societies. NAFLD is the most common liver disorder in Western industrialized countries, affecting 20 to 40 percent of the general population (16).

Estimates of current prevalence range from 5 to 30 percent in the Asia-Pacific region, depending on the population studied(17).

PATHOGENESIS: The pathogenesis of nonalcoholic fatty liver disease has not been fully elucidated. The most widely supported theory implicates insulin resistance as the key mechanism leading to hepatic steatosis, and perhaps also to steatohepatitis. Othershave proposed that a "second hit," or additional oxidative injury, is required tomanifest the necroinflammatory component of steatohepatitis. Hepatic iron, leptin, anti-oxidant deficiencies, and intestinal bacteria have all been suggested as potential oxidative stressors.

CLINICAL COURSE AND PROGNOSIS: Relatively few patients have been observed prospectively to document the natural history of NASH. NASH is generally considered to be a clinically stable disorder and has a markedly better prognosis than alcoholic steatohepatitis. A population-based study in the United States found that patients with nonalcoholic fatty liver disease had slightly lower overall survival than expected for the general population (standardized mortality ratio of 1.34, 95% CI 1.003-1.76)(17) . Higher mortality was associated with

advancing age, impaired fasting glucose, and cirrhosis. NASH may be an important underlying cause of cryptogenic cirrhosis, particularly among older, diabetic women(18).

Independent predictors of fibrosis progression included diabetes mellitus, a low initial fibrosis stage, and a higher body mass index. Elevated liver enzymes were also a predictor of progression(19). Approximately 38 to 50 percent of patients with alcoholic hepatitis progress to cirrhosis over a seven-year period (20,21); comparable values for NASH are much lower at 8 to 26 percent (22,23,24) . NASH is also associated with higher 5- and 10-year survival rates than alcoholic hepatitis (67 versus 38 percent and 59 versus 15 percent, respectively). Patients who developed cirrhosis from NASH may also be at increased risk for hepatocellular carcinoma (25,26).

Clinical features and diagnosis: Most patients with NASH are asymptomatic although fatigue, malaise, and vague right upper abdominal discomfort bring some patients to medical attention(27). The most common presentation is elevation of liver aminotransferases detected on routine laboratory testing. Hepatomegaly is a frequent finding.Serum AST and ALT are elevated in almost 90 percent of patients. The AST/ALT ratio is usually less than 1; this is much lower than the ratio in alcoholic hepatitis, which is usually above 2 and averaged 2.85 in one report and 2.6 in another . Alkaline phosphatase is less frequently elevated and hyperbilirubinemia is uncommon (28). Ultrasonography often reveals a hyperechoic texture or a bright liver because of diffuse fatty

infiltration(29) . However, this is a nonspecific finding and should not be used to make the diagnosis of NAFLD.

Both CT and MRI can identify steatosis but are not sufficiently sensitive to detect inflammation or fibrosis(30).

Liver biopsy is the only way to confirm or exclude the diagnosis of NASH (31,32) A histologic scoring system has been proposed that can assist in diagnosis of nonalcoholic fatty liver disease and may be useful for assessing the response to therapy(33).

TREATMENT: There is no proven effective therapy for NASH, although modification of risk factors, such as obesity, hyperlipidemia, and poor diabetic control is generally recommended.Weight loss and increased physical activity can lead to sustained improvement in liver enzymes, histology, serum insulin levels, and quality of life(34). Cryptogenic chronic liver disease with elevated Alkaline phosphatase. Possible Primary biliary cirrhosis (PBC) is an uncommon condition found throughout the world that primarily affects middle-aged women. Most patients are identified in an asymptomatic phase, but gradually they develop symptoms of pruritus, fatigue, and symptoms of associated syndromes or end-stage liver disease.

Typical laboratory findings are elevations of alkaline phosphatase levels Modest elevations of aspartate aminotransferase (AST) and alanin aminotransferase (ALT) levels, and positive antimitochondrial antibody test

results. The characteristic pathologic features of PBC are destruction and drop-out of intrahepatic bile ducts, chronic portal tract inflammation, cholestasis, and progressive fibrosis, cirrhosis, and portal hypertension.

Treatment options include ursodeoxycholic acid (UDCA), cholestyramine, replacement of fat-soluble vitamins, management of complications of portal hypertension, and liver transplantation for end-stage disease.

Pathogenesis and Genetics: The etiology and pathogenesis of PBC are unknown. A variety of etiologies have been postulated, including infectious causes; however, the most common view is that the disease falls within the category of autoimmune diseases (35).

The rationale for this disease is that multiple features of PBC support a Primary autoimmune pathogenesis, including the histologic features of the liver bile duct lesions; characteristic autoantibodies; strong female predominance; an association, although weak, with MHC class II genes; the frequent association with other autoimmune syndromes.

AMAs are the most distinctive specific immunologic characteristic of PBC and, not surprisingly, have been the focus of numerous studies. As noted in the preceding text, AMAs can inhibit the enzymatic activity of their target antigens in vitro; however, it is not known whether this occurs in vivo.

Autoantibodies may potentially cause tissue injury, regardless of whether they alter the function of their target antigen; therefore, this particular issue does not resolve the question of the pathogenicity of AMAs. AMAs do fix complement; however, remarkably little attention has been directed at determining whether there is specific deposition of AMAs at sites of tissue injury in the liver.

A number of attempts to induce biliary disease in anima l models have met with limited success. In one study, immunization of mice with PDC-E2 resulted in an antibody response, however, without evidence of biliary disease (36). In other model systems, the presence of multiple positive and negative genetic susceptibility genes is necessary in addition to environmental triggers to produce autoimmune disease(37).

Considerable information has emerged to define the nature of both the B-cell and T-cell responses to the autoantigens recognized by AMAs. There is overlap between epitopes recognized by B cells and both CD4+ T cells and CD8+ T cells, and there is enrichment for specific antigen-reactive clones within the liver(38) .Cytokines produced in liver infiltrates are dominated by TH1 cytokines but also contain TH2 cytokines, providing a mixed picture. Furthermore, expression of adhesion molecules and chemokines, such as fractalkine, by biliary epithelial cells provide further details on potential mediators involved in the formation of inflammatory lesions within the liver. However, the precise sequence of events and specific effector mechanisms contributing to bile duct injury are as yet undefined, and the possibility that the inflammatory

lesions are entirely secondary in nature has not been eliminated. Other animal models have been examined that might contribute insights into the pathogenesis of PBC. In an interesting example of serendipity, elimination of a specific diabetes-determining locus in the nonobese diabetic (NOD) mouse eliminates diabetes but results in a mouse with a characteristic spontaneous development of a unique liver phenotype consisting of peribiliary infiltrates, autoantibodies, and cystic biliary lesions(39).

The striking female predominance of PBC obviously links the pathogenesis of the disease to the genetic basis of sex determination, as for many other autoimmune diseases. However, the specific causal mechanisms are currently unclear.

One recent population-based study confirmed that autoimmune conditions are found in approximately one half of patients and that the prevalence of autoimmune disease in first-degree family members is 14% (40), consistent with the thesis that PBC shares common genetic susceptibility traits with other autoimmune conditions.

More recently, an international study evaluated 16 twin pairs with PBC, of whom 8 were confirmed to be monozygotic (41).

Table-1: Epidemiology and Genetics-

Incidence	2–24/1,000,000
Prevalence	19–240/1,000,000
Median age (y) (range)	50 (20–90)
Female	90%
Environmental risk factors	Unknown
Associated autoimmune syndromes	50%
Familial clustering	1%–2%
Twin concordance rate	Monozygotic 0.63/Dizygotic 0.0
MHC class II	Weak, variable associations

MHC, major histocompatibility complex.

Autoantibodies:

Extensive studies have refined diagnostic testing forAMAs, which are found in up to 95% of patients (8). Cloning and characterization of the M2 autoantigens recognized by PBC sera have led to the identification of four major components of a family of mitochondrial antigens that contain lipoic acid and are members of the 2-oxoacid dehydrogenase (2-OAD) multimeric enzyme complexes (42,43,44,45). These includepyruvate dehydrogenase complex (PDC), 2-oxoglutarate Dehydrogenase Complex (OGDC), and branched-chain 2-oxoacid dehydrogenase complex (BCOADC)(46,47).

Approximately 95% of sera of patients with PBC reacts with both the PDC core dihydrolipoamide acetyl transferase (E2) structure and the E3-

binding protein (E3BP) by immunoblot or enzyme-linked immunosorbent assay (ELISA), these two subunits being completely cross-reactive(48,49,50,). PBC sera also react, at a much lower frequency, with other components of PDC, including the E1α and E1β subunits (51). Sera also react with the E2 subunits of OGDC and BCOADC with a frequency of 90% and 50%, respectively.

Pathology

The gross pathologic features of PBC are not specific for the disease and includebile staining of the liver, enlargement, fine nodularity, and, eventually, a grossly cirrhotic appearance.

The characteristic microscopic hepatic abnormalities include portal inflammation with destruction and disappearance of intrahepatic bile ducts, abnormal bile duct proliferation, fibrosis, and cirrhosis.

In stage 1, Portal lesions are characterized by damage to bile ducts. The duct is typically surrounded by a dense lymphocytic infiltrate, which may also include histiocytes, plasma cells, eosinophils, and, occasionally, true epithelioid giant cells.

In stage 11 of the disease is characterized by the appearance of abnormal proliferating bile ductules without duct lumens, disappearance of normal bile ducts, and extension of the portal inflammation into the hepatic parenchyma.

In stage 3, there is a substantial increase in fibrosis, and fibrous septa may Link portal tracts. Lymphocytic infiltrates remain in portal tracts, but bile ducts may be difficult to identify or completely absent.

Stage 4 is characterized by frank cirrhosis with regenerative nodules.

Clinical Features:

In order of frequency, the most common symptoms of PBC are fatigue, pruritus, and jaundice. Occasionally, patients have right upper quadrant abdominal pain. Many other symptoms may be present, including that of associated syndromes, Such as dry mouth and eyes, Raynaud's phenomenon, and arthralgias.

As the disease progresses, the most common physical findings are hepatomegaly, splenomegaly, excoriations, skin hyperpigmentation, jaundice, and xanthelasma (52).

Occasionally, patients are brought to attention because of xanthoma, xanthelasma, increasing skin pigmentation, or fracture. Much less commonly, the presenting symptoms may be due to decompensated liver disease, including ascites, edema, bleeding, or encephalopathy.

Routine Laboratory Tests:

The routine laboratory abnormalities in PBC are typical of chronic cholestatic Syndromes and chronic liver disease.

Table -2

Alkaline phosphatase	2 to 2.5 times upper limit of normal
AST, ALT	<5 times upper limit of normal
Bilirubin	Normal in early phase, progressive rise late phase
Cholesterol, HDL cholesterol	Elevated
Serum IgM	Elevated
Antimitochondrial antibodies	Present titer >1:40 in 95% of patients
Other autoantibodies	ANA, RF, SMA, and others commonly found

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; IgM, immunoglobulin M; ANA, antinuclear antibodies; RF, rheumatoid factor; SMA, smooth muscle antibody.

Complications:

Table-3

Emotional disturbance: Debilitating pruritus and fatigue
Metabolic bone disease
Portal hypertension: Ascites, edema, encephalopathy, varices
Malabsorption: Fat-soluble vitamins
Hyperlipidemia
Hepatocellular carcinoma
Asymptomatic urinary tract infection
Distal renal tubular acidosis

THERAPY:

UDCA is the only U.S. Food and Drug Administration (FDA)-approved medical therapy for PBC, which is generally agreed to ameliorate serum biochemical abnormalities and potentially delay progression of disease to death or need for transplantation, although controversy remains about the utility of the drug. A number of mechanisms have been proposed by which UDCA is therapeuticin PBC, including anticholestatic effects and anti–apoptotic actions (53,54).

And also therapies directed at complications, including osteoporosis, fat-soluble vitamin deficiency, and complications of portal hypertension, such as ascites, edema, variceal bleeding, and hepatic encephalopathy.

PART - I

Studies on Non-Alcoholic Steatohepatitis

Material and methods

Study design:

This was a retrospective case-control analysis.

Sample size:

We studied 100 subjects with 50 subjects in each arm. This was an arbitrary number and was not based on any calculation because there were no previous studies that could be used to determine sample size.

Subjects:

This study was conducted in the Department of Gastroenterological Sciences, Christian Medical College, Vellore. There were a total of 100 subjects, 50 subjects were cases and 50 were controls.

The reports of liver biopsies obtained in the Department between June 2004 and July 2007 were examined. All sequential cases of those with histological evidence of Steatohepatitis were chosen and their records examined. Those patients with other putative causes like Hepatitis B (this included patients

who had a positive IgG anticore antibody), Hepatitis C and alcohol were excluded. This group formed the cases for the study. From the records of the same years a further 50 patients were chosen arbitrarily if they had a known cause of the disease. Therefore liver biopsy was the basis of the diagnosis in the first 50 (i.e. cases) and a definite disease aetiology the basis of choosing the second 50 (i.e. controls).

The next stage was extracting from the records the clinical features and investigations of this group (both cases and controls) at the time of that admission. The MELD score at the time of presentation was calculated on all subjects. The following steps were undertaken.

The clinical findings and investigational reports of the cases were compared with those in the controls to look for any significant differences. The clinical findings on presentation that were recorded for the study were the presence of jaundice , ascites and pedal oedema. Other clinical findings were not included in the comparative analysis. Encephalopathy and spontaneous bacterial peritonitis was recorded if it was the reason for admission. A past history of either was disregarded.

Among the investigations, the indices of liver function, haematological parameters such as haemoglobin and platelets, ESR and Anti nuclear antibody (if available) were compared in the two groups. Further the ratio such as SGOT/SGPT was also compared. Other investigations such as imaging and endoscopy were not included in the study.

Based on the histology reports the cases were classified into those with mild, moderate and severe parenchymal disease. A correlation with the MELD score was attempted.

Statistical Methods:

Descriptive statistics like mean and SD were presented for normally distributed continuous variables and median with interquartile range for non-normally distributed continuous variables The results between the two groups were compared for statistically significant difference. For the categorical variables chi-square test was used. For continuous variable with normal distribution, t- test was used. For continuous variable with non-normal distribution, Mann-Whitney's U test was used. P value of <0.05 was considered significant. The statistical analysis was done using SPSS software for windows version 16.

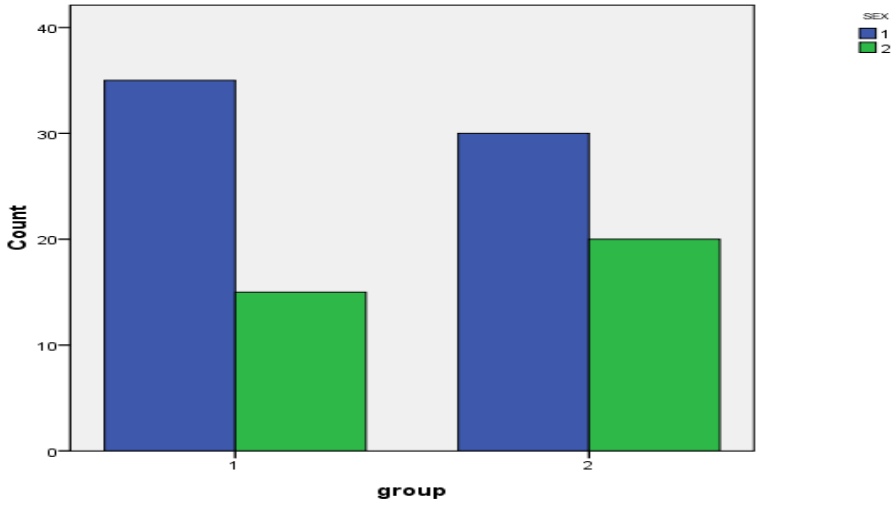
Results

GENDER and AGE: There were 35 males and 15 female in group-1 and 30 males and 20 females in group-2. There were no significant differences in these numbers between patients and controls. The ages of the cases of steatohepatitis varied between 7 – 67years with mean ±SD=45.1±10.426.The ages of the control group varied between 14-62 years with mean ± SD =39.49 ± 31.826.

Table-4

Gender	Male	Female	Total
Group-1(cases)	35(70%)	15(30%)	50(100%)
Group-2(control)	30(60%)	20(40%)	50(100%)

GENDER:



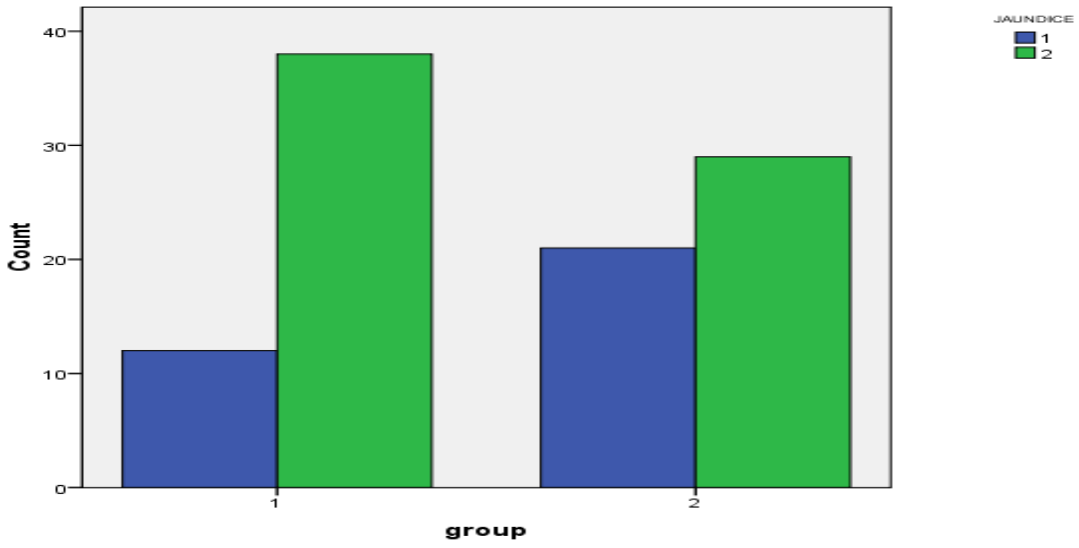
CLINICAL FINDINGS:

JAUNDICE: 12(24%) patients in gr-1 and 21(42%) patients in Gr-2 were jaundiced, so jaundice is more common in the control group as compared to cases, which is significant statistically(P<0.05).

Table-5

Jaundice	yes	No	Total
Group-1(cases)	12(24%)	38(76%)	50(100%)
Group-2(control)	21(42%)	29(58%)	50(100%)

JAUNDICE:

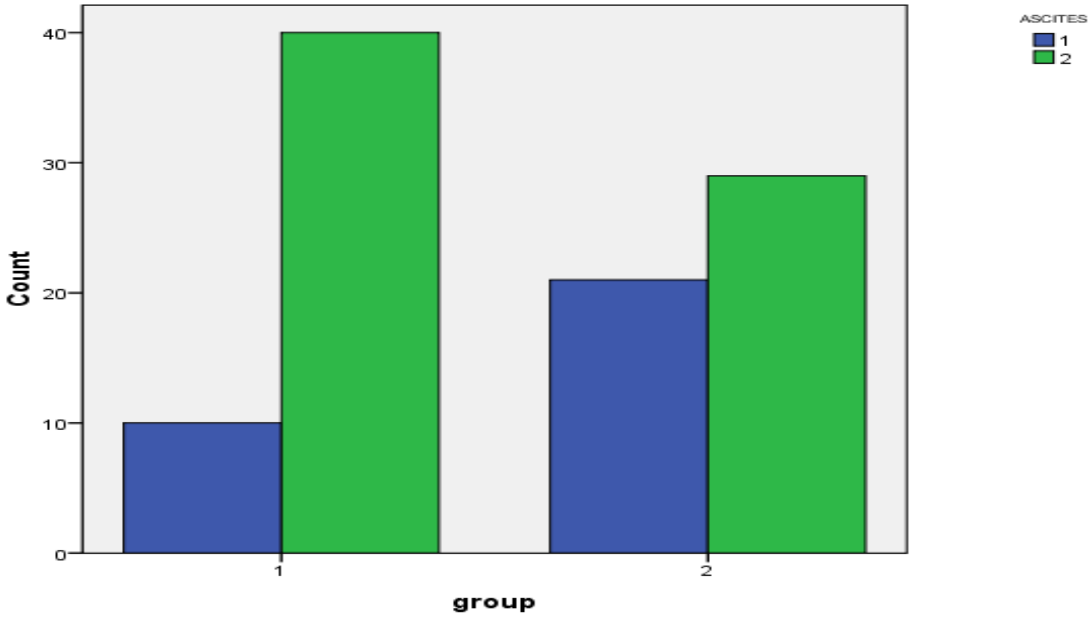


ASCITES: 10(20%) patients in Group-1 and 21 (42%) patients in Groups-2 had ascites. Ascites is more common in the control group and this finding is statistically significant(P<0.05).

Table-6

Ascites	yes	No	Total
Group-1(cases)	10(20%)	40(80%)	50(100%)
Group-2(control)	21(42%)	29(58%)	50 (100%)

ASCITES:

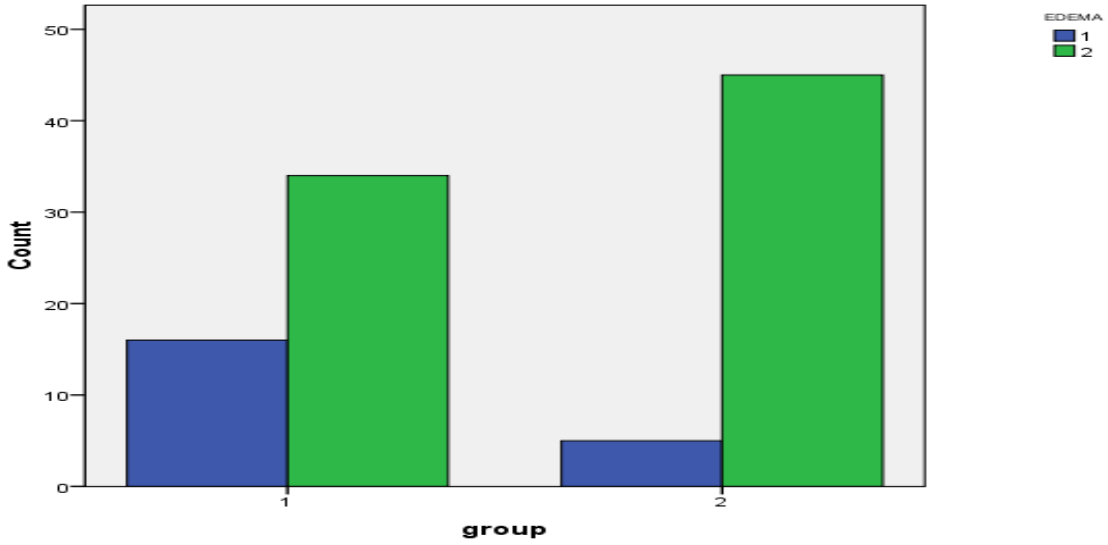


EDEMA: 16(32%) patients from gr-1 and 5(10%) patients from gr-2 had bilateral pedal edema and this difference is statistically significant (P<0.05).

Table-7

Edema	Yes	No	Total
Group-1(cases)	16(32%)	34(68%)	50(100%)
Group-2(control)	05(10%)	45(90%)	50(100%)

EDEMA:



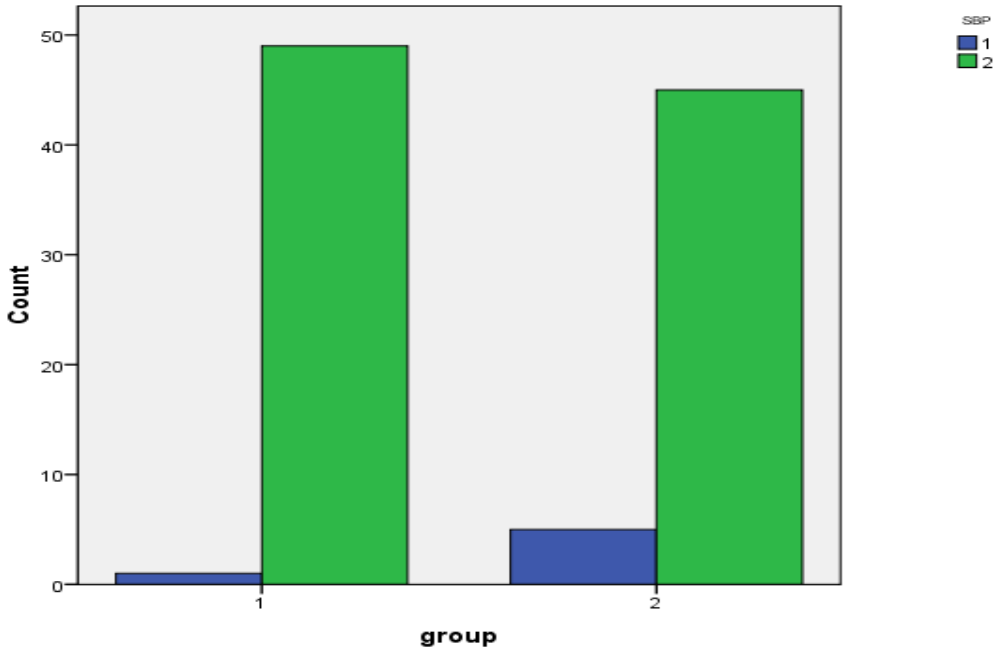
COMPLICATIONS:

Spontaneous bacterial peritonitis: 1(2%) patient from Gr-1 and 5(10%) patients from Gr-2 had SBP. The control group had a higher incidence of SBP, but this did not reach statistical significance.

Table-8

SBP	Yes	No	Total
Group-1(cases)	1(2%)	49(98%)	50(100%)
Group-2(control)	5(10%)	45(90%)	50(100%)

SBP:

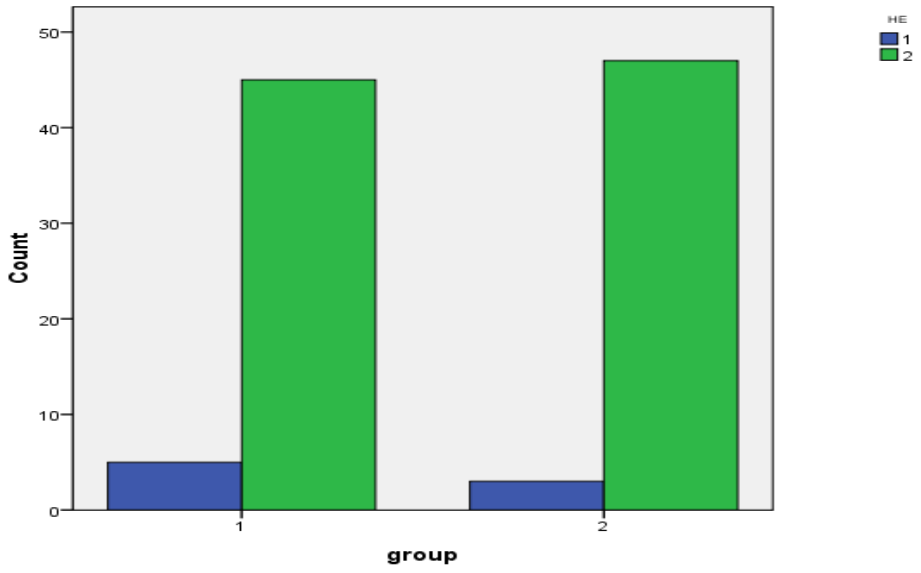


HEPATIC ENCEPHALOPATHY: 5(10%) patients in gr-1 and 3(06%) patients from gr-2 had hepatic encephalopathy. Here, the incidence of hepatic encephalopathy was more in the cases, but again this was not statistically significant.

Table-9

HE	Yes	No	Total
Group-1(cases)	05(10%)	45(90%)	50(100%)
Group-2(control)	03(06%)	44(94%)	50(100%)

Hepatic Encephalopathy:

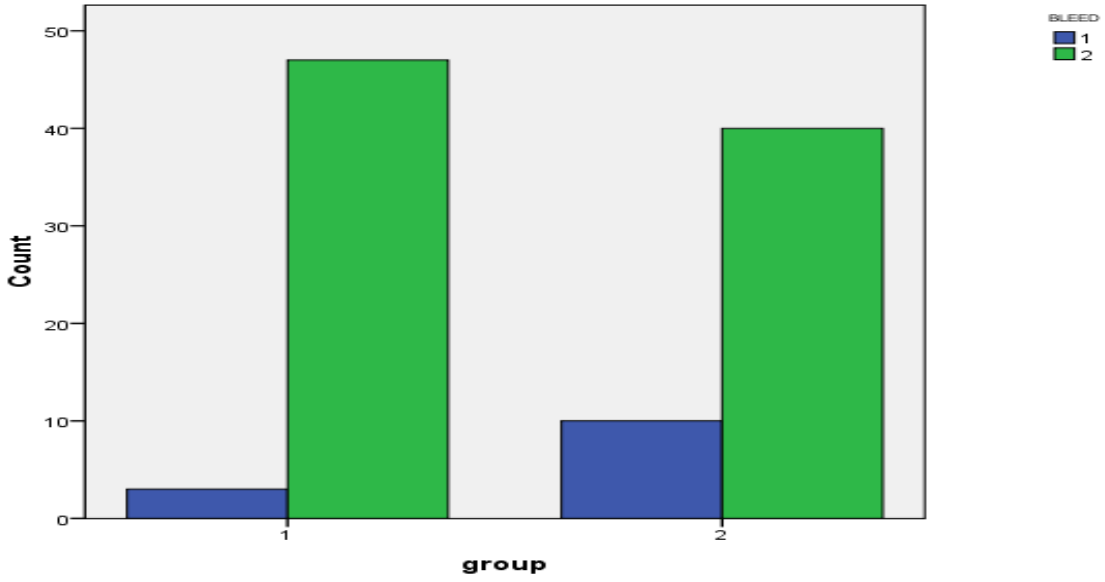


BLEED: 3(6%) patients in Gr-1 and 10(20%) patients from Gr-2 had upper g.i. bleed, control group more variceal bleed as compared to cases, which is significant statistically(P<0.05).

Table-10

Bleed	Yes	No	Total
Group-1(cases)	03(06%)	47(94%)	50(100%)
Group-2(control)	10(20%)	40(80%)	50(100%)

Gastrointestinal bleed:



Composite table 5-10

Indices	Cases(n=50)	Control (n=50)	Significant
Jaundice	12(24%)	21(42%)	*
Ascites	10(20%)	21(42%)	*
Edema	16(32%)	05(10%)	*
SBP	01(2%)	05(10%)	-
HE	05(10%)	03(06%)	-
Bleed	03(06%)	10(20%)	*

* → statistically Significant ($p<0.05$)

SBP= Spontaneous bacterial peritonitis, HE = Hepatic encephalopathy

INVESTIGATIONS AND LABORATORY PARAMETERS:

These laboratory investigations (ref. Table -11) did not show any Significant differences between cases and control except ESR(<0.05).

T – test:

Table-11

Group Statistics

	GROUP	N	Mean	Std. Deviation	Std. Error Mean
ALB	1	50	3.392	.8121	.1149
	2	50	3.256	.8553	.1210
GLO	1	50	4.496	.9337	.1320
	2	50	4.272	.8347	.1180
PT	1	50	14.514	4.9422	.6989
	2	50	15.436	4.3863	.6203
APTT	1	50	36.132	14.2593	2.0166
	2	50	36.418	9.4658	1.3387
HB	1	50	10.710	2.5453	.3600
	2	50	11.138	2.5136	.3555
TC	1	50	5588.00	3241.670	458.441
	2	50	6850.00	4321.623	611.170

ALB= Albumin, GLO=Globulin, PT=Prothrombin time, HB= Hemoglobin

aPTT= activated partial thromboplatin time, TC= Total count.

Table-12

Independent Samples Test

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
ALB	Equal variances assumed	.815	98	.417	.136	.1668	-.1950	.4670
	Equal variances not assumed	.815	97.738	.417	.136	.1668	-.1950	.4670
GLO	Equal variances assumed	1.265	98	.209	.224	.1771	-.1275	.5755
	Equal variances not assumed	1.265	96.794	.209	.224	.1771	-.1275	.5755
PT	Equal variances assumed	-.987	98	.326	-.922	.9345	-2.7765	.9325
	Equal variances not assumed	-.987	96.637	.326	-.922	.9345	-2.7768	.9328
APTT	Equal variances assumed	-.118	98	.906	-.286	2.4204	-5.0893	4.5173
	Equal variances not assumed	-.118	85.163	.906	-.286	2.4204	-5.0984	4.5264
HB	Equal variances assumed	-.846	98	.400	-.428	.5059	-1.4319	.5759
	Equal variances not assumed	-.846	97.985	.400	-.428	.5059	-1.4319	.5759
TC	Equal variances assumed	-1.652	98	.102	-1262.00	764.001	2778.135	254.135
	Equal variances not assumed	-1.652	90.881	.102	-1262.00	764.001	2779.620	255.620

Mann-Whitney Test:

Table-13

Ranks

	GROUP	N	Mean Rank	Sum of Ranks
MELD	1	50	49.81	2490.50
	2	50	51.19	2559.50
	Total	100		
TB	1	50	47.41	2370.50
	2	50	53.59	2679.50
	Total	100		
DB	1	50	49.90	2495.00
	2	50	51.10	2555.00
	Total	100		
SGOT	1	50	48.43	2421.50
	2	50	52.57	2628.50
	Total	100		
SGPT	1	50	48.15	2407.50
	2	50	52.85	2642.50
	Total	100		
ALP	1	50	54.30	2715.00
	2	50	46.70	2335.00
	Total	100		
PLATELETHB	1	50	48.01	2400.50
	2	50	52.99	2649.50
	Total	100		
TC	1	50	45.86	2293.00
	2	50	55.14	2757.00
	Total	100		
ESR	1	50	56.95	2847.50
	2	50	44.05	2202.50
	Total	100		
SGOT/SGPT	1	50	52.87	2643.50
	2	50	48.13	2406.50
	Total	100		

MELD= model for end-stage liver disease, TB= Total bilirubin , DB= Direct bilirubin ALP= Alkaline phosphatase

Table-14

Test Statistics

	MELD	TB	DB	SGOT	SGPT
Mann-Whitney U	1215.500	1095.500	1220.000	1146.500	1132.500
Wilcoxon W	2490.500	2370.500	2495.000	2421.500	2407.500
Z	-.238	-1.066	-.208	-.714	-.810
Asymp. Sig. (2-taile	.811	.286	.835	.475	.418

Table-15

Test Statistics[a]

	ALP	PLATELETHB	TC	ESR	SGOT/SGPT
Mann-Whitney U	1060.000	1125.500	1018.000	927.500	1131.500
Wilcoxon W	2335.000	2400.500	2293.000	2202.500	2406.500
Z	-1.310	-.858	-1.600	-2.239	-.817
Asymp. Sig. (2-tailed)	.190	.391	.110	.025	.414

a. Grouping Variable: GROUP

For all non normally distributed data a Mann whitney test was done for the above investigation and laboratory parameters including MELD scores to see if there is any statistically significant difference between the groups and was found to be significant only ESR at $P<0.05$.

Model for end-stage liver disease (MELD):

There was no difference in the MELD score between the cases and controls.

Table -16

	MELD
Mann-Whitney U	1215.502
Wilcoxon W	490.500
Z	-.238
Assymp. Sig.(2-tale)	.811

(Also ref. to table-14)

Below is the frequency data (ref. Table-17)

MELD score frequency data (cases and control):

Table-17

MELD Score	**Cases (No. of patients)**	**Controls(No. of patients)**
1-5	12	15
6-10	19	15
11-15	16	14
16-22	03	06
	Total =50	Total =50

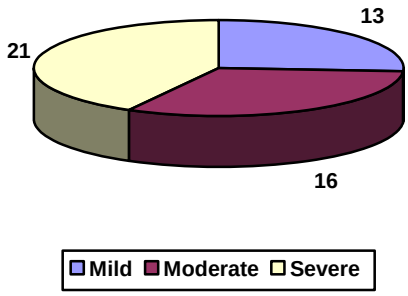
Comparison of MELD score in relation to histological liver injury:

When the MELD score was below 6, there were very few cases with severe injury. When MELD scores were above 6 , the histological damage was much more but did not show a progressive increase thereafter (ref. table-18).

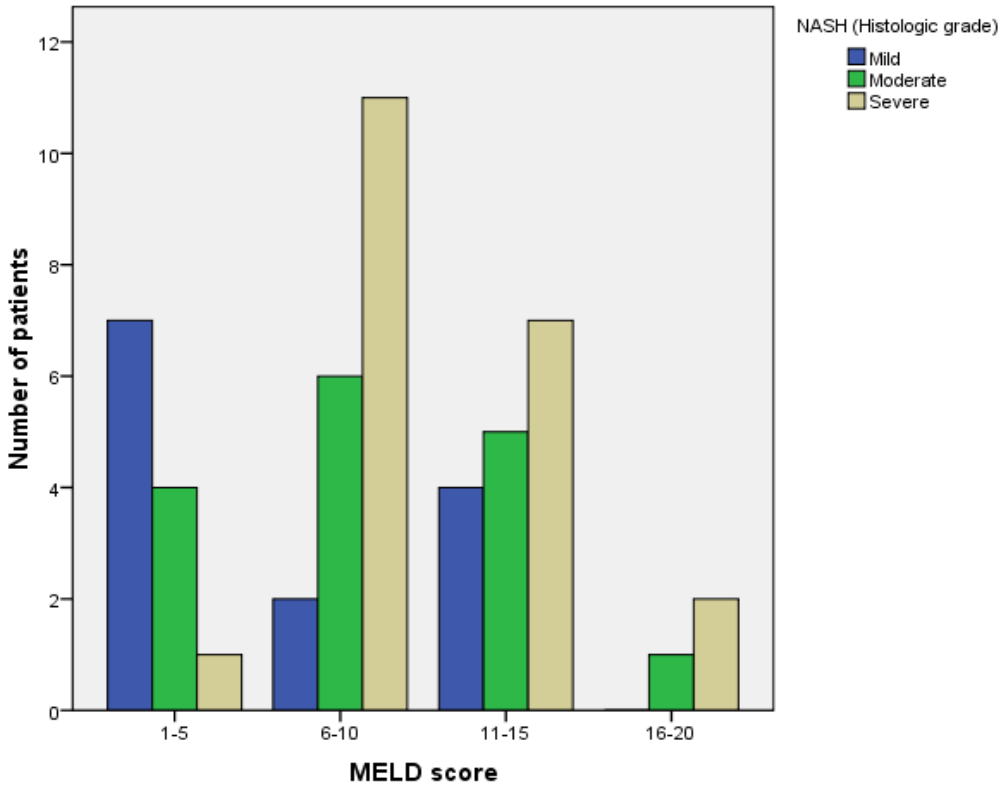
Table-18

	MELD SCORES			
NASH	1-5	6-10	11-15	16-20
Mild	07(58.34%)	02(10.53%)	04(25.00%)	00(0.00%)
Moderate	04(33.34%)	06(31.58%)	05(31.58%)	01(33.33%)
Severe	01(08.34%)	11(57.89%)	07(43.75%)	02(66.33%)

Distribution of histological severity of the disease according to the MELD score



Graph: Comparision of histologic grades (NASH) with MELD score.



Conclusion

1) This study used cases and controls with similar MELD scores, gender ratios and age ranges. Therefore these are fairly well matched cases and controls.

2) In this study of chronic liver disease with steatohepatitis, we found that group-2 (diseased controls) were more likely to have Jaundice, ascites and gastrointestinal bleeding. The cases were more likely to have pedal edema.These differences were statistically significant.

3) All investigational parameters between the two groups were not significantly different except for the ESR.

4) MELD score showed no correlation with histological findings in the Steatohepatitis cases it was observed that with MELD scores below 6 there were few patients with severe histological damage. However the damage did not proportionately increase above 6.

Part - II

Title : Serum Anti-Mitochondrial Antibody (AMA) in Cryptogenic Chronic Liver disease with elevated Serum Alkaline Phosphatase levels.

AIM

- To determine how frequently the Anti mitochondrial antibody test is positive in patients with chronic liver disease with high serum alkaline phosphatase levels.

- To identify the discriminatory value of clinical features in patients who tested positive for AMA from those with a negative test.

Material and Methods

Study design: Retrospective case-control study.

Study population and sample size:

This was a retrospective study where 120 patients with chronic liver disease for whom the anti mitochondrial test (AMA) was done because of a raised alkaline phosphatase (ALP) either at presentation or on initial follow up formed the population from which cases and controls were drawn. 10 patients who tested AMA positive (and this was the entire AMA positive group of the 120

cases) served as cases and 20 subjects that were randomly chosen from the AMA negative group served as controls.

In order to do this we used the following method of identifying the total AMA positive population.The records of the serological laboratory between January 2004 and February 2005 were scrutinized and all those for whom an AMA test was requested were chosen for inclusion. All of these had chronic liver disease with an elevated serum alkaline phosphates level.

All subjects who tested positive for the AMA were included as cases. Of those in this population who had elevated alkaline phosphatase but tested negative for AMA a sample of subjects was drawn to act as controls.

This was random but was intentionally skewed towards the female gender to match the gender ratio of the cases. A random selection of 20 test negative controls was heavily skewed towards the male sex. Therefore a selection of 2 males and 18 females were made randomly from gender separate groups.

The next stage was examining the records and investigations of both these groups at admission. The following steps were undertaken.

1) The clinical findings and investigational reports of the cases were compared with those in the controls to look for any significant differences.

2) The clinical findings on presentation that were recorded for the study were the presence of jaundice, ascites and pedal oedema. Other clinical findings were not included in the comparative analysis. Encephalopathy and spontaneous bacterial peritonitis was recorded if it was the reason for admission. A past history of either was disregarded.

3) Among the investigations, the indices of liver function, haematological parameters such as haemoglobin and platelets, ESR and Anti nuclear antibody (if available) were compared in the two groups. Further the ratio such as SGOT/SGPT was also compared. Other investigations such as imaging and endoscopy were not included in the study

Statistical Methods:

Descriptive statistics like mean and SD were presented for normally distributed continuous variables and median with interquartile range for non-normally distributed continuous variables. The results between the two groups were compared for statistically significant difference. For the categorical variables chi-square test was used. For continuous variable with normal distribution, t- test was used. For continuous variable with non-normal distribution, Mann-Whitney's U test was used. P value of <0.05 was considered significant. The statistical analysis was done using SPSS software for windows version 16.

Results

AMA was requested for 120 patients during the period between January 2004 to January 2005. Out of total 120 patients 95 were females and 25 were males.

The age range was between 18-70Years (Mean ± SD=43.63 ± 13.11) Only 10 patients were tested positive for AMA and rest were negative. In the Positive group were nine Females and one Male; age ranges from 18 – 70 years (Mean ± SD=43.8 ± 13.63).

Alkaline Phosphatase levels:

The range of_ALP levels for all 120 cases was 56 to 1266 IU/L with mean ± SD=303.83 ±267.34IU/L; ALP value for AMA positive cases(n=10) range 132 -1266 IU/L with a medial value =267.50 IU/L and a mean ± SD=368.40 ± 340.08 IU/L. ALP for Controls group (n=20) range from 133 to 853IU/Lwith a median value =204.50 IU/L and mean ± SD = 294.06 ± 189.78).

Characteristics of patients:

There were no significant differences in age, sex and MELD score between AMA positive and AMA negative groups.

(Table-19)

Characteristics of patients.

	Study group AMA(+)	Control group AMA (-)	*P value*
No of patients.	N=10	N=20	-
Age in years.	18-70 (Mean=38)	18-62 (Mean=42.7)	ns
Sex	M=1/F=9	M=2/F=18	ns
MELD	8-10	7-11	ns

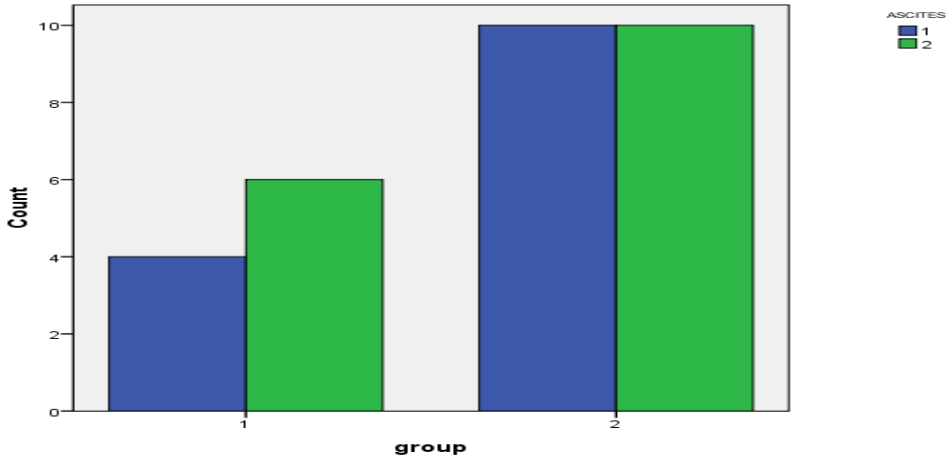
CLINICAL FEATURES:

ASCITES: 4(40%) patients in group -1 and 10(50%) patients in group -11 were having ascites , rest were negative both clinically and radiologically.

Table- 20

ASCITES	Yes	No	Total
Group -1(AMA+)	4(40%)	6(60%)	10(100%)
Group-2(AMA-)	10(50%)	10(50%)	20(100%)

Ascites:

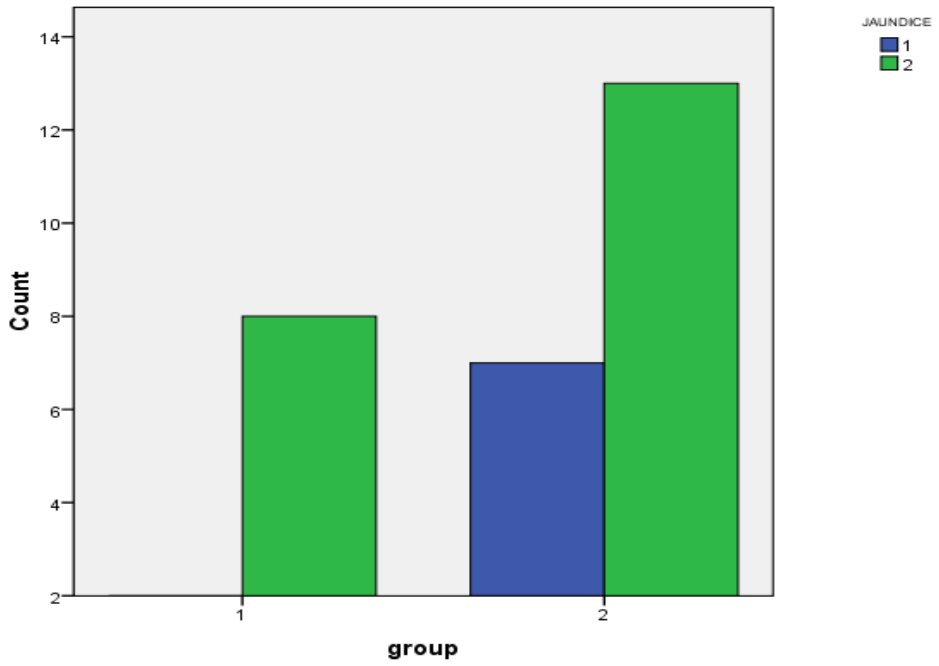


JAUNDICE: 2(20%) patients from gr-1 and 7(35%) from gr-11 were having Jaundice, this was not statistically significant .

Table-21

Jaundice	Yes	No	Total
Group-1(AMA+)	2(20%)	9(90%)	10(100%)
Group-2(AMA-)	7(35%)	13(65%)	20(100%)

Jaundice:

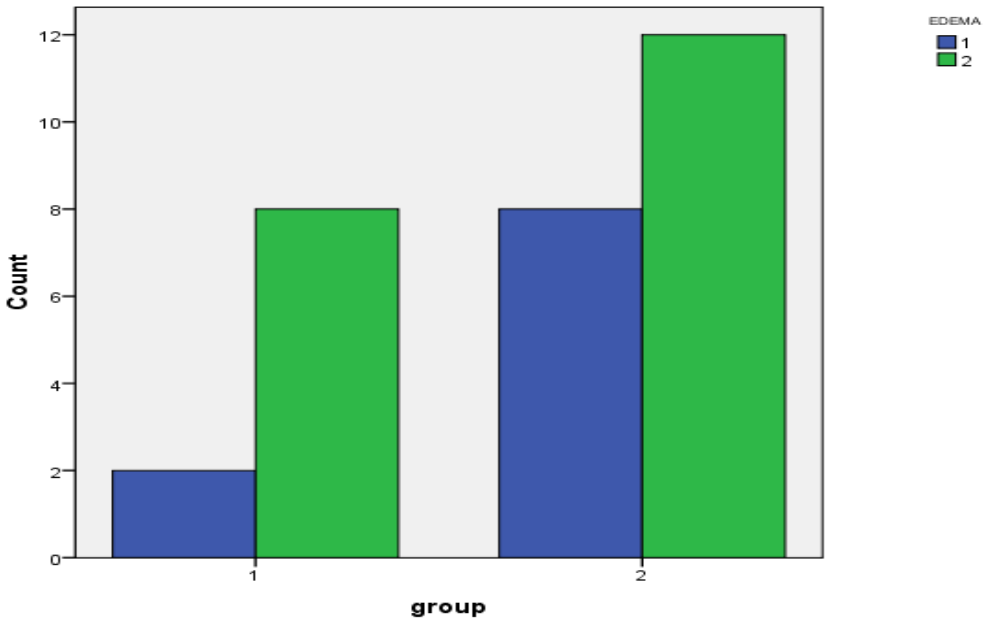


EDEMA: Pedal edema was seen in 2(20%) patients in gr-1 and 8(40%) patients in gr-2, this was not statistically significant.

Table-22

Edema	Yes	No	Total
Group-1(AMA+)	2(20%)	08(80%)	10(100%)
Group-2(AMA-)	8(40%)	12(60%)	20(100%)

Edema:



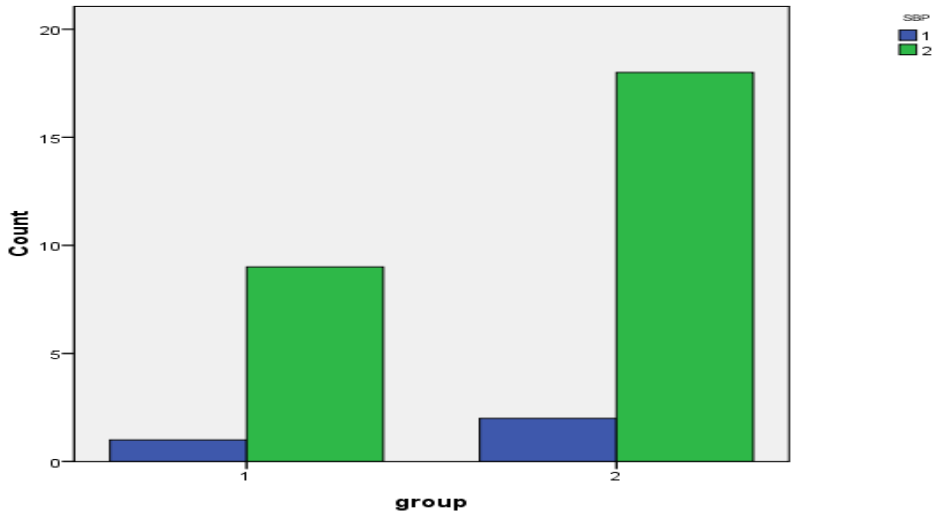
COMPLICATIONS:

Spontaneous bacterial peritonitis: There were 1(10%) in gr-1 & 2(10%) in gr-11 having SBP, this was not statistically significant.

Table-23

SBP	Yes	No	Total
Group-1(AMA+)	1(10%)	9(90%)	10(100%)
Group-2(AMA-)	2(10%)	18(90%)	20(100%)

SBP:

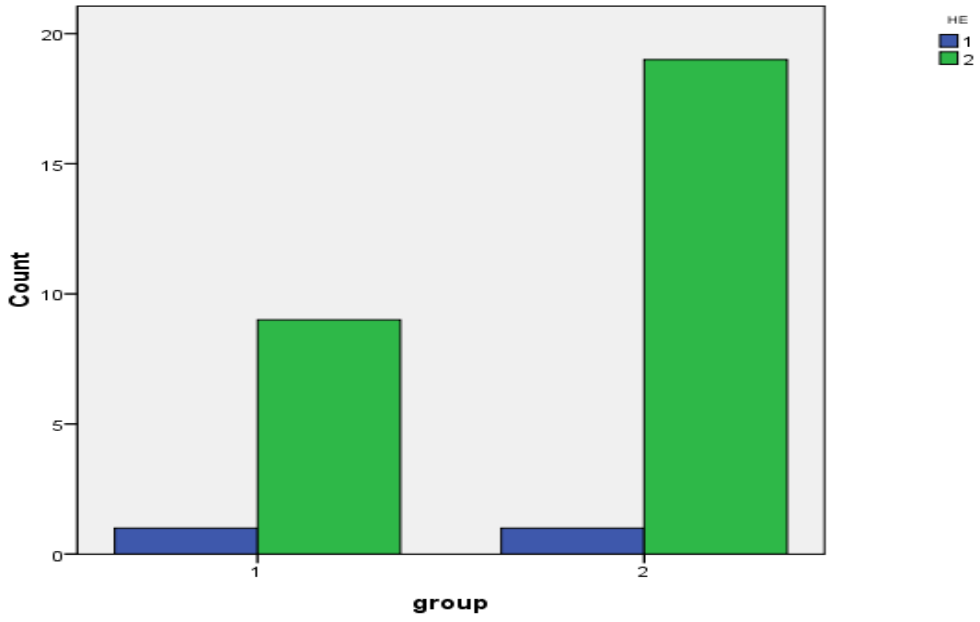


Hepatic encephalopathy: 1(10%) patient from group-1 and 1(05%) patient from group-2 developed hepatic encephalopathy, this was not statistically significant.

Table-24

HE	Yes	No	Total
Group-1(AMA+)	1(10%)	9(90%)	10(100%)
Group-2(AMA-)	1(05%)	19(95%)	20(100%)

Hepatic encephalopathy:

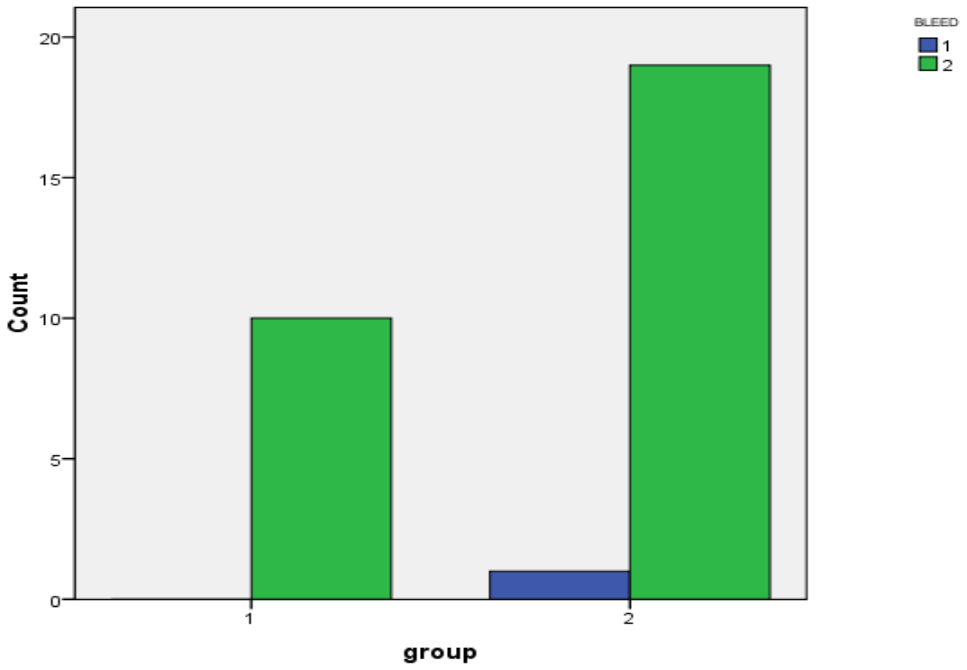


BLEED: There was 1(05%) patient who had esophageal variceal bleed in gr-2 but there was no episode of bleed in gr-1, this was not statistically significant.

Table-25

Bleed	Yes	No	Total
Group-1(AMA+)	0	10(100%)	10(100%)
Group-2(AMA-)	1(05%)	19(95%)	20(100%)

Bleed:



Composite Table-20-25

Indices	AMA positive	AMA negative	s/s[p<0.05]
Ascites	4(40%)	10(50%)	ns
Jaundice	2(20%)	7(35%)	ns
Edema	2(20%)	8(40%)	ns
SBP	1(10%)	2(10%)	ns
HE	1(10%)	2(10%)	ns
Bleed	0	1(10%)	ns

s/s=statistically significant, ns= not significant SBP=spontaneous bacterial peritonitis, HE= hepatic encephalopathy.

Investigation and Laboratory parameters:

The two groups did not show significant difference for most of the parameters except TB and aPTT (ref. Table-21).

Table-26

	Group-1(AMA+)		Group-2(AMA-)		Mean differ.
	Mean	**± SD**	**Mean**	**± SD**	
TB	2.3	3.4	5.555	7.8	3.255 **
DB	1.18	2.13	3.525	5.945	2.345
Alb: Glo:	3.33 4.31	0.636 0.735	3.135 3.83	0.701 1.128	-0.205 -0.28
SGOT	97.6	65.501	168.4	278.683	70.8
SGPT	75.8	55.838	135	314.547	59.2
ALP	360.4	346.176	265.55	210.654	-94.85
PT	12.63	5.81	13.56	2.08	0.93
aPTT	23.16	16.366	35.87	5.648	12.71 **

** significant at P<0.05

TB= Total bilirubin, DB=Direct bilirubin,Alb= albumin, Glo=globulin,

ALP=alkaline phosphatase, PT= prothronbin time, aPTT= activated partial thromboplastin time.

Independent samples t-Test:

Table-27

Parameters	Groups(AMA+ And AMA-)	N	Mean	SD	t-Value	df
ALB	1	10	3.38	0.561	0.959	0.346
	2	20	3.14	0.701	1.034	0.312
GLOBULIN	1	10	4.310	0.7355	0.128	0.899
	2	20	4.255	1.2344	0.152	0.881
PT	1	10	12.630	5.8071	-0.647	0.532
	2	20	13.560	2.0775	-0491	0.634
HB	1	10	10.410	2.1605	-0.759	0.454
	2	20	11.060	2.2369	-0.768	0.452
TC	1	10	6480	2766.386	-0.702	0.489
	2	20	7360	3437.778	-0.756	0.458
SGOT/SGPT	1	10	1.3962	0.47843	-O.546	0.589
	2	20	1.5176	0.61377	-0.594	0.558

HB= hemoglobin, TC= Total count

Table-28

Test Statistics[b]

	MELD	TB	DB	SGOT	SGPT	ALP	APTT	PLATELATE
Mann-Whitney U	73.500	54.000	62.000	100.000	95.500	80.000	49.000	99.000
Wilcoxon W	128.500	109.000	117.000	310.000	305.500	290.000	104.000	154.000
Z	-1.170	-2.028	-1.680	.000	-.198	-.880	-2.245	-.044
Asymp. Sig. (2-taile	.242	.043	.093	1.000	.843	.379	.025	.965
Exact Sig. [2*(1-taile Sig.)]	.248[a]	.044[a]	.100[a]	1.000[a]	.846[a]	.397[a]	.024[a]	.983[a]

a. Not corrected for ties.

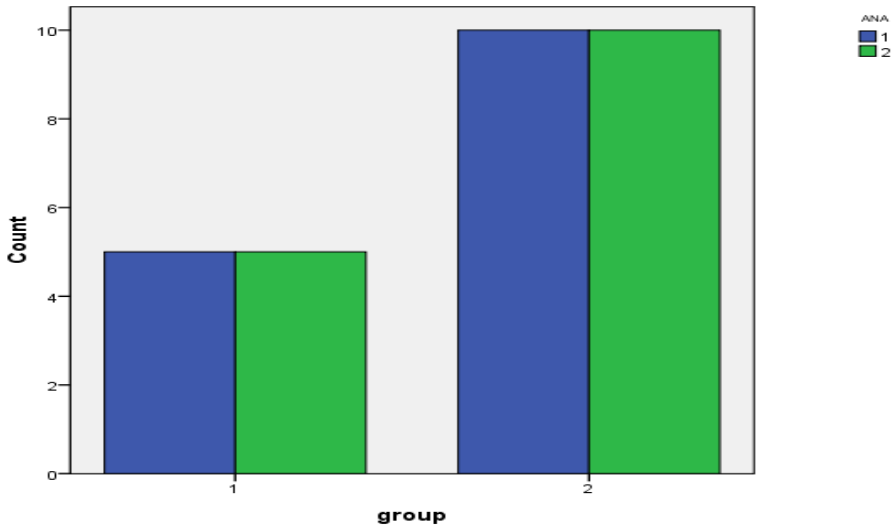
b. Grouping Variable: GROUP

For all non normally distributed data a Mann Whitney test was done for the various parameter to see if there is any statistically significant difference between the gps and was found to be significant only for TB and APTT at P<0.05.

Other features:

ANA: ANA were positive 50% in both the groups, there was no significant statistically.

ANA POSITIVITY



Liver biopsy and histology:

Liver biopsy were available on 3 out of 10 AMA positive and on 11 out of 20 AMA negative patients. The rest did not undergo liver biopsy. In the AMA positive group, none of them had characteristics of Primary biliary cirrhosis.

In AMA negative patients, biopsy findings were similar ; however one patient had features suggestive of primary biliary cirrhosis and another had granulomas on histology (ref. table-22 &23).

Comparison of USG abdomen and liver histology between the AMA positive and AMA negative groups(table 29&30):

Table -29

USG/ Liver Bx

AMA+(n=10)		AMA- (n=20)	
USG abdo.	**Liver bx**	**USG abdo.**	**Liver bx**
1)Coarse,Shrunken & vr+	**-**	**1)Coarse&vr+**	**-**
2)Irregular&surfae nodularity	**-**	**2)Coarse**	**Micronodular cirrhosis**
3)Coarse&lobulatd	**Sinusoidal congestion& pericellular fibrosis**	**3)Mildly Coarse & enlarged**	**Moderate periportal inflammation with periportal & bridging fibrosis**
4)Coarse	**Bridging fibrosis, nudularity &focal bile duct damage**	**4) Coarse &vr+**	**-**
5)Coarse & lobulated	**-**	**5) Coarse &vr+**	**Focal portal inflammation &fibrosis**
6)Coarse	**-**	**6)Early CLD**	**Chronic cholestasis.**

Table – 29 contd..

AMA+ (n=10)		AMA-(n=20)	
USG abdo.	**Liver bx**	**USG abdo.**	**Liver bx**
7)Coarse , nodular & vr+	**-**	**7)Nodular& vr+**	**-**
8)vr+, coarse &nodular	**-**	**8) Coarse &vr+**	**Granulomatous inflammation with periportal bridging fibrosis**
9)Coarse	**9) early focal bridging fibrosis&moderate periportal inflammation.**	**9) Early CLD**	**-**
10)Shrunken, coarse& nodular		**10)Coarse &nodular**	**-**
		11) Coarse &irregular margin	**-**
		12) Shrunken, coarse	**Cirrhosis with focal activity.**

Table-30

USG / liver biopsy in AMA – ve patients:

USG abdomen	**Liver bx**
13)Shrunken coarse& Micronodular cirrhosis	Micronodular cirrhosis
14)Coarse & irregular surface	Portal inflammation with destruction &disappearance intrahepatic bile ducts , abnormal bile duct proliferation, fibrosis & cirrhosis and f/s/o primary biliary cirrhosis.
15) Coarse & irregular surface	Cirrhosis with mild steatosis
16) Coarse & irregular surface	-

17)Diffusely Coarsing & irregular surface	Bridging fibrosis with nodularity & mild inflammation
18)Coarse & irregular surface	-
19) Coarse & shrunken	Early focal bridging fibrosis & moderate periportal inflammation
20) Coarse & shrunken	-

Conclusion

1) AMA was rarely positive (about 8.33%) in this group of cryptogenic chronic liver disease with high ALP values.

2) The clinical picture and investigational results were not different from AMA negative controls.

3) The only discriminating investigations are a high Total Bilirubin, and activated Partial Thromboplastin Time in cases which are AMA positive.

Discussion

Cryptogenic liver disease in the late stages is often overlooked because the condition requires transplantation and this mode of therapy is widely available in the West. However this is not always possible in poorer societies. There are new treatments that are emerging which may rescue people with advanced disease. The best example is the success that new therapies have had in such conditions such as HBV infection. It is possible that in the future we may similarly be able to treat other conditions in late stages without resorting to liver transplantation.

It is therefore important to study patients who are passed off as cryptogenic disease only on the grounds that the serological markers of viral infection and a history of alcohol abuse is absent. We chose two groups from such a population of patients.

The first was steatohepatitis. This was possible because of fortuitous availability of liver biopsy material. The second was those with high alkaline phosphatase levels to examine if the AMA test could be used to detect PBC in this group. It was also hoped that the clinical picture, or investigational results would help in making a diagnosis of NASH and/or PBC before a biopsy or test is conducted.

There are many possible causes for cryptogenic liver disease. We have looked at only two groups: a) those which had biopsy evidence of

Steatohepatitis and b) those with high alkaline phophatase or putative PBC. There are possibly many other causes. These would include cryptogenic autoimmune liver disease for which there is no reliable autoimmune marker. It may also include occult HBV infection or copper storage disorders. It could also be non cirrhotic liver disease which presents with the syndrome of chronic liver failure. The problem is that in many of these conditions, the liver disease is so advanced that the signs of the original cause may have disappeared. This scenario is the accepted opinion in NASH.

The findings of this retrospective study on patients histologically proven to have advanced NASH has demonstrated that among this population with non viral, non alcoholic cryptogenic disease are a number of cases of steatohepatitis. In the period of three years there were 50 cases. This is an underestimation as there were many who did not undergo a liver biopsy as they were either too advanced or for other reasons. Further as the steatosis disappears with advancing disease a number may have escaped detection.

The first question was whether clinical or laboratory parameters would help arrive at a diagnosis. Although there were significant differences in the number of cases with jaundice, ascites, gastrointestinal bleeding and peripheral oedema between patients and controls, none of these can be used alone or together to make a clinical diagnosis of advanced NASH. Among the laboratory tests the ESR was different. This is found surprisingly higher in the NASH group

rather than controls. Again this cannot be used for the diagnosis of the condition. However it may point to the fact that there is ongoing inflammation.

The clinical findings of more ascites and gastrointestinal bleeding in the control group - while the pedal oedema is higher in NASH - is a strong suggestion that portal hypertension is lower in the group with NASH. This seems particularly relevant as the MELD scores and serum albumin levels were not different in the two groups.

It appears from the above finding that in the NASH group, hypo-albuminemia is the cause of pedal edema. In the control group the low albumin combined with a high hydrostatic portal pressure results in Gastro Intestinal bleeds and ascites. This requires confirmation by wedged hepatic pressure. The difference in the number of patients with Jaundice is unexplained but may point to other protective mechanisms.

With regard to the second question about correlation between the histological severity and the MELD scores, it was found that although there was a trend to higher MELD scores with increasing severity , this seemed to level off after a MELD score of 6.Therefore MELD scores could not be used to predict the nature of the histological damage. What are the implications of these findings? A liver biopsy is essential for the diagnosis of NASH in advanced disease. There is a need to study the nature of portal hypertension in patients with NASH.

The studies on those with high Alkaline phosphatase levels showed that the AMA test was very rarely positive. The majority of patients who tested positive were women who also were positive for the ANA test. Therefore the conclusion that can be drawn is that PBC is either a rare disease or that it is falsely negative in many of our patients.

This has to be proven by other means such as by examining histology in this group. The group who tested positive for AMA was not different from those that were negative either on the basis of clinical or investigational findings, which makes a case for a large population of occult PBC in this population.

These studies make a case for liver biopsies in advanced liver disease. This and other investigations and studies in cryptogenic liver disease may prepare us for the therapies that may appear in the future.

Final Conclusion

1) Patients with advanced NASH cannot be clinically distinguished from patients with other chronic liver disdease . Therefore biopsy is essential.

2) Patients with steatohepatitis have less chance of developing ascites and having a gastrointestinal hemorrhage.

3) They have a lesser chance of gastro intestinal bleeds and edema. This may reflect differences in portal hypertension

4) There was no histological correlation with MELD scores.

5) AMA is rarely positive (about 8.33%) in cryptogenic chronic liver disease with high ALP values.

6) The clinical picture is not different from that of AMA negative controls.

7) The only discriminating investigations are a high Total Bilirubin, and activated Partial Thromboplastin Time in cases with AMA positive.

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Investigations

1. CBC profile, ESR, ANA

2. Serum biochemistry

3. virological study

4. Imagings

    U/S Abdomen,

5. Per cut. / TJLB liver biopsy

6. Histologic examination

    Dry weight copper and special stains as clinical situation.

Appendix -1 – master sheet

Abbreviations

M= Male

F= Female

HB= Hemoglobin (g%)

TC=Total count

ESR in mm/hr

ALB= Albuminin g%

SBP= spontaneous bacterial peritonitis

HE= Hepatic encephalopathy, CC=cryptogenic cirrhosis

MELD= Model for End stage Liver Disease

TB= Total bilirubin

DB= Direct bilirubin

GLO= Globulin

SGOT=serum glutamic –oxaloacetic transaminase

SGPT=Serum glutamic-pyruvic transaminase

ALP=Alkaline phosphatase

PT=Prothrombin time

aPTT =Activated prothrombin time

ANA=antinueclear anti body

Male-1, Female-2

Ascites present-1, abscent-2

SBP present-1, abscent-2

HE present-1, abscent-2

Jaundice present-1, abscent-2

Bleed present-1, abscent-2

Edema present-1, abscent-2: ANA (+)=1,(-)=2

Appendix-2 (NASH SCORING)

[University Pathologists - Staging and Grading – Liver]

NONALCOHOLIC STEATOHEPATITIS STAGING

Stage	Histologic criteria
1	Zone 3 perivenular perisinusoidal/pericellular fibrosis, focal or extensive
2	As above with focal or extensive periportal fibrosis
3	Bridging fibrosis, focal or extensive
4	Cirrhosis

REFERENCE

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NASH STAGING AND GRADING - Nonalcoholic Steatohepatitis Clinical Research Network scoring system (Nonalcoholic Steatohepatitis Activity Score - NAS), 2005

ACTIVITY SCORE

Histologic feature	Grade	Description
Steatosis	0	<5%
	1	5-33%
	2	33-66%
	3	>66%
Lobular inflammation	0	None
	1	<2 foci/200x field
	2	2-4 foci/200x field
	3	>4 foci/200x field
Ballooning	0	None
	1	Few balloon cells
	2	Many cells/ prominent ballooning

NAS > 5 = NASH, NAS<2 = no evidence of NASH, NAS 3-4 = borderline/possible

FIBROSIS STAGING

Stage	Histologic criteria
1	Zone 3 perivenular perisinusoidal/pericellular fibrosis, focal or extensive · 1A - delicate perisinusoidal fibrosis · 1B - dense perisinusoidal fibrosis · 1C - portal-only fibrosis
2	As above with focal or extensive periportal fibrosis
3	Bridging fibrosis, focal or extensive
4	Cirrhosis

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