STUDY ON THE PRESENTATIONS OF CHRONIC LIVER DISEASE IN CORRELATION TO THEIR ETIOLOGIES

DISSERTATION

Submitted to

T. N. DR. M.G.R. MEDICAL UNIVERSITY

in

Partial fulfillment of the requirement

for the award of

D.M. (MEDICAL GASTROENTEROLOGY)

MADRAS MEDICAL COLLEGE

Dr. M.G.R. Medical university, Chennai

Feb 2006

CERTIFICATE

This is to certify that the dissertation titled **"*Study on the presentations of chronic liver disease in correlation to their etiologies*"** is a bonafide work done by Dr. G. Venkatraman, Post graduate in Medical Gastroenterology from June 2003 to February 2006 under my supervision and submitted the same for the ensuing D.M. (Medical Gastroenterology) higher speciality examination, February 2006, to be conducted by TN. Dr. MGR medical university, Chennai.

Dean
Madras Medical College
Chennai

Prof. S. Barnaba Durairaj, MD., DM
Professor and Head,
Department of medical gastroenterology
Madras Medical College,
Chennai – 600 003.

ACKNOWLEDGEMENT

I am greatly indebted to our Professor and Head of the Department **Dr.S. Barnaba Durairaj M.D. D.M.,** for his poignant guidance, valuable suggestions and painstaking efforts in correcting the manuscript.

I thank **Dr.P. Padmanaban, M.D., D.M.,** Professor, for his enthusiastic support throughout the study.

My thanks are also due to **Dr. P. Ganesh, Dr. Narayanaswamy, Dr. D. Suresh, Dr. K. Premkumar**, Assistant Professors in the department for their guidance and encouragement.

I recall with gratitude about the concern shown by my former Professors, **Dr. B. Ramathilagam,M.D., D.M., Dr. S. Rajanikanth, M.D., D.M., and Dr.N. Dinakaran M.D., D.M.**

I thank my colleagues and friends for their constant enthusiasm, and timely help.

I express my thanks to **Dr. Kalavathy Ponniraivan, MD.,** Dean, Madras Medical College, Chennai for permitting me to do this study.

Last but not the least I thank all the patients for their cooperation, throught the study in the benefit of others despite their illness.

CONTENTS

INTRODUCTION

Chronic liver disease (CLD) and its sequelae form a major part of gastroenterological workload in any large hospital. As well known, it is a disease with very high degree of morbidity and mortality. In the past, CLD was broadly divided into alcoholic and post hepatitis varieties. Over the years, with the advent of newer techniques for identification of viruses, biochemical markers, and newer histopathological methods, it is now possible to enlarge the classification and attach more informative labels to such cases. Even among virus related CLD, a large group so far labeled as Non A, Non B, identification of Hepatitis C has become an established practice. Transfusion associated NANB hepatitis has a new perspective now. Despite these transformations, a small proportion of patients fail to demonstrate any known viral marker raising further questions.

There are now well documented studies on the beneficial effects of antiviral agents in the management of chronic hepatitis B and C. Hence it becomes almost mandatory to determine if a given case of CLD is virus related or not, so that a decision regarding the use of such antiviral agents can be made. Another problem, which is assuming importance, is co-existence of more than one hepatotropic virus. While the co-infection or superinfection of HBV and Hepatitis D Virus (HDV) is well studied, the significance of the presence of viral makers of HBV and HCV in the same patient is not yet clear. Which agent is responsible for the observed liver damage and how the presence

of one virus alters the biological behavior of other, are questions which have already caught the attention of hepatologists worldwide.

The frequent demonstration of viral markers in alcoholic liver disease (ALD) raises fresh dilemmas. Golding et al., stress the importance of establishing the presence or absence of Hepatitis viruses before dubbing the case as Alcoholic liver disease. Conditions which have been earlier passed off as Cryptogenic liver cirrhosis or as ALD need to be reevaluated now in the light of prevalence of high degree of viral markers in them.

Reports have begun to appear regarding the histopathology of Chronic Hepatitis C. Though not pathognomonic, the appearances are claimed to be distinct and characteristic, Hence there is need for correlation of HCV markers with histopathology and substantiating such observations.

With reference to HBV, the immunological aspects have been so well studied and understood that there is a distinct clinical significance for the presence or absence of each viral antigen and antibody. Moreover, the recent demonstration that even patients with anti HBe, who were believed to have a good prognosis till now, can still harbor HBV DNA and may develop severe disease, has given fresh insight into this aspect.

Another important aspect in CLD is its prevention and control. Now that effective vaccination against HBV is available, identification of viral etiology is of great value in protecting the family members and other contacts against the disease.

Even in an established case of chronic hepatitis B, there may be sudden exacerbation or unexplained clinical deterioration, which may be related to alteration in serological status or superinfection with Delta or HCV. .

Hence in summary, more accurate categorization of CLD based on known etiological markers is essential for the following reasons:

i. Presence of viral markers in CLD presumed to be of non viral etiology..
ii. Presence of multiple viral markers as well as non viral etiological factors in the same patient.
iii. Detection of Hepatitis C as an important cause of CLD as well as transfusion related hepatitis.
iv. Availability of effective anti viral drug regimens.
v. Availability of prophylactic measures to prevent the disease in family contacts

BACKGROUND AND LITERATURE REVIEW

Role of Viruses in Chronic liver Disease:

The viruses associated with CLD, Cirrhosis and Hepatocellular carcinoma are Hepatitis B,C and D as per our current knowledge. Blumberg discovered the '***Australia Antigen'*** in 1965 in an Australian aborigine. Since then, there has been a steady evolution of knowledge regarding hepatitis viruses and their mechanisms of liver injury. It is extremely fascinating to know that these viruses by themselves are not cytopathic and yet they are responsible for devastating hepatic injury.

HEPATITIS B VIRUS

Virology

Hepadnaviruses

HBV is a member of a family of distinct viruses, known as the ***Hepadnaviridae***, which infect humans and a few animal species (duck, ground squirrel, and woodchuck). Characteristics of these viruses include the presence of partially double-stranded DNA that is surrounded by an outer lipoprotein envelope and an inner core composed of nucleocapsid proteins. The virus encodes a polymerase that catalyzes by reverse transcription both the generation of DNA complementary to the viral RNA template and the synthesis

of positive-strand viral DNA from the negative-strand DNA template of the virus these viruses are all predominantly hepatotropic.

Structure:

HBV is a remarkably compact virus with four *open reading frames (ORF)* (S,P,C,and X protein, respectively). Intact HBV virions are 42 nm in diameter and are readily visualized by electron microscopy. HbsAg,or S protein, which is 24 kD in size, is the major envelope protein of the virus.S1 region, is believed to play a role in binding virus to a receptor on the hepatocyte surface. Within the envelope is a 27-nm structure known as the nucleocapsid core, which consists of 180 copies of the viral core protein, or hepatitis B core antigen (HBcAg),surrounding the viral DNA and the virally encoded polymerase. The *viral polymerase* functions as both a reverse transcriptase for synthesis of the negative DNA stand from genomic RNA and an endogenous *DNA polymerase*. The HBV polymerase, is encoded by the *P gene* of the virus, the function of the *X protein* appears to function as a transcriptional activator that influences the transcription of HBV genes by regulating the activity of transcriptional promoters.

Genomic Organization

Despite its small size (3.2 kb),HBV encodes four major proteins: the surface, core, and X proteins and the polymerase. This compactness is achieved by the use of overlapping ORFs so that more than one half of the nucleotides are used in a different frame for the transcription of different viral messenger RNAs (mRNAs). The precore region contains a signal sequence that directs the protein to the endoplasmic reticulum where host proteases cleave much of the C-terminus of the protein to

form HbeAg, which is subsequently excreted from the cell. These so-called “precore mutants” in humans are associated with active viral replication (readily detectable HBV DNA in serum), lack of HbeAg production, and progressive liver disease. In light of the cross-immunoreactivity between HBcAg and HbeAg, a possible function of HbeAg is to divert the immune response of the host away from virally infected hepatocytes that express HBeAg on their surfaces. HbeAg is also a marker of active viral replication. Production of high levels of HBeAg is indicative of synthesis of large amounts of full-length genomic mRNA, which in turn reflects active viral replication.

Replication

The life cycle of HBV includes the following steps.

1. Viral binding and entry
2. Viral uncoating in the cytoplasm
3. Synthesis of complete double-stranded DNA in the nucleus
4. Synthesis of genomic or pregenomic RNA and viral transcripts necessary for viral protein production
5. Translation of viral transcripts
6. Encapsidation
7. Reverse transcription and synthesis of DNA strands
8. Envelopment

In contrast to classical retroviruses such as HIV, integration of HBV DNA into the host genome is not necessary for viral RNA synthesis, and HBV transcripts are synthesized entirely from episomal DNA. The unique life cycle of this family of viruses affords opportunities for the development of antiviral agents.

Epidemiology

Incidence and Prevalence

Chronic hepatitis B is a common disease with an estimated global prevalence of over 300 million carriers, or approximately 5% of the world's population. In the Far East the Middle East, Africa, and parts of South America, the prevalence is high, with HBeAg positivity rates ranging from 8% to 15%. Regions of intermediate prevalence (2%-7%) include Japan, parts of South America, Eastern and Southern Europe, and parts of central Asia. Prevalence is lowest (<2%) in the United States and Canada, Northern Europe, Australia, and the southern part of South America.

Transmission

HBV is parenterally transmitted via blood or blood products or by sexual or perinatal exposure, the same routes as for HIV.

Perinatal and Early Childhood Transmission

Infants born to HBeAg-positive mothers who have high levels of viral replication (HBV DNA level>80 pg/mL) have a 70% to 90% risk of perinatal acquisition in the absence of interventions. The risk of mother-to-infant of transmission from HBeAg-negative mothers is substantially lower (10%-40%).

Sexual Transmission

Sexual activity is probably the single most important mode of HBV transmission in areas of the world such as North America, where the prevalence of infection is low.

Heterosexual sex, now accounts for the majority of cases of HBV infection (26%) with an identifiable risk factor in the United States. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk of HBV infection. Studies of sexual and household contacts of HBV carriers have shown that 0% to 3% of the spouses or sexual partners and 4% to 9% of the children are HBsAg positive.

Injection Drug Use

In the United States and Western Europe, injection drug use remains a very important mode of HBV transmission (23% of all cases)

Other Modes of Transmission

Other risk factors for HBV infection include working in a health-care setting, transfusions and dialysis, acupuncture, tattooing, travel abroad, and residence in a an institution.

Pathogenesis
Immune Pathogenesis

Clinical observations suggest that the immune response of the host is more important than viral factors in the pathogenesis of liver injury caused by HBV, Chronic HBV carriers who have normal liver enzyme levels and normal or near-normal liver histologic studies, despite high levels of viral replication. Infants with immature immune systems who acquire HBV infection at birth have a high rate of chronic infection and replication yet typically have only mild liver injury. Conversely, HBV induced fulminant hepatic failure is associated with a vigorous immune response, low serum levels of virus, and massive hepatocellular necrosis. It has been widely accepted that CTLs are responsible for destruction of virally infected hepatocytes and for viral clearance. However,, the number of CTLs involved is generally much fewer than the number (10^{11}) of virally infected hepatocytes. Thus, secondary non-antigen-specific immune responses, such as those mediated by inflammatory cytokines, may be more important for viral clearance than the CTL-mediated mechanism. Recent data point to the importance of tumor necrosis factor-α (TNF-α) and gamma-interferon as prime mediators of this non-antigen-specific clearance of HBV.

Chronic Infection

With chronic infection, HBsAg, HBeAg, and HBV DNA remain positive for 6 months or longer. After the acute phase of infection, serum ALT levels fall but often remain persistently abnormal (from 50 to 200 U/L). IgM anti-HBc titers typically fall to undetectable levels after 6 months IgG anti-HBc persists during the acute and chronic phases of disease. With time, there may be a spontaneous loss of HBV DNA and HBeAg, frequently in association with a flare of serum ALT levels and seroconversion to anti-HBe positivity. Spontaneous loss of HBeAg is rare. Anti-HBs may be detected simultaneously with HBsAg in serum in fewer than 10% of cases. The presence of anti-HBs is associated with immunity to HBV infection. Isolated anti-HBs is more likely to be acquired by vaccination than by natural infection, in which both anti-HBs and IgG anti-HBc are typically present. Fifty percent of patients with chronic HCV infection are anti-HBc positive .

Diagnosis

Serologic assays for the diagnosis of acute and chronic infection .HBV DNA quantification is generally performed by signal or target amplification tests: the liquid hybridization test uses a liquid phase to hybridize I-HBV DNA after the sample HBV DNA has been denatured. The lower detection limit of the assay is 1-2 pg/mL or $6x10^5$ copies/mL. The RNA -DNA hybrid assay has a sensitivity of 0.018 pg/ mL or 5 $x10^5$ copies/mL.The branched DNA assay the sensitivity limit is 7 x 10^5 DNA equivalents/mL. The polymerase chain

reaction (PCR) assay is based on the amplification of viral DNA, performed automatically in the Cobas analyzer after the manual extraction of viral DNA performed automatically in the (Cobas Amplicor HBV Monitor the sensitivity of the Cobas-Amplicor assay is between 100 and 400 copies/mL, and that for the Taqman method is as low as 10 copies/mL; both assays are more sensitive than the branched DNA and Genostics assays.

Complications

Patients with chronic HBV infection are at risk of developing long-term complications of portal hypertension and hepatic decompensation, such as variceal bleeding, ascites, and hepatorenal syndrome, as well as HCC. Patients with chronic liver disease, particularly those with established cirrhosis, are at increased risk of developing HCC.the risk of developing HCC is increased 10- to 390-fold in patients with chronic HBV infection compared with those who are HBsAg negative and is greater in those who acquired HBV infection perinatally than in those who acquired the infection as adults. Cirrhosis of the liver is present in more than 90% of patients with HCC related to HBV suggesting that the presence of cirrhosis is a risk factor for HCC development. Chronic inflammation associated with active viral replication, together with ongoing cellular proliferation and regeneration associated with cirrhosis, is likely a predisposing factor that leads to cellular transformation and frank malignancy.

Co infection with Other Viruses

Risk factors for acquisition of HBV infection are similar to those for acquisition of HIV infection. Both viruses have an increased prevalence in persons with multiple sexual partners and in injection drug users. In contrast, sexual transmission of HDV and HCV is relatively inefficient. Markers of prior or active HBV infection are present in more than 80% of patients with HIV infection, approximately 10% of whom are HBsAg positive. Conversely, HIV infection can be prevalent in patients with chronic HBV infection.

Pathology

As with other chronic viral hepatitides, HBV infection is associated with a predominantly lymphocytic infiltrate that may or may not be confined to the portal tracts. Characteristic of chronic HBV infection is the presence of ground-glass hepatocytes, in which the cytoplasm is stained pink with hematoxyline and eosin, reflecting the massive overproduction of HbsAg in these chronically infected cells/

Natural History

Chronic HBV infection is usually defined as detectable hepatitis B surface antigenemia for a period of six months or more. The risk of chronic infection is related to two major factors: the age at which infection is acquired and the immune state of the host. Detection of HBV DNA by sensitive molecular techniques. Infectious virions can clearly be detected in patients who

are HBsAg negative. The risk of chronicity after acute HBV infection is low in immunocompetent adults. The reported risk of chronic infection after acute exposure in adults ranges from less than 1% to 12%, but the consensus is that the risk of chronicity is less than 5%. The risk of chronic infection is greatly increased in patients who have a reduced ability to recognize and clear viral infection (e.g. patients on chronic hemodialysis, those on exogenous immunosupperssion following solid organ transplantation, and those receiving cancer chemotherapy).patients with concomitant HIV infection are also at significant risk of developing chronic infection (20%-30%)the risk of chronicity after neonatally acquired infection is extremely high (up to 90%). Presumably because neonates have an immature immune system. Cirrhosis develops in 15% to 20% of them within 5 years, even if histologic liver damage is initially mild. Many patients with chronic HBV infection have normal serum aminotransferase levels, normal or near-normal liver histology findings, and no symptoms. These “inactive carriers” appear to be immunologically tolerant of the virus, and their prognosis is excellent.

HEPATITIS C VIRUS

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis and HCC and a major indication for liver transplantation worldwide. The most striking feature of this virus is its ability to induce persistent infection is at least 85% of infected persons despite a vigorous humoral and cellular host immune response.

Virology

Structure

HCV consists of a positive-strand RNA surrounded by the core (nucleocapsid),which is surrounded by two envelope proteins (E1 and E2). The negative strand of HCV RNA , is produced as a replicative intermediate during replication and is acknowledged to be a marker of ongoing viral replication.

Genomic Organization

HCV is a single-stranded positive-sense RNA virus that belongs to the Flaviviridae family. The positive-strand RNA of HCV has three potential function:1)as a template for synthesis of negative-strand RNA during replication,2)as a template for translation of viral proteins, and 3) as genomic RNA to be packaged into new virions. In contrast to HBV,HCV has no DNA intermediate and therefore cannot integrate into the host genome.

Genotypes and Quasispecies

Like many RNA viruses HCV has an inherently high mutation rate that results in considerable heterogeneity throughout the genome. Genotype, which refers to genetically distinct groups of HCV isolates that have arisen during the evolution of the virus. First variant cloned by Choo and associates is designated type 1a.although some studies have reported an association between genotype 1 b and more severe liver disease, other studies have failed to find such an association. The second component of genetic heterogeneity is known as Quasispecies. Quasispecies are closely related yet heterogeneous sequences of the HCV genome within a single infected person that result from mutations during viral replication. The highest proportion of mutations has been found in the E1 and E2 regions at both the nucleotide (1.2%-3.4%) and the amino acid (1.4%-2.7%)levels particularly in the hyper variable region (HVR) at the amino terminal end of E2. The quasispecies nature of HCV may be one of the mechanisms by which the virus escapes immune responses

Epidemiology

Incidence and Prevalence

The worldwide seroprevalence of HCV infection, based on antibody to HCV (anti-HCV),is estimated to be 3%.

Transmission

The modes of transmission of HCV infection can be divided into percutaneous (blood transfusion and needlestick inoculation) and nonpercutaneous (sexual contact, perinatal exposure). The latter group may represent occult percutaneous exposure. Overall, blood transfusion from unscreened donors and injection drug use are the two best documented risk factors. In contrast to the high efficiency of perinatal transmission of HBV from mothers to infants, the efficiency of perinatal transmission of HCV is low with a risk estimated to range from 0% to 10% the prevalence of anti-HCV positivity in pregnant women ranges from 0.7% to 4.4%, Perinatal transmission occurs exclusively from mothers who are HCV RNA positive at the time of delivery. The risk posed to the infant from breast-feeding is believed to be negligible.

Clinical Manifestations and Diagnosis

Acute and Chronic Infection

HCV accounts for approximately 20% of cases of acute hepatitis. Acute infection is, however, rarely seen in clinical practice, because the vast majority of patients experience no clinical symptoms. HCV RNA appears in the blood within 2 weeks of exposure and is followed by an increase in serum aminotransferase levels several weeks later. HCV infection is self-limiting in only 15% of patients in whom HCV RNA in serum becomes undetectable and ALT levels return to normal. Approximately 85% of infected patients do not

clear the virus by 6 months, and chronic hepatitis develops. Of these the majority will have elevated or fluctuating serum ALT levels, whereas one third have persistently normal ALT values.

Diagnostic Tests for Hepatitis C
I. Serologic assays

1. Screening assays

   EIA-1

   EIA-2

   EIA-3

2. Confirmatory assays (RIBA)

II. Virologic assays

1. HCV RNA detection

   Qualitative

   Quantitative

   PCR –based methods

   BDNA assays

2. Genotyping
3. Analysis of HCV quasispecies

Diagnosis

Selection of Serologic and Virologic Tests

Initial diagnostic testing of HCV infection is currently made by detecting specific antibody by second or third- generation EIA tests for low-risk patients,

a negative EIA test result is sufficient to exclude HCV infection. For high risk, recently exposed, or immunocompromised patients (including HIV-infected, chronic hemodialysis, and transplant patients), further confirmatory testing is required. For years, confirmation was performed using RIBA testing. PCR testing has supplanted the RIBA test as the confirmatory test of choice.

Pathology

The range of histologic findings in patients with chronic HCV infection is broad, from minimal periportal lymphocytic inflammation to active hepatitis with bridging fibrosis, hepatocyte necrosis and frank cirrhosis. Steatosis, lymphoid aggregates, and bile duct damage are frequent in the liver biopsy specimens from patients with HCV infection, but there is considerable overlap with the histologicfindings in patients with chronic HBV infection and autoimmune hepatitis. The Histologic Activity index (HAI) developed by Knodell and associates is still used to quantity the degree or liver damage .A simplified system in which inflammation is graded from 0 t 4 and fibrosis is staged from 0 to 4 has been developed by Scheuer and colleagues. Recent consensus conferences have stated that a liver biopsy is mandatory in patients with chronic hepatitis C and elevated serum aminotransferase levels so that correct grading and staging can be performed. This information is particularly relevant when considering antiviral therapy or when other causes of liver disease may be present.

Natural History

Infection with HCV, once established, persists in the vast majority of patients. Disease progression is largely silent, and patients often are identified only on routine biochemical screening or blood donation.

Studies of Patients with End-Stage Disease
(Cross-Sectional Studies)

Evaluation of patients with established chronic hepatitis, cirrhosis, or non-HBC HCC with use of antibody assays provides evidence for an association between HCV infection and liver disease. Anti-HCV is detectable in 8% to 69% of patients with cryptogenic cirrhosis, with most studies finding rates of approximately 50%. Anti-HCV is also detectable in 6% to 76% of persons with HCC.

Prospective Studies

These studies provide unequivocal evidence of the progressive nature ofchronic HCV infection. Infections often can be sub clinical, and in symptomatic patients, fatigue is the most frequent complaint. Cirrhosis, defined histologically or clinically is present in 8% to 42% of patients. In approximately 10% of cases, decompensated disease was present, with splenomegaly, ascites, coagulopathy, and esophageal varices. If the data from all these studies are combined, we can conclude that when the entire population

of HCV-infected persons is observed, only a small percentage have serve outcomes during the first two decades of infection.

Factors Associated with Disease Progression

Age (older than 40 years),male gender, and increased alcohol intake (>50 g daily) have been documented as variables associated with disease progression. Immunosupperssion is clearly linked with more aggressive disease. Other less consistently documented prognostic factors include mode of transmission (higher disease progression when infection is through blood transfusion than through injection drug use) and coinfection with HBV.

ALCOHOLIC LIVER DISEASE

EPIDEMIOLOGY

Alcohol is one of the most openly available and generally consumed mood-altering substances. Patterns of intake vary among different geographic regions. Men who drink more than 80 g of ethanol per day are at substantial risk for development of clinical liver disease. Liver disease in women who drink excessively is two to four times more likely to develop than in men who drink excessively. The risk of liver disease begins at relatively low levels of alcohol consumption (30 g/day), this finding has led to a general recommendation that the maximal safe level of ethanol consumption is 20 g/day of ethanol, or two “drinks” per day, even among those who ingest large amounts of alcohol(more than 60 g /day),serious liver disease develops in only approximately 1 in 10. When disease occurs, it can take many form, ranging from steatosis, to alcoholic hepatitis, to hepatic fibrosis or cirrhosis, because disease severity does not correspond to classic dose dependency, other factors are likely to play an important role in pathogenesis. These factors may be hereditary, environmental, or both.

ETHANOL METABOLISM

Hepatic metabolism

The liver is the primary site of ethanol metabolism. Within the liver, ethanol can be oxidized by three enzyme systems: the alcohol dehydrogenases

(ADHs), cytochrome P-4502E1 (CYP2E1), and catalase. Both ADH and CYP2E1 convert ethanol to acetaldehyde. Acetaldehyde is then oxidized to acetate, primarily by a low- $K_{m.}$

Aldehyde dehydrogenase in hepatocyte mitochondria designated ALDH2. Acetaldehyde is a highly reactive and potentially toxic metabolite of ethanol. If the ability of the lover to remove acetaldehyde is decreased, acetaldehyde can accumulate in the liver and in the circulation. Acetaldehyde produces symptoms of flushing and tachycardia and can provoke circulatory collapse. Persons who are homozygous for the mutant enzyme experience severe side effects from acetaldehyde and thus rarely consume ethanol.

Gastric Metabolism:

Although the liver is the primary site of alcohol metabolism, ethanol can also be oxidized in the gastrointestinal tract. Gastric ADH has been implicated in a so-called gastric first-pass metabolism of ethanol; by oxidizing ethanol directly in the stomach, this enzyme may limit the amount of ingested ethanol that is delivered to the portal circulation.

Gastric ADH activity is lower in women than in men and can also be inhibited by certain drugs, such as aspirin and histamine H_2 receptor blockers.

PATHOGENESIS OF ALCOHOLIC LIVER INJURY
Toxic and Metabolic Mechanisms

Redox Alteration.ADH – mediated ethanol oxidation is accompanied by the reduction of oxidized nicotinamide-adenine dinucleotide(NAD^+) to reduced NAD (NADH) . Excess NADH shifts the redox state of hepatocytes, which in turn affects other NAD^+ -dependent processes, including lipid and carbohydrate metabolism. One consequence of the redox shift is hepatic steatosis.

NADH also interferes with gluconeogenesis. In patients who have underlying carbohydrate malnutrition can lead to profound hypoglycemia.

Chronic ethanol consumption, however, can prolong the redox shift by damaging hepatocyte mitochondria and preventing reoxidation of NADH to NAD^l.

OXIDANT STRESS:

Ethanol oxidation leads to formation of several free radical species in the liver, including the hydroxyethyl radical, the superoxide anion(O_2^-), and the hydroxyl radical ($OH^·$). These free radicals can inflict oxidative damage on a wide range of intracellular compounds. Radical formation has classically been considered a consequence of ethanol oxidation by CYP2E1.Recurited leukocytes can also contribute to alcohol-induced oxidant stress. Neutrophils, which figure prominently in alcoholic hepatitis, are stimulated by ethanol to produce superoxide radical.

The effects of radicals on the liver may be amplified if ethanol also reduces antioxidant defenses. Chronic alcohol consumption causes depletion of several antioxidants in the liver, including vitamins A and E and glutathione.S-adenosyl-methionine (SAMe), a glutathione precursor, can replete mitochondrial glutathione stores and correct some of the functional alterations.

HYPOXIA:

Another means by which ethanol may preferentially damage pericentral hepatocytes is through tissue hypoxia. The hypermetabolic state enhance the portal-to-central oxygen gradient, leaving pericentral hepatocytes in a state of relative hypoxia, Pericentral hypoxia has been documented in human alcoholics.

EFFECTS OF ACETALDEHYDE:

If acetaldehyde reaches a high enough concentration, it can become a substrate for the enzymes aldehyde oxidase and xanthine oxidase, which produce free radicals. Acetaldehyde impairs mitochondrial beta oxidation of fatty acids can also react with specific amino acid residues on cellular proteins to form acetaldehyde-protein adducts. The adducts localize preferentially to the pericentral zone, where liver injury is most pronounced. Aldehyde-protein adducts may contribute to alcoholic liver disease by forming neoantigens that stimulate immune responses or by promoting hepatic collagen synthesis. Acetaldehyde-induced impairment of protein secretion implicated as the major

event underlying hepatocellular swelling ("ballooning") in alcoholic liver disease.

IMMUNE AND INFL. MECHANISMS OF LIVER INJURY
KUPFFER CELL ACTIVATION AND CYTOKINE PRODUCTION:

Kupffer cells, which are the resident macrophages of the liver, produce oxidants and cytokines for the purpose of host defense. Chronic ethanol consumption causes abnormal activation of Kupffer cells. In this setting, the cytokines and oxidants elaborated by these cells can inflict damage on the liver itself.

Among the many compounds produced by activated Kupffer cells, tumor necrosis factor (TNF), transforming growth factor-β(TNF-β), and superoxide appear to be the most pertinent to alcoholic liver injury.

IMMUNE RESPONSES TO ALTERED HEPATOCELLULAR PROTEINS:

If hepatocellular proteins form adducts with either aldehyde or hydroxyethyl radicals, they can be altered sufficiently to provoked immune responses.

Mechanisms of Fibrosis

Liver fibrosis is a serious and potentially irreversible consequence of chronic ethanol use. Fibrosis occurs in only 10% to 15% of alcoholics but can be found in almost 50% of alcoholics who have evidence of liver disease. Central to the pathophysiologic mechanisms of alcoholic liver fibrosis is activation of hepatic stellate cells.Activated stellate cells are the principal

collagen producing cells of the liver. They are responsible for the perisinusoidal fibrosis that is characteristic of alcoholic liver disease.

COFACTORS IN THE DEVELOPMENT OF ALCOHOLIC LIVER DISEASE:

Heritable Factors

Studies have focused on polymorphisms in ADH, CYP2E1, and ALDH which together cause a wide range of ethanol elimination rates. Asians who inherit the slower allele, ADH2*, tend to consume more alochol[65] and experience more liver disease than those with ADH2*2. Unlike ADH, the CYP2E1 allele associated with liver disease is the one that encodes the more active enzyme.

For ALDH, the mutant allele ALDH2*2 has been implicated in the development of alcoholic liver disease. ALDH2*2 homozygotes have a strong aversion to ethanol caused by acetaldehyde toxicity. Among patients who have alcoholic liver disease, those with TNFA-A had twice the frequency of steatohepatitis of those without TNFA-A.

Gender

Women are more susceptible to serious alcoholic liver injury than men. They also exhibit a tendency toward disease progression even with abstinence. One theory implicates the reduced levels of gastric ADH in women as a causative factor. Accelerated alcoholic liver injury in women also may be related to gender-specific differences in fatty acid metabolism.

Diet and Nutrition.

In human beings, however, alcoholic liver injury appears to be influenced strongly by nutritional status.Both undernutrition and overnutrition have been implicated as risk factors in the development of alcoholic liver disease. Obesity is now well recognized as an independent risk factor for hepatic steatosis and steatohepatitis. When alcohol consumption is superimposed on obesity, the risk of liver disease rises almost six fold.

In addition to fat and calories, dietary iron can influence the development of liver disease in alcoholics. Chronic ethanol ingestion promotes absorption of iron from the intestine, and over time hepatic iron stores increase. It can contribute to liver disease by enhancing the oxidant stress.

Coexistent Viral Hepatitis.

18% to 25% of alcoholics are infected with the hepatitis C virus (HCV) . In alcoholics with liver disease, the frequence of HCV infection is even higher. The combination of alcohol and HCV infection significantly accelerates the progression of liver disease over that seen with either insult alone. This association may be related to the effects of alcohol on HCV replication or on the host immune response to the virus.

Like HCV, hepatitis B virus(HBV) accelerates the progression of alcoholic liver disease. Epidemiologic surveys indicate that HBV infection hastens mortality in alcoholics.

DIAGNOSIS:

Alcohol should be strongly suspected as a cause of liver disease in any patient who consumes more than 80 g/day of ethanol. Confounding factors such as gender or HCV infection warrant consideration of alcoholic liver disease even in individuals who consume two drinks of ethanol daily.

Laboratory Findings.

Atleast 75% of patients with alcoholic liver disease have a macrocytic anemia. Leukocytosis is also common. AST and ALT levels rarely exceed 300U/L and do not correlate well with disease severity.

The AST/ALT ratio often exceeds 2 in patients who have alcoholic liver disease. A ratio greater than or equal to 3 strongly suggests alcoholic liver disease.

The high AST/ALT ratio in alcoholics with liver disease has been attributed to pyridoxine deficiency, which cause a reduction in the AST and ALT content of hepatocytes, and disproportionate reduction in ALT content.

The serum bilirubin level and prothrombin time(PT) are useful predictors of the severity of liver disease in alcoholics. Maddrey and associates also used the bilirubin level and PT to generate a “discriminant function” that identifies patients who have a significant short-term mortality rate:

Disciminant function= 4.6x(PT in seconds – control in seconds) + bilirubin(mg/dL)

A discriminant function greater than 32 predicts a 1-month mortality rate of approximately 50%.

Histology

Liver biopsy is viewed by many authorities as the standard for diagnosing alcoholic liver injury useful for diagnosis and for prediction of prognosis. Among the most common histologic features of alcoholic liver disease are (1) steatosis ,(2)ballooning degeneration of hepatocytes, (3) presence of Mallory's bodies, (4)neutrophilic inflammation, and (5) pericellular fibrosis .

Steatosis is present in 60% to 95% of alcoholic liver disease patients. Macrovesiculur steatosis is the rule.

Fibrosis, which occurs in 50% to 75% of patients who have alcoholic liver injury, begins with deposition of connective tissue around the terminal hepatic venule and then extends into the hepatic parenchyma("chicken-wire" fibrosis).The cirrhosis that evolves is micronodular.

In 1991, Chedid and colleagues definded four histologic categories of alcoholic liver disease for the purpose of predicting survival (1) fatty liver (FL), (2) alcoholic hepatitis(AH), (3) cirrhosis, and (4) cirrhosis with alcoholic hepatitis(C+AH). The two main criteria used to stratify patients into the four categories were hepatic inflammation (absent in FL and present in AH) and cirrhosis (present in C and C+AH but not in FL or AH)

COMPLICATIONS

Ascites, gastrointestinal hemorrhage, and encephalopathy, hypoalbuminemia and hypoprothrombinemia.When alcoholic liver disease progresses to cirrhosis, hepatocellular carcinoma (HCC) may develop. Alcohol alone appears to be an independent risk factor for HCC, albeit a weaker one than viral hepatitis. Among alcoholics, men older than 50 years of age appear to be most vulnerable to development of HCC.

NONALCOHOLIC STEATOHEPATITIS:

Nonalcoholic steatohepatitis (NASH) is a form of chronic hepatitis with histological features of alcohol-included liver disease that occurs in persons who do not consume a significant amount of alcohol.

NASH is considered to be part of the spectrum of NAFLD. NAFLD is an uncommon disorder that occurs most often in middle-aged obese women. Obesity, hyperglycemia, and hyperlipidemia are commonly associated with NAFLD and are thought to be predisposing conditions, Other identified risk factors include total parenteral nutrition, protein-calorie malnutrition, jejunoileal (J-I) bypass, and use of certain drugs.

Ultrasonographic surveys of the general population demonstrate fatty liver in about 25% of adults in the United States. NAFLD is the most common explanation for an elevated serum alanine aminotransferase (ALT) level in blood donors. The prevalence of NAFLD in the general population parallels the prevalence of obesity and insulin resistance, the most common risk factors for

this type of liver disease. Progression to cirrhosis does occur. Advanced fibrosis or cirrhosis on liver biopsy specimens in up to two thirds of patients with NAFLD who were older than age 45 years and had type 2 diabetes, obesity or hypertriglyceridemia. Clinical manifestations of portal hypertension, hepatic failure, and hepatocellular carcinoma may develop in patients with NAFLD and significant hepatic fibrosis. In a 1999 series, liver disease was the second leading cause of health in patients with NAFLD.

ETIOLOGY

Divided into two broad categories: (1) drugs and toxins and (2) metabolic abnormalities either acquired or congenital. Most published series of patients with NAFLD emphasize a predisposition to steatohepatitis in middle-aged women who are obese or who have non-insulin-dependent diabetes mellitus or hyperlipidemia.

Several mechanisms have been proposed as causes of NASH, including amino acid imbalance, hyperglycemia (caused by diabetes mellitus or excessive administration of glucose), excessive circulating levels of anabolic (eg. insulin) relative to catabolic (eg..leptin) hormones, and endotranslocation). Each of these processes can shift metabolism to favor net lipogenesis rather than lipolysis, because hepatitis and cirrhosis are much less frequent than steatosis can provoke histologic progression to cirrhosis or that an additional insulates necessary to produce this outcome.

Similarities in the histologic features and natural histories of alcoholic liver disease and NAFLD suggest that common mechanism may be involved in the pathogenesis of these two disorders that chronic oxidative stress also may be involved in the pathogenesis of NAFLD.

Inpatients with NAFLD, the content of ethanol in the breath increases progressively with body mass index and is independent of serum liver enzyme levels or histological evidence of fibrosis. These findings suggest that endogenous ethanol might promote the earliest stage of obesity-related NAFLD (i.e., steatosis).

Serum and adipose tissue levels of TNF-α are increased in obesity, steatohepatitis occurs commonly in obese patients who have undergone j-1 bypass surgery and in patients receiving long-term total parental nutrition (TPN);

Most patients with NAFLD are asymptomatic. NAFLD is often diagnosed after serum liver biochemical abnormalities are noted during routine laboratory testing. Hepatomegaly has been noted in up to three fourths of patients in several studies. Stigmata of portal hypertension appear to occur less frequently, although splenomegaly was noted at the time of diagnosis in about 25% of the patients in one series.

Laboratory Findings

The suspicion of NAFLD is usually prompted by abnormal serum liver biochemical findings. Increased serum alanine aminotransferase(ALT) and

aspartate aminotransferase(AST) activity is the predominant laboratory abnormality. Usually the levels of ALT, AST, or both, are mildly to moderately increased, and the serum levels of these enzymes cannot be used to predict the histologic severity of hepatic inflammation or fibrosis. Patients with NAFLD usually have an AST/ALT ratio of less than 1.Increases in serum alkaline phosphatase and gamma-glutamyl transpeptidase (GGTP) levels are not uncommon in patients with NAFLD. Hyperbilirubinemia, prolongation of the prothrombin time and hypoalbuminemia are noted infrequently in most series.

Abnormal serum lipid profiles and elevated serum glucose concentrations are also common in patients with NAFLD and have been reported in 25% to 75% of cases.

A diagnosis of NAFLD can be established only in patients who do not consume significant amounts of alcohol. Incidence of alcohol-induced liver disease begins to increase only after certain “threshold” levels of alcohol consumption (i.e.20 g/day of ethanol in women and 80 g/day of ethanol in men) are exceeded habitually.

There is no perfect test to identify alcohol use, particularly in the context of underlying liver disease. The ratio of dTf to total Tf appears to be the best single marker of chronic excessive alcohol consumption in such patients. A dTf/Tf ratio of 1.3% or greater was a reliable indicator of excessive chronic alcohol consumption.

Testing to exclude viral hepatitis has become a prerequisite for the diagnosis of NAFLD. Although HCV infection does not cause NAFLD,

NAFLD may increase the severity of HCV-related liver damage. Regression analysis has identified hepatic steatosis as an independent predictor of cirrhosis in patients with chronic hepatitis. Obesity-related steatosis has the same ominous prognostic implications as alcohol-induced steatosis in patients infected with HCV.

Noninvasive imaging techniques, including ultra-sonography, computed tomography, and magnetic resonance imaging (MRI), can identify hepatic steatosis. Of these, phase contrast MRI appears to be the most promising, because its results correlate well with the degree of histologic steatosis. None of these imaging techniques is sufficiently sensitive to detect hepatic inflammation, fibrosis, or cirrhosis. Therefore liver biopsy remains the best diagnostic test for confirming the clinical suspicion of NAFLD and staging the severity of liver injury and fibrosis.

A 1999 study from the Mayo Clinic suggested that older age, obesity, diabetes mellitus, and a serum AST/ALT ratio greater than 1 can help identify a subset of patients with NAFLD who are most likely to have severe fibrosis indicated on liver biopsy.

HISTOLOGIC FEATURES

The major histologic features of NAFLD resemble those of alcohol-induced liver disease and include steatosis (fatty liver), steatohepatitis (fatty liver plus parenchymal inflammation with or without accompanying focal necrosis), and variable degrees of fibrosis (including cirrhosis). As in alcoholic

liver disease, steatosis in NAFLD is predominantly macrovesicular and generally distributed diffusely throughout the liver lobule. As in patients with alcohol-induced liver disease, fibrosis can progress to cirrhosis in NAFLD. In both, steatosis is the principal early finding, but episodes of steatohepatitis can punctuate the course, and eventually cirrhosis develops in some affected persons.

Role of Liver Biopsy

The combination of the history, physical examination, noninvasive blood tests, and imaging studies is useful for excluding other diseases as an explanation for abnormal liver enzyme levels. Liver biopsy is seldom necessary simply to diagnose NAFLD, which is currently a diagnosis that is made by excluding other causes of chronic hepatitis in patients with fatty liver. Liver biopsy is the most sensitive and specific means to stage patients with NAFLD.

PROGNOSIS

Emerging evidence suggests that clinically significant liver disease is probably no more rare in NAFLD than in chronic hepatitis C or other types of chronic hepatitis. Evidence from Caldwell and colleagues in 1999 suggests that NAFLD is likely to have been the underlying liver disease for most patients with cryptogenic cirrhosis.NAFLD may be as important a cause of cirrhosis in the United States as alcohol or hepatitis C infection.

Study on the presentations of chronic liver disease in correlation to their etiologies in inpatients in Government General Hospital, Chennai.

AIM

To study about the various clinical, biochemical and pathological presentations of chronic liver disease and to estimate their relative frequencies.

1. To estimate the contribution of hepatitis virus B & C in causing chronic liver disease in an urban setup
2. To find out the relative prevalence of NASH in patients diagnosed to have chronic liver disease (CLD)

OBJECTIVE

The study was conducted with following specific objectives in mind.

1. What is the relative frequency of clinical, biochemical, pathological abnormalities found in chronic liver disease of various etiologies
2. What is the prevalence rate of HBV and HCV in patients with CLD
3. Should NASH be considered to be an emerging risk for CLD in urban population ?

PATIENTS & METHODOLOGY

The study was conducted in Government General Hospital, Chennai, during the period September 2003 to August 2004.

100 consecutive adult patients admitted to the male and female wards of Medical Gastroenterology ward diagnosed to have CLD (by criteria given below) were included in the study.

INCLUSION CRITERIA

1. Symptoms of parenchymal liver disease for more than 6 months in the form of continuous or fluctuating jaundice, unexplained asthenia, poly arthralgia, fever, anorexia, pruritus in association with significant elevation of transaminases or histopathological evidence of chronic hepatitis
2. High SAAG ascites with evidence of portal hypertension by clinical, and ultra sound examination complemented by Doppler whenever required
3. Evidence of portal hypertension with histopathological evidence of cirrhosis or chronic active hepatitis
4. Persistent elevation of transaminases in patients symptomatic over a period of 6 months

Portal hypertension was deemed to exist in the presence of esophageal or gastric varices during GI endoscopy, anterior abdominal wall veins with flow away from the umbilicus, ultra sound evidence of collateral venous circulation and portal vein diameter of > 1.1 cm with or without splenomegaly.

EXCLUSION CRITERIA

1. Patients diagnosed as acute viral hepatitis, resolving within 6 months
2. Liver secondaries with known or unknown primaries.
3. Obstructive jaundice as evidenced by ultrasound, or ERCP

4. Cases diagnosed to have Budd-Chiari syndrome, VOD, EHPVO or non cirrhotic portal hypertension.
5. Portal hypertension without ascites where liver biopsy could not be done
6. Cases where EHPVO or NCPF could not ruled out with certain after exhaustive testing

Thus 100 consecutive cases satisfying the above criteria were included in the study comprised of males and females. The youngest was aged 15 years and the oldest being 65 years. All patients hailed from Chennai city or its suburbs within 75 km radius.

A careful and complete history, as well as clinical examination as per proforma was performed. Following investigations were done for all patients.

Complete blood counts, Bleeding time, Clotting time, Prothrombin time, Urinalysis, Stool examination for parasite and occult blood, Blood Sugar, Urea, Electrolytes, Creatinine, Serum Bilirubin SGOT, SGPT, Alkaline Phosphate, Total protein, Albumin, Globulin, Ascitic fluid protein, albumin, SAAG, amylase, cell count and cytology, Chest X ray, USG abdomen, Doppler study of portal venous system (in selected cases), UGI endoscopy, liver biopsy (in selected cases).

Samples were sent to Department of Microbiology, Madras Medical College and to Dr.A.L.Mudhaliar Post Graduate Institute of Basic Medical Sciences, Taramani, Chennai for testing viral markings for HBV and HCV.

Liver biopsies were done using biopsy gun under ultrasound guidance with standard precaution after obtained informed consent. Liver biopsy could not be done in some patients due to massive ascites, coagulopathy, HE or other moribund state. The histopathology examination and reporting were done by Institute of Pathology, Madras Medical College.

Amount of ethanol intake, frequency and type were noted and so also about other drug usage.

In selected patients where Wilson's disease was suspected slit lamp examination for KF ring and serum Ceruloplasmin level were estimated. Alfa protein levels were estimated in patients when imaging studies have shown presence of nodule or clinical suspicion of HCC was strong.

DEFINITIONS

Non alcoholic fatty liver disease (NASH) is defined as a form of chronic hepatitis with histological features of alcohol induced liver disease who do not consume significant amount of alcohol.

Obesity is diagnosed when BMI (Body mass index) is more than 30

Diabetes mellitus is diagnosed when fasting blood sugar exceeds 126 mg/ dl or when post-prandial blood sugar level exceeds 180 mg/dl

Hepatitis B was diagnosed when HbsAg found positive. HbeAg and anti HbeAg were done in selected cases.

Hepatitis C infection was diagnosed with positive anti- HCV antibodies and abnormal LFT. HCV RNA was done in selected cases

(ELISA, Zhongshan Biotech Co. China)

HCC (Hepatocellular Carcinoma) was diagnosed with USG/ CT findings and elevation of Alfa feto protein levels. Liver biopsy was done in some patients whenever feasible.

Wilson's disease was diagnosed by positive KF ring in cornea and low serum ceruloplasmin levels.

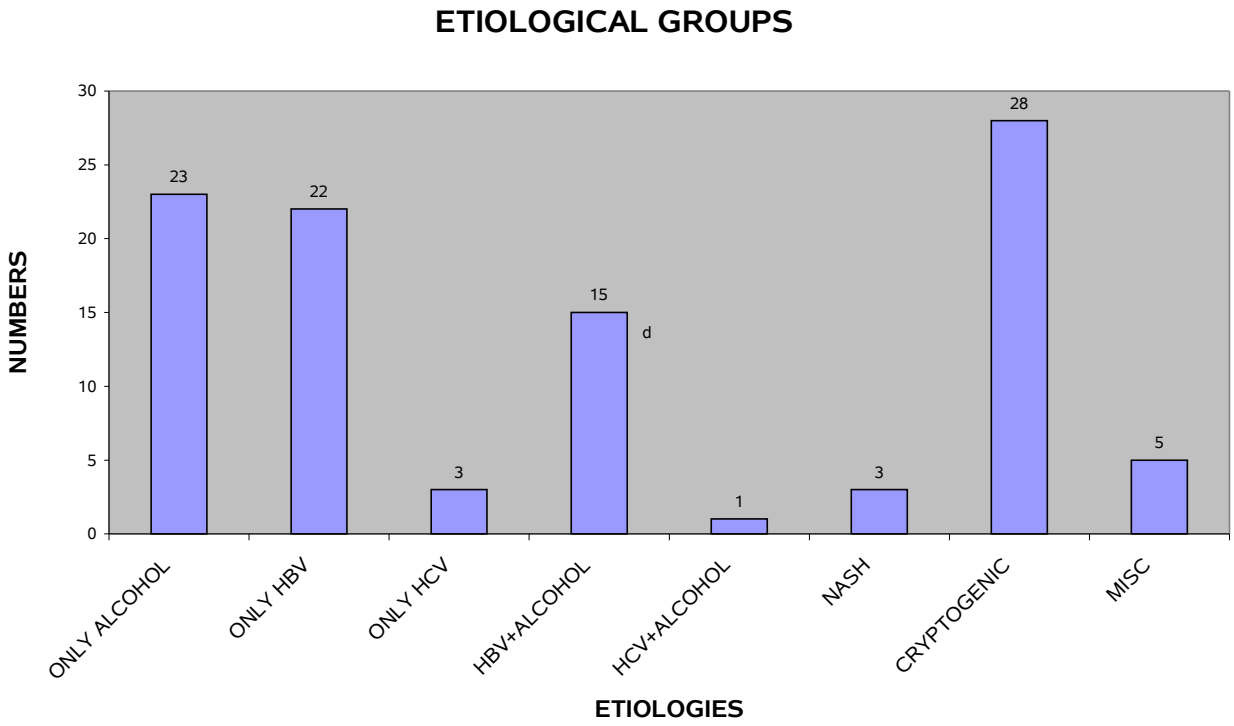
RESULTS

The 100 patients of chronic liver disease could be classified on clinicopathological as well on etiological basis. On clinic pathological grounds the patients could be divided as follows:

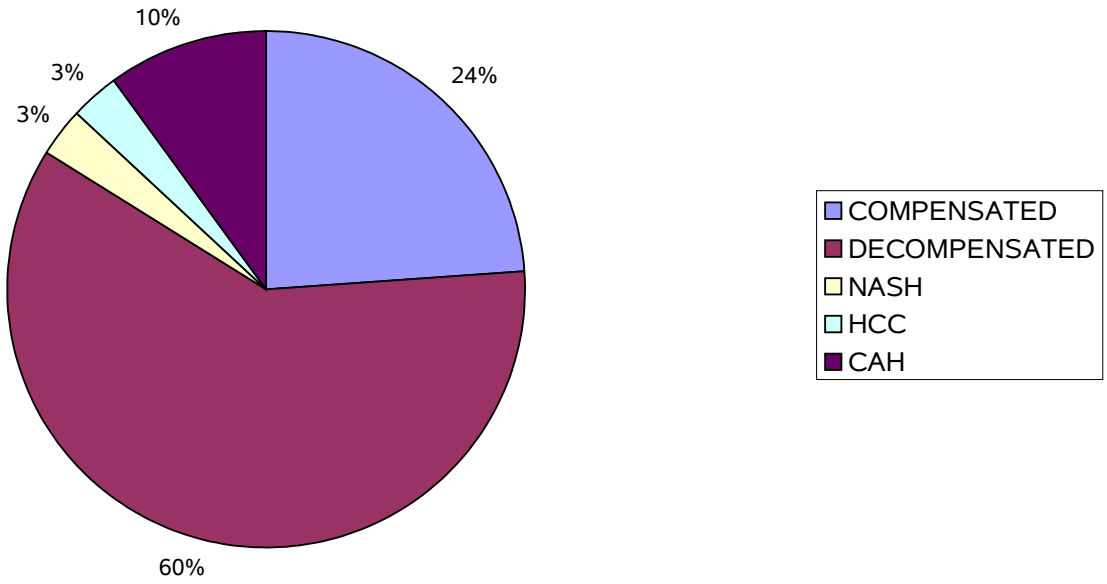
Decompensated cirrhosis	-	60
Compensated cirrhosis	-	24
NASH	-	3
HCC	-	3
CAH	-	10
Total	-	100

Based on the evaluation of viral markers, alcoholic intake, previous blood transfusion and copper studies in a few cases, the patients could be grouped into several categories as follows:

Group A	:	Positive for only alcohol	-	23
Group B	:	Positive for only HBV	-	22
Group C	:	Positive for only HCV	-	3
Group D	:	Postive for both HBV & alcohol	-	15
Group E	:	Positive for both HCV & alcohol	-	1
Group F	:	NASH	-	3
Group G	:	Cryptogenic	-	28
Group H	:	Miscellaneous - Wilson's Disease	-	5
		Total		100


ETIOLOGICAL GROUPS
NUMBERS
30
25
20
15
10
5
0
23
22
3
15
d
1
3
28
5
ONLY ALCOHOL
ONLY HBV
ONLY HCV
HBV+ALCOHOL
HCV+ALCOHOL
NASH
CRYPTOGENIC
MISC
ETIOLOGIES

CLINICOPATHOLOGICAL GROUPS



Each etiological group could be split up into the clinicopathological groups as already mentioned.

On comparing the variables, in the age group of 15-35 yrs, alcohol was the only etiology detected, where as both HBsAg and anti HCV antibodies were not found. In the age group of 36 – 60years alcohol was the only aetiology in 15 patients, both alcohol and HBV were present in 15 patients and both alcohol and HCV were present in 1 patient. In the age group of > 60years, alcohol was the only etiology in 3 patients and again in this age group, there was no viral markers detected.

When comparing alcohol abuse and sex, alcohol alone had been detected as etiological factor in 21 male patients and 2 females and combined HBV & alcohol was present in 13 male patients and 2 female patients. Alcohol and HCV was detected as the etiological factor in 1 male patient.

Regarding distribution of various etiological factors in the 3 age groups, HBV alone was responsible in 5, 16 and 1 in the age groups of 15-35, 36-60 and >60 years respectively. Both HBV and alcohol was more common in the age group of 36 – 60years, cryptogenic cirrhosis was more common in the age group of 36-60 years, where it is present in 17 patients. Cryptogenic cirrhosis was present in 3 and 8 patients respectively in the age group of 15-35 years and >60 years respectively. NASH was common in the age group of 36 – 60 yrs. All the 3 cases in this study belonged to that age group. Regarding Wilson's

disease , all the 5 patients belonged to the age group of 15 – 35 years. All HCV related cirrhosis belonged to the age group of 36 – 60 years.

Regarding the sex and etiological factors, cryptogenic cirrhosis was present in 20 female and 8 male patients. Wilson's disease was present as aetiology in 2 females and 3 male patients. All the three NASH patients were females. All HCV related cirrhosis were found in male patients. All HCV related CLD were found in the age group of 36 – 60 years.

Regarding NASH, all the 3 patients belonged to the age group of 36 – 60 years. All the 3 NASH patients were females.

In the characterization of Wilson's disease by age and sex, it was all found in the age group of 15 – 35 yrs, and 3 patients were male and 2 patients were female.

Among the 3 cases of HCC recorded alcohol abuse was present in one patient only, even that was along with HBV. In HCC, HBV was present in all the 3 cases. In 2 patients HBV alone was present as the etiological factor and in 1 patient it was present along with alcohol**.**

In NASH, all the 3 patients were obese. Hypertriglyceredemia was present in 2 out of 3 patients and diabetes was present in one patient.

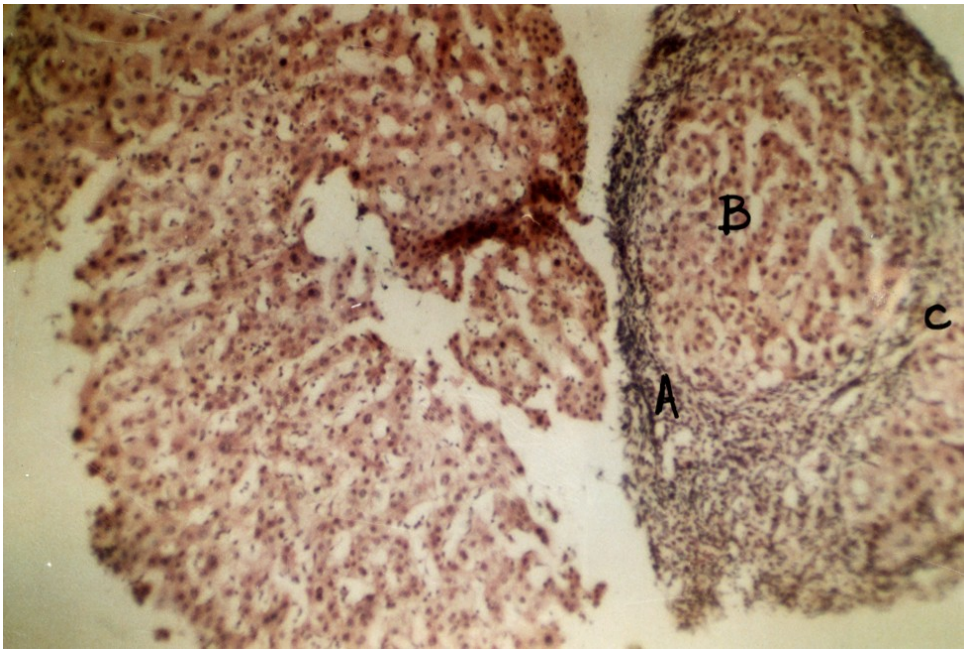
In HCV related CLD, thrombocytopenia was present in all the 4 cases.

In cryptogenic cirrhosis, Hypertriglyceredemia was found in 16 out of 28 patients and diabetes was found in 13 out of 28 patients. Promiscuity was found in 3 patients of cryptogenic cirrhosis. 15 out of 28 patients with cryptogenic cirrhosis were obese.

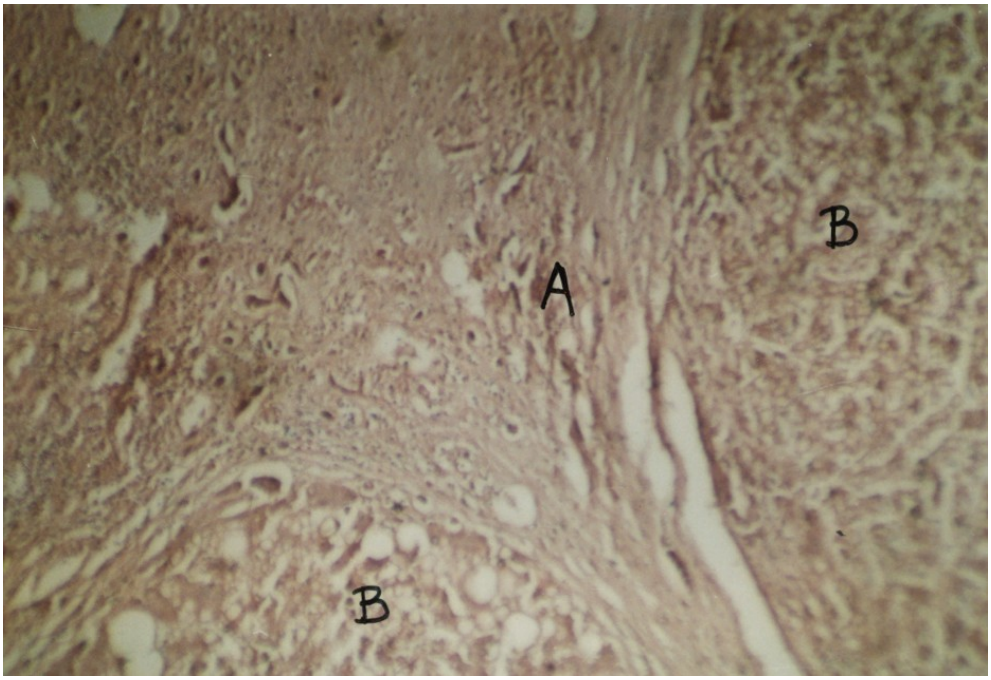
13 patients received blood transfusions in the past. The transfusions were mostly for UGI bleeding, post surgery or unknown indications. It was not possible to ascertain whether blood transfused came from related donors or professional donors. All the 13 had some identifiable etiological factor to explain the CLD.

Histopathology

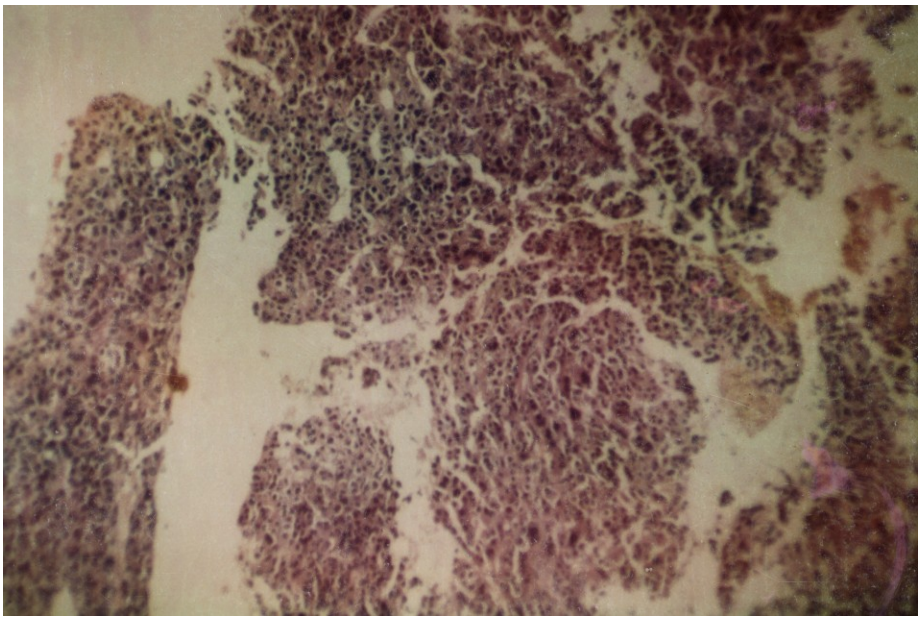
Liver biopsy was performed in 49 patients. Satisfactory results were available in 41 patients. The rest being reported as “inadequate tissue”. The histopathology reports were HCC in 3, chronic active hepatitis with (or) without fatty change in 13 and varying grades of cirrhosis with (or) without fatty change in the rest.



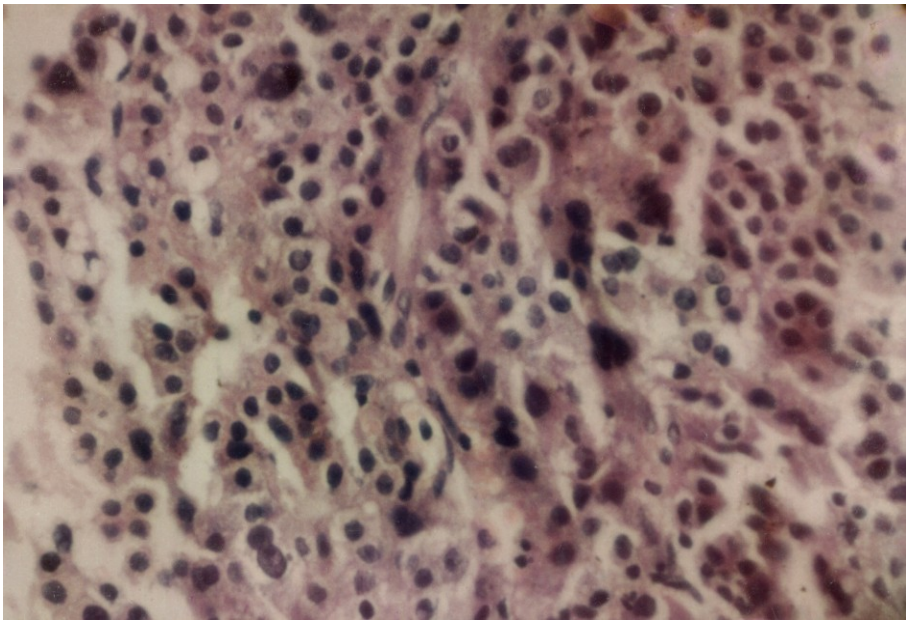
CIRRHOSIS WITH ACTIVITY – Showing inflammatory cell activity (A) in addition to regenerating nodule (B) and minimal fibrosis (C)



CIRRHOSIS – Showing periportal fibrosis (A) and regenerating nodules (B)



HEPATOCELLULAR CARCINOMA – Showing malignant hepatocytes in a sinusoidal arrangement



HEPATOCELLULAR CARCINOMA – High Power View

Table – 1 : Age distribution among various study groups

		ALCOHOLIC				
		ALCOHOL ONLY	NON ALCOHOLIC	ALCOHOL +HBV	ALCOHOL + HCV	Total
AGE	15-35	5	11	0	0	16
	36-60	15	41	15	1	72
	>60	3	9	0	0	12
Total		23	61	15	1	100

Table – 2 : Prevalence of CLD due to HCV in various age groups

		AGE			
		15-35	36-60	>60	Total
HCV	+ve	0	4	0	4
	-ve	16	68	12	96
Total		16	72	12	100

Table – 3 : Distribution of NASH in different age groups

		AGE			
		15-35	36-60	>60	Total
NASH	Present	0	3	0	3
	Absent	16	69	12	97
Total		16	72	12	100

Table – 4 : Distribution of NASH in both sexes

		SEX		
		F	M	Total
NASH	Present	3	0	3
	Absent	29	68	97
Total		32	68	100

Table – 5 : Obesity and NASH

		BMI		
		obese	nonobese	Total
NASH	Present	3	0	3
	Absent	7	90	97
Total		10	90	100

Table - 6 : NASH and Hypertriglyceridemia

		HYPER TRIGLY		
		Present	Absent	Total
NASH	Present	2	1	3
	Absent	15	82	97
Total		17	83	100

Table – 7 : NASH and Diabetes

		DIABETES		
		Present	Absent	Total
NASH	Present	1	2	3
	Absent	16	81	97
Total		17	83	100

Table – 8 : Hypertryglyceridemia in Cryptogenic cirrhosis

		HYPER TRIGLY		
		Present	Absent	Total
CRYPTOGENIC	Present	16	12	28
	Absent	14	58	72
Total		30	70	100

Table – 9 : Cryptogenic cirrhosis and Diabetes

		DIABETES		
		Present	Absent	Total
CRYPTOGENIC	Present	13	15	28
	Absent	17	55	72
Total		30	70	100

Table – 10 : Prevalence of obesity in Cryptogenic cirrhosis

		BMI		
		Obese	Nonobese	Total
CRYPTOGENIC	Present	15	13	28
	Absent	8	64	72
Total		23	77	100

Table – 11 : Cryptogenic cirrhosis in various age groups

		AGE			
		15-35	36-60	>60	Total
CRYPTOGENIC	Present	3	17	8	28
	Absent	13	55	4	72
Total		16	72	12	100

Table – 12 : Cryptogenic cirrhosis in both sexes

		SEX		
		F	M	Total
CRYPTOGENIC	Present	20	8	28
	Absent	12	60	72
Total		32	68	100

DISCUSSION

As set out under objectives of the study, the data collected from 100 patients was analysed with respect to the presence and distribution of various virological markers, clinicopathological features and other etiological factors.

From the results, it is seen that 39 out of 100 were positive for significant alcohol consumption, 37 had markers for HBV, 4 of them were positive for anti HCV antibodies and 31 were negative for alcohol, HBV, HCV and blood transfusion.

Alcoholic Liver Disease and Hepatitis B & C

39 out of the 100 patients admitted using significant amounts of alcohol over a period of not less than 10 years. Only in 23 patients, alcohol was the sole recognizable etiological factor and these patients could be safety labeled as having alcohol induced chronic liver disease.

In the remaining 16 patients, there were additional etiological markers noted .15 patients had HBV and 9 patients had received blood transfusions in the past. Among the 9 patients with H/O Blood transfusion, alcohol was the sole etiological factor in 3 patients and HBV was the additional etiological factor in the remaining 6 patients. The question that arises in this group of 16 patients having multiple etiological factors, is the relative contribution of each of them in the production and continuation of CLD. Even if we assume that in 6 patients out of these 16 patients, the presence of hepatitis B viral markers is

incidental due to blood transfusion, in the remaining 9 patients there is no past h/o blood transfusion.

While there is no controversy in the role of alcohol in inducing acute alcoholic hepatitis and fatty liver, there is scope for discussion with reference to CLD and alcohol, especially HCC. The observation of patients developing chronic sequalae despite total abstinence from alcohol at an early stage, though attributed to immune mediated mechanisms, is not fully explained. Ever since viral markers become available, investigators started probing the probable relationship between HBV and alcohol and recently HCV and alcohol (11,12,13). The question of which is incidental (or) co pathogen is not yet resolved. While there are several papers devoted to this subject, the paper by Mendenhall et al (14) based on a multicenter VA cooperative study is worth reviewing. 350 patients with CLD were analyzed for the presence of HBV and HCV markers and compared with two other groups namely 1) no alcoholism nor liver disease 2) alcoholism only. While HBV prevalence was similar in all the three groups, HCV prevalence was significantly higher in those with liver disease. The study of clinical characters like age, alcohol intake, bilirubin, transaminase, alkaline phosphatase, prothrombin time did not show any difference between HBV positive and HBV negative groups. On the other hand mean bilirubin values and prothrombin time showed significant elevation in HCV positive patients in comparison with HCV negative. Histological comparisons were done using twelve criteria (six for alcoholic hepatitis and six for chronic viral hepatitis). Ito cell activity ,cirrhosis and all features of viral

hepatitis were significantly more marked in HCV positive cases. They conclude that HCV and HBV markers in alcoholic liver disease have different clinical significance. Anti HCV antibodies correlate with clinical severity and histopathological changes, while HBV antibodies do not. HCV infection has an adverse effect on long term survival.

In our study, 23 out of 39 patients with alcohol consumption did not have any viral markers. Gross comparison of histopathological appearance of these two groups i.e. (HBV positive, HBV negative ALD) did not reveal any notable differences in features, hence it appears that alcohol is a common etiological factor in CLD. In many cases coexistence of hepatitis B was noted and in a small percentage of cases blood transfusion and hepatitis C were associated. With regard to the presence of Hepatitis B markers it looks as though the clinical picture and outcome do not differ much in the two groups. The number of patients positive for HCV is too small to draw any conclusions as to how alcoholic liver disease will be modified by coexisting HCV.

Regarding virological markers in ALD, serological HBsAg may be absent and serum Hepatitis B virus DNA testing may be necessary to diagnose hepatitis B infection (15). Positive second generation ELISA tests usually correlate with a positive hepatitis C virus RNA and allow diagnosis of complicating Hepatitis C disease (16). Alcohol aggravates liver damage due to hepatitis C virus (16). Viremia increases and the natural history is adversely affected (17,18). The relative risk of developing cirrhosis is increased. In our study, we have not performed HBV DNA in alcohol positive and HBsAg negative patients. Performing HBV DNA in this group may have yielded more HBV positive patients with alcoholic etiological background. Due to lack of resources we did not perform HBV DNA which is the limitation of this study.

CLD and Hepatitis C:

One of the main aims of this study is to estimate the prevalence of hepatitis C virus infection in patients with CLD in general and alcohol related CLD in particular. HCV accounts for 20% of acute cases, 70% of cases of chronic hepatitis, 40% cases of end stage cirrhosis, 60% of cases of hepatocellalar carcinoma and 30% liver transplants. Only 4 patients out of 100 had HCV antibodies implying previous infection with HCV. All cases due to HCV were found in the age group of 35-60 yeas. All the 4 of them were male. one patient (case no. 25) had also h/o alcohol consumption. None of the patients had concomitant HBV infection. H/o blood transfusion in the past was present in none of the 4 patients. All the 4 patients presented with decompensated liver disease. None of them presented with HCC. According to Dinis - Ribeiro M et al, Older patients, with a shorter time interval between HCV infection and diagnosis, and namely those with markers of HBV infection represent patients with higher risk for progression to hepatic cirrhosis (19). In our study, the patients with HCV related CLD all belonged to the age group of 35-60 years with mean age of 51 years. Our patients were relatively elderly, when compared to HBV related and alcohol related CLD. According to Fattorich G et al, patients with HBV infection may present with cirrhosis about 10 years earlier than those with HCV infection. HCV infection also tends to be associated with a higher risk of decompensation(20). In our study, HBV alone

is the etiological factor in 5,16 and 1 patients respectively in the age group of 15-35, 36-60 and > 60 years.

All the 15 patients with both alcohol and HBV as the etiological factor belonged to the 36-60 years age group. The mean age of the patients with HBV infection was 39 years which is again less than that of those with HCV related CLD. Among the 37 patients with HBV. Only 8 of them belonged to compensated liver disease. All of the 15 patients with alcohol as the additional etiological factor belonged to decompensated liver disease. Caldwell et al reported 30% incidence of anti HCV antibody in ALD, compared to 2% in healthy controls and other studies have recorded 25-52% incidence (14). In our study only 1 patient out of 39 with ALD had anti HCV positivity (2.6%) which is far lower than the figures from American and Western European studies.

In two Indian studies, Prof B.N. Tandon and colleagues (13) have reported about 45% incidence of HCV in CLD and Amarpurkar et al from Bombay have shown 17% incidence in their cases (12).

Severity of the liver disease:

All patients with cirrhosis were classified into child's criteria into child A,B and C categories. The main etiological groups (Alcohol only, HBV only, HCV only, HBV + Alcohol, HCV + alcohol) were further divided according to child's grading. No statistically significant difference could be made out from these figures. In other words, alcohol and hepatitis B, whether acting singly or in combination do not by themselves have any mitigating or aggravating effect on the clinical status. If the incidence of hepatitis C was higher,certainly the picture would have been different because long standing HCV infection is known to produce more severe liver disease, especially in ALD as per the studies discussed earlier.

HCC and Viral markers:

The role of hepatitis B in HCC is well established since long time. The presence of HBV markers in HCC parallels the prevalence of HBV in the general population. In countries like Taiwan, Hong Kong, Uganda, about 80-90% of HCC are related to HBV. In Japan, when anti HCV was estimated, 70% of Japanese with HCC were anti HCV positive according to Nishioka (quoted in 14). While similar high prevalence was reported in the Western Europe (11), some what lower figures are reported in United States (30-54%) and Africa (34-54%). In China, Tsai JF et al suggested that by multivariate analysis, both HBsHg and anti HCV were important and independent risk factors for HCC (odds ratio, 6.52 and 4.59 respectively) (10). Asian race and patient age <50 years were found to be independent predictors for HBs Ag positivity, while a history of blood transfusion was the only predictor for anti HCV positivity in HCC patients. Chronic HBV infection was the major etiological factor in Asian - American HCC patients, while chronic HCV infection and alcoholism were major etiological factors in Caucasian HCC patients in the USA. (9). In our study, 3 patients were found to have HCC. 2 patients had HBV alone as the etiological factor and1 patient had both HBV and alcohol as the etiological factor. None of 3 patients (patient No: 19,37 and 57) had markers for HCV. None of them had blood transfusions.All the 3 had been detected in patients with cirrhotic background. Since IV drug abuse has been suggested as the main mode of transmission, this may account for low prevalence of anti HCV in HCC patients. It also reflects the low prevalence of HCV infection in our study

on CLD. Although anti HCV is some what less sensitive than HCV RNA assay, HCV RNA assay in 16 patients with features of steatohepatitis in our study did not detect more number of cases attributable to HCV.

Non alcoholic steato hepatitis (NASH)

One of the main aim of this study is to evaluate the contribution of NASH to CLD. Obesity, diabetes and hypertriglyceredemia are the main risk factors associated with NASH. The prevalence of NASH in Thai patients with non HBV, non HCV chronic hepatitis was 76.1%, while the liver biopsy can add the diagnostic yield especially in the group of unexplained chronic hepatitis with obesity, diabetes mellitus and dyslipidemia (8).

In our study, all the 3 NASH patients were female in the age group of 36-60 years. Among them, all of them were obese with BMI of > 30. Two out of 3 patients had hypertriglyceredemia and 1 patient had diabetes. HCV RNA assay was done in 16 patients including who were diagnosed as NASH, who demonstrated variable degrees of steatosis and steatohepatitis on USG and elevated liver enzymes and HPE. All the 16 cases were HCV RNA negative. The same risk factors like obesity etc are associated with advanced hepatic fibrosis in patients with chronic hepatitis C. (5,6,7). All the patients had clinical jaundice and hepatomegaly. None of them had varices on endoscopic examination. In our study, NASH contributed to only 3% cases of chronic liver disease. We do not know, in how many patients in cryptogenic cirrhosis group it contributed to the progression to cirrhosis since the some of the patients with

cryptogenic cirrhosis, the same risk factors like obesity, diabetes mellitus, and female gender are also present.

Cryptogenic cirrhosis:

The aetiology is unknown and this represents a heterogeneous group. Cirrhosis can be difficult to ascribe to NASH if histologic features have been lost (or) obscured by cirrhotic nodules. Such nodules have been shown occasionally to have focal fatty changes (3). Serial biopsy studies have established the progression of NASH to a stage of "bland" cirrhosis. The loss of fatty infiltration may be the result of altered blood flow or decreased sinusoidal permeability and lipoprotein delivery as the liver becomes fibrotic. Results of epidemiologic and familial studies have strongly suggested that many cases of "cryptogenic" cirrhosis are the result of such a process (2). The significantly increased frequency of steatosis and steatohepatitis after transplantation for cryptogenic cirrhosis further supports this relation.

Frequency varies in different parts of the World. In the UK, it is about 5-10%, whereas in other areas like France and urban USA, the proportion is lower. The advent of testing for hepatitis B and C transferred many previously designated cryptogenic cirrhosis to the post hepatitic group. Estimation of serum smooth muscle and mitochondrial antibodies and better interpretation of liver histology separate others into the autoimmune chronic hepatitis - PBC group. Some of the remainder may be alcoholics who deny alcoholism.

According to Caldwell SH et al, 70% of cryptogenic cirrhosis were female. 74% of them had H/o obesity and/or diabetes. Both diabetes and obesity were significantly more common in the cryptogenic cirrhotic patients compared with the cirrhotic patients with PBC (or) hepatitis C. In contrast, the prevalence of obesity and diabetes was similar to the NASH patients who were, on average, a decade younger. He concluded that NASH plays an under recognized role in many patients with cryptogenic cirrhosis, most of whom are older type 2 diabetic and obese females (2).

Patients with advanced cryptogenic cirrhosis are more likely to be obese and diabetic compared with age and sex matched patients with advanced cirrhosis. This supports the hypothesis that NASH may be an etiological factor in some of the patient with CC (1,4).

In our study, Cryptogenic cirrhosis was present in 20 females and 8 males. It was more common in the age group 36-60 years, where it is present in 17 patients. It was present in 3 and 8 patients respectively in age groups 15-35 and >60 years

Hypertriglyceridemia was found in 16 out of 28 patients. Diabetes in 13 out of 28 patients and 15 patients were obese (BMI > 30). Promiscuity was associated in 3 patients.The risk factors of NASH were present in majority of patients with Cryptogenic cirrhosis. It may be speculative that NASH may be a predisposing factor in the development of cirrhosis in these patients.

SUMMARY

An attempt was made to detect the presence of etiological markers especially for HBV, HCV and alcohol in 100 patients of Chronic liver disease. The various clinical and pathological features were correlated with etiological factors. An attempt was made to find out the relative prevalence of NASH in patients diagnosed to have chronic liver disease (CLD).

The study could have been more informative and authoritative if some of the following technologies were routinely available - kits for HBV DNA, HCV RNA, electron microscopy and markers for autoimmune liver disease.

CONCLUSIONS

1. Chronic liver disease is a major health problem in this area.
2. 60 out of 100 patients with CLD were related to Ethanol and HBV either singly or in combination.
3. The incidence of HCV infection is very low in this part of the world (4%).
4. A sizeable proportion (28%) belongs to the group of cryptogenic cirrhosis which calls for a lot of research in this sphere to detect new viruses or environmental factors responsible.
5. The prevalence of NASH in patients with CLD is 3%
6. Obesity, Hypertriglyceredemia and Diabetes were present in patients with NASH either singly or in combination.
7. Coexistence of HBV and Alcoholic liver disease does not alter the histopathology or clinical severity of the illness .
8. In view of the too small number of HCV cases, we could not document the histopathological characteristics claimed to be distinctive of HCV infection.

BIBLIOGRAPHY

1. Poona vala A, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patient with cryptogenic cirrhosis: A case control study: Hepatology 2000; Oct: 32 (4 pt 1) 689-92.
2. Caldwell SH et al.Cryptogenic cirrhosis: Clinical characterization and risk factors for underlying disease. Hepatology 1999: 29: 664-669.
3. Bashist B et al. CT demonstration of rapid changes in the fatty infiltration of liver (Radiology 1982;142: 691-692).
4. Duseja A, Nanda M, Das A, Bansali A et al. Prevalence of obesity,diabetes mellitus, hyperlipidemia, in patients with cryptogenic liver cirrhosis.Trop gastroenterol. 2004 Jan-Mar;25(1)15-7.
5. Younossi ZM et al. Obesity and NASH in chronic hepatitis C: J Clinical gastroenteriol 2004 Sep. 38 (8): 705-9.
6. Monto A, Alonzo J et al. Steatosis in chronic hepatitis C; Relative contributions of obesity, diabetes mellitus and alcohol: Hepatology. 2002. Sep: 36 (3): 729-36.
7. Sollis - Herruzo JA, Perez - Carreras et al. Factors associated with the presence of NASH in patients with

chronic hepatic C infection; Am J Gastroenterol 2005 may 100 (5): 1091-8.

8. Kladchareon N, Tree Prasertsuk S et al. The prevalence of NASH in Thai patints with non-HBV, non-HCV chronic hepatitis. J Med Assoc. Thai 2004 Sep: 87 Suppl. 2: 529-34.

9. Hwang SJ et al. Evaluation of hepatitis B and C viral markers: Clinical significance in Asian and Caucasian patients with HCC in the USA; J Gasteroenterol hepatol. 1996 Oct; 11 (10) : 949-54.

10. Tasai JF. Chang WY et al. Hepatitis B and C virus infections as risk factors for liver cirrhosis and cirrhotic HCC: a case -control study. Liver, 1994 April: 14 (2): 98-102.

11. Bruix J, Calvert X et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with HCC and hepatic cirrhosis - Lancet 1989, 2: 1004-1006.

12. Amrapurker DN, Kumar A, Parikh SS. Hepatitis C virus infection in chronic liver disease in Bombay. Ind JGE 1992, 11; 162-163.

13. Gupta H, Irshad M, Jashi YK, Acharya SK, Tandon BN, Hepatitis C virus antibody in acute and chronic liver disease in India. Scand J inf dis 1990, 22: 627.

14. Genesca J, Esteban J et al. Blood borne Non A Non B Hepatitis: Hepatitis C seminars in Liver disease, 1991, 11: 147-163.

15. Zignega AL, Forschi M, Laffi G et al. “Inapparent” hepatitis B virus infection and hepatitis C virus replication in alcoholic patients with (or) without liver disease. Hepatology 1994; 19: 577.

16. Fong T-L, Kanel GC, Conrad A et al. Clinical significance of concomitant hepatitis C infection in patients with alcoholic liver disease. Hepatology 1994; 19:554.

17. Cromir SL, Jenkins PJ, Bowen DS et al. Chronic hepatitis C: Effect of alcohol on heaptic activity and viral titre. J. Hepatol. 1996: 25: 821.

18. Grellier LFL, Dusheiko GM. The role of hepatitis C virus in alcoholic liver disease. Alcohol 1997; 32: 103.

19. Dinis - Ribeiro M, Ranalho F et al. Factors associated with development of cirrhoses in patients with HCV chronic infection - Hepatogastroenterology 2005 Jan - Feb; 52 (61): 176-9.

20. Fattovich G, Pantalena M et al. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis.