"COMPARISION OF FIBROMETER TEST WITH FIBROSCAN AND ITS CORRELATION WITH LIVER BIOPSY IN DETECTING LIVER FIBROSIS IN PATIENTS WITH HEPATITIS B INFECTION"

DISSERTATION SUBMITTED FOR

DM MEDICAL GASTROENTEROLOGY

BRANCH- IV

AUGUST 2015

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY, CHENNAI, TAMILNADU.

CERTIFICATE

This is to certify that this dissertation entitled **"COMPARISION OF FIBROMETER TEST WITH FIBROSCAN AND ITS CORRELATION WITH LIVER BIOPSY IN DETECTING LIVER FIBROSIS IN PATIENTS WITH HEPATITIS B INFECTION"** submitted by **Dr. B. SAJEETH MANIKANDA PRABU** to the Faculty of Medical Gastroenterology, the Tamilnadu Dr.MGR Medical University, Guindy, Chennai-600032, in partial fulfilment of the requirement for the award of DM Degree, Branch IV (Medical Gastroenterology) is a bonafide work carried out by him under my direct supervision and guidance, during the academic year 2012 to 2015.

Prof. Dr. P. Ganesh, M.D., D.M
Professor and HOD/Guide,
Department of Medical Gastroenterology,
(DDHD,GPH, Annanagar),
Kilpauk Medical College,
Chennai

Dr. Narayana Babu M.D., Dch
Dean,
Kilpauk Medical College,
Chennai

DECLARATION

I **Dr. B. SAJEETH MANIKANDA PRABU** declare that I carried out this work on **"COMPARISION OF FIBROMETER TEST WITH FIBROSCAN AND ITS CORRELATION WITH LIVER BIOPSY IN DETECTING LIVER FIBROSIS IN PATIENTS WITH HEPATITIS B INFECTION"** at the Department of Medical Gastroenterology, Govt Peripheral Hospital and Kilpauk Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any university, board either in India or abroad.

This is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulation for the D.M. Degree examination in Medical Gastroenterology.

Govt. Kilpauk Medical College **Dr.B. SAJEEETH MANIKANDA PRABU**

Chennai.

05.04.2015

ACKNOWLEDGEMENTS

I am greatly indebted to my guide **Dr. P .Ganesh, M.D., D.M.,** Professor and Head of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai, for giving me the chance to undertake this dissertation work under his guidance. Also I express my deep sense of gratitude for his constant encouragement, directions, periodical discussions, rigorous reviews and precious suggestions that helped in the shaping of my dissertation.

I express my sincere gratitude to **Dr. T.Rajkumar Solomon, M.D., D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai, for giving me permission to do this dissertation and also his kind encouragement continuous guidance and patronage for doing this study.

I consider it as my privilege to thank my beloved teacher **Dr.A.Murali M.D., D.M** Professor of Medical Gastroenterology, Govt Royapettah Hospital, Kilpauk Medical College for his enlightening ideas, encouragement, philosophical view and ever available help for finishing my study.

I also thank **Dr. S. Jeevan Kumar, M.D., D.M.,** Retired Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai for his encouragement and suggestions for my study.

I am extremely grateful to **Dr. Narayanan Babu M.D., Dch,** Dean, Kilpauk Medical College, Chennai, for granting me permission to do this dissertation.

I am also extremely thankful to **Dr. K. Narayanasamy M.D., D.M,** Professor and Head, Department of Hepatology, Madras Medical College, Chennai for permitting me to use the Fibroscan facility in the department, for his initiative support and periodic discussions that helped me to complete this study.

I am also extremely thankful to **Dr. R. Balamurali, M.D., D.M., Dr.G.Ramkumar, M.D.D.M, and Dr.K.Muthukumaran, M.D., D.M.,** Assistant Professors, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai for their opinion and moral support for doing this dissertation.

I extend my gratitude to **Dr.Chezhian D.M., Dr.Senthil D.M., Dr.Shanthi D.M**., Assistant Professors, Department of Hepatology for their opinion and moral support for doing this dissertation.

I am also very thankful to my colleagues and all my **Fellow Residents** who have helped me in this dissertation.

I am also thankful to **Dr.R.Ravanan, M.Sc., M.Phil., Ph.D,** Associate Professor and Head, Department of Statistics, Presidency College, Chennai for his help in the statistical analysis of my dissertation work.

I thank all the patients who voluntarily participated in this study, without whom this study would not have seen the light of the day.

I also thank all the paramedical staff attached to Govt. Peripheral Hospital, Anna Nagar, Chennai who have helped me in doing this dissertation work.

Above all I thank God the Almighty for his blessings to do this work.

Finally, I would like to thank my wife, without whose co-operation this work would not have been possible.

ABBREVIATIONS

HBV – Hepatitis B Virus

HCV – Hepatitis C Virus

HIV – Human Immunodeficiency Virus

ORF – Open Reading Frame

DNA – Deoxy Ribonucleic Acid

RNA – Ribonucleic Acid

ALT – Alanine aminotransferase

PCR – Polymerized Chain Reaction

HBsAg – Hepatitis B Surface antigen

HBcAg – Hepatitis B Core Antigen

HBeAg – Hepatitis B envelope antigen

Anti-HBe – Antibody to envelop anigen

PNALT – Persistently Normal ALT

cccDNA – covalently closed circular DNA

ECM – Extracellular matrix

HSC – Hepatic Stellate Cells

TGF – Transforming Growth Factor

PDGF – Platelet Derived Growth Factor

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID No.03/01/2015 Dt.20.01.2015
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "Comparision of Fibrometer virus with Fibroscan and its Correlation with liver biopsy in Detecting Liver Fibrosis in patients with Hepatitis B Infeation."-For Project Work-submitted by Dr.B. Sajeeth Manikanda Prabu, DM Medical Gastroenterology, PG KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

20/1/15

CHAIRMAN,
Ethical Committee
Govt Kilpauk Medical College,Chennai

GOVT. KILPAUK MEDICAL COLLEGE
20 JAN 2015
Kilpauk, Chennai-10.

20/1/2015

A Compliance Ethical Committee

TABLE OF CONTENTS

INTRODUCTION	**-**	**1**
REVIEW OF LITERATURE	**-**	**3**
AIM OF THE STUDY	**-**	**49**
MATERIALS AND METHODS	**-**	**50**
OBSERVATIONS AND RESULTS	**-**	**57**
DISCUSSION	**-**	**67**
CONCLUSION	**-**	**73**
LIMITATIONS	**-**	**74**
BIBLIOGRAPHY	**-**	**75**

APPENDIX

PLAGIARISM CERTIFICATE

PROFORMA

MASTER CHART

INTRODUCTION

INTRODUCTION

Hepatitis B is a major health problem affecting more than 350 million people globally. Hepatitis B carriers are defined as persons positive for HBsAg for more than 6 months[1, 4]. The spectrum of the disease and the history of chronic HBV infection are various and variable, ranging from inactive carrier state to chronic hepatitis which evolve into cirrhosis in upto 20% of the cases with hepatic insufficiency and portal hypertension being one of the serious complication. Chronic carriers of HBV are at 100 times increased risk of developing hepatocellular carcinoma than non-carriers[5.]

The chance of developing sequelae is about 15% to 40% over lifetime, hence evaluation and early treatment is required. The prognosis and management of HBV related liver disease depends on the degree of liver fibrosis. HBV infected patients with ALT values close to the upper limit of normal may have abnormal histology and can be at increased risk of mortality from liver disease especially those above age of 40yrs.

Liver biopsy is needed to assess the degree of liver damage. Liver biopsy is an invasive procedure and its diagnostic accuracy decreases because of sampling error and inter-observer variation. Therefore a need for alternate method to detect liver fibrosis has lead to the development of non invasive modalities like Serum markers, Fibroscan and Fibrometer. Fibroscan accurately assesses fibrosis and could avoid liver biopsy in patients with Chronic Hepatitis B infection[6]. Fibrometers are blood tests for liver fibrosis to assess fibrosis stage

and area of fibrosis. They have 90% predictive value in a higher proportion of patients than other usual blood tests [7].

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Hepatitis B infection is a potentially life threatening problem worldwide. It can cause both acute and chronic infection. Chronic infection can be either asymptomatic or may be associated with chronic inflammation of liver leading to cirrhosis over a period of time.

EPIDEMIOLOGY:

The prevalence of HBV infection is different in various parts of the world. Indian sub - continent has been categorized as region of intermediate risk with lifetime risk of infection being 20% to 60%[2]. Horizontal transmission occurs among any age. Based on the prevalence of HBsAg countries are defined as having high risk (≥8%), high intermediate (5-7%), low intermediate (2-4%) and low risk (<2%)[8].

INFECTIVITY:

The modes of transmission of infection is by percutaneous and mucous membrane exposure to infected blood and body fluids principally semen and vaginal fluid. The virus is 50 to 100 times more infectious than HIV and 10 times more infectious than Hepatitis C virus (HCV). HBV replicates primarily in hepatocytes but are also found in adrenal glands, testes, colon, nerve ganglia and skin[9].

The most common mode of transmission of infection is during perinatal period or in early childhood. Utero-placental transmission accounts for less than 2% of perinatal infection. Risk of perinatal transmission is highest among HBeAg positive mothers. The risk of transmission of virus is about 70% to 90% among mothers who are HBeAg positive and about 5 – 20% in mothers who are negative for envelope antigen. There virus does not spread by breast feeding[10].

Hepatitis B virus can survive on inanimate surfaces for atleast one week, so transmission can occur even in the absence of visible blood residue[11].

VIROLOGY:

Hepatitis B virus is a small 3.2kilobase DNA virus that belongs to Hepadnaviridae. **Baruch S. Blumberg** first identified Australian Antigen in mid 1960's, subsequently was referred as hepatitis B surface antigen. Later electron microscopic characterization of these particles called Dane particles as Hepatitis B virus was done by D.S.Dane in early 1970. Other viruses in the family are woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus (GSHV), avian duck hepatitis virus (DHBV) and heron hepatitis virus (HHBV). All viruses have the same features in common including the small enveloped hepatotropic virion with complete coding and incomplete noncoding strand[3].

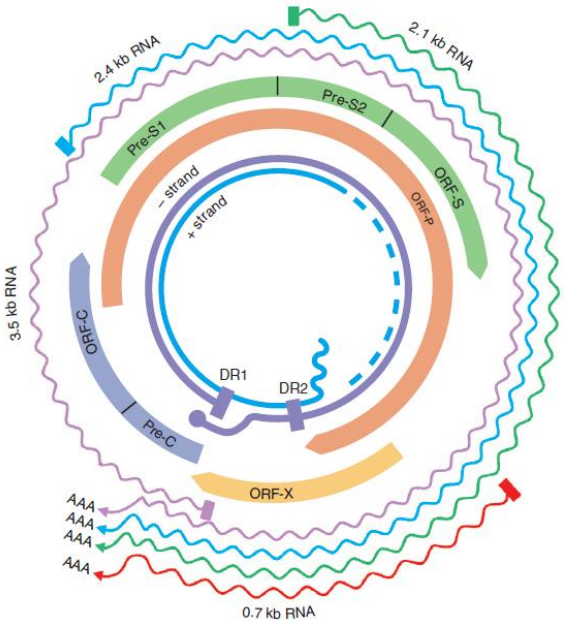


Fig 3: Molecular structure and organization of HBV

The hepatitis B genome is composed of four open reading frames (ORF). The major gene components are Core, Surface, X and Polymerase genes. The core gene is responsible for production of HBeAg. Surface gene encodes PreS1, PreS2 and S protein. X gene has transactivating properties and is responsible for hepatic carcinogenesis. Polymerase gene plays a vital role in packaging and DNA replications including RNA and DNA dependent DNA polymerase.

LIFE CYCLE OF HBV:

HBV binds to the surface of the hepatocyte via its envelope. The receptor for the entry of virus has not been identified. Once the virus penetrates the hepatocyte, the viral surface protein is removed and the HBV nuclear material

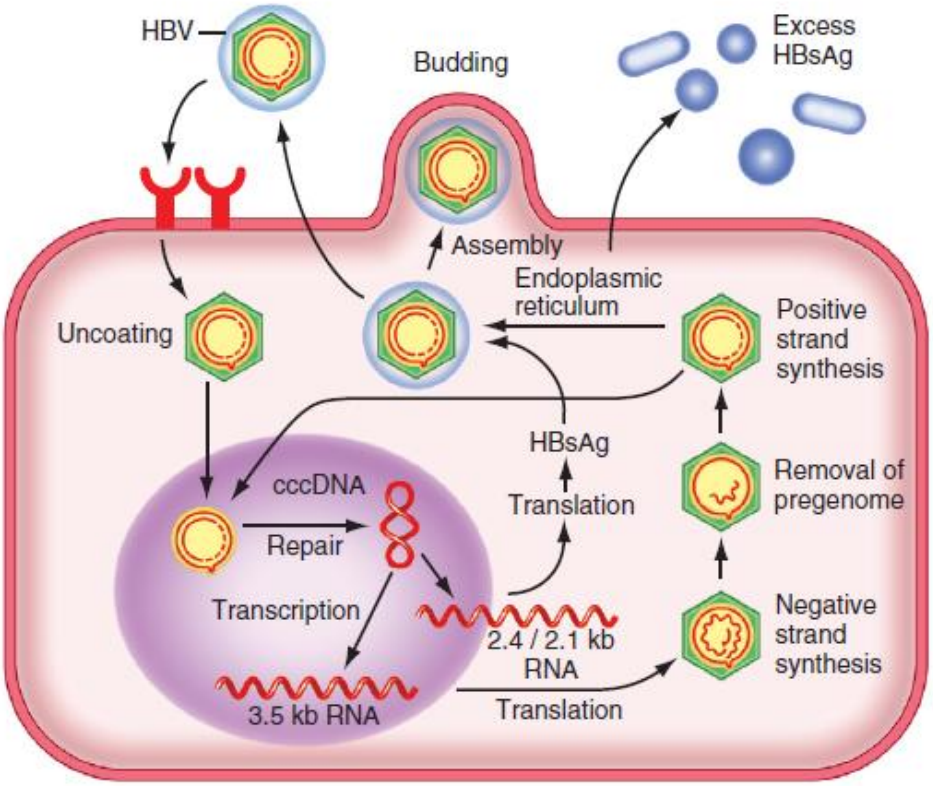


Fig 4: Life cycle of HBV

migrates from the cytoplasm to the nucleus to deliver the viral genome (rcDNA). Within the nucleus, the rcDNA is converted to covalently closed circular DNA (cccDNA), a viral mini - chromosome which serves as a template for the transcription of four messenge RNAs (mRNA) (3.5,2.4,2.1 and 1kb). These transcripted RNA's are transported to the cytoplasm and translated into seven viral proteins viz the core protein (HBcAg), the pre-C/C protein which gets processed into secreted HBeAg, the viral polymerase (Pol), the three envelope proteins (large, medium and small surface proteins bearing HBsAg) and the X protein (HBx).

The core proteins assemble in the cytoplasm and encapsidate both the pgRNA and the viral Pol to from nucleocapsid. HBV replication occurs through RNA intermediate and requires active viral reverse transcriptase and polymerase enzyme. The mutation of HBV is estimated to be 10^{13} to 10^{15} point mutations per day[12]. Multiprotein complexes including caperone proteins are also packaged into nucleocapsid. PgDNA gets reverse transcripted into neg(-) strand DNA followed by synthesis of pos(+) strand DNA. The mature nucleocapsid can migrate either into the nucleus to deliver viral genome and enrich cccDNA or to endoplasmic reticulum to be secreted as new HBV virion.

GENOTYPES[13, 14]:

The virus has ten genotypes designated A to J and numerous subtypes[13]. Among the ten, genotypes B, C & D are common in Southeast Asia and in India.

Genotype A is predominant in United States and Northern Europe. Genotype B and C are seen distributed in Eastern Asia and Far East. Genotype D is seen widely distributed throughout the world, Mediterranean region, North Africa, Eastern Europe, Indian sub continent, Middle East and Arctic continent. Genotype E is confined to Western and Sub Saharan Africa. Genotype G, H etc are seen in indigenous population in Central and South America. There is an association between HBV and precore, core promoter variant. The most common precore variant ($G_{1896}A$) is predominantly found in genotype B, C and

D and rarely in genotype A. This accounts for the high prevalence of HBeAg negative chronic hepatitis in Asia. The most common core promoter variant ($A_{1762}T$, $G_{1764}A$) is found in genotype A, C and D.

Several studies suggest that these genotypes are associated with progression of disease and with rate of recovery. Studies from Asia showed that genotype B is associated with low prevalence of HBeAg, earlier seroconversion, sustained remission after seroconversion and less active liver disease when compared with genotype C. Genotype C is associated with rapid progression of disease subsequently leading to early development of cirrhosis and hepatocellular carcinoma.

CLINICAL OUTCOMES:

After exposure to virus people tend to develop either Acute or Chronic hepatitis. The principal determinant of clinical outcome is the age at which the person becomes infected.

Acute Hepatitis B:

Usually two third of patients with acute infection have asymptomatic or subclinical course that goes unrecognized. Rest of the individual have mild to moderate course with some develop acute liver failure. The incubation period rnages from few weeks to six months (average 60 to 90 days). Hepatitis B induced acute liver failure accounts for % of death, related to the infection[15].

Rapid elimination of virus results in HBsAg clearance from serum. Hence diagnosis of acute infection requires testing for anti – HbcIgM. The rate of spontaneous survival in HBV related liver failure is 20%. Liver transplantation has increased the survival rate upto 60%.

Chronic Hepatitis B:

Perinatal exposure of BV leads to chronic carrier state in 95% of individuals. But in contrast 30% of children exposed at the age of 5 years develop chronicity. Only 2- 5% of exposed adults become chronically infected[16].

PATHOGENESIS:

HBV is not a cytopathic virus. The cellular immune response is a principal arm involved in pathogenesis of disease. The immunological response to HBV infection consist of an innate response by natural killer cells and interferon and an adaptive response involving HLA class II restricted CD_4 cells and HLA class I restricted CD_8 cytotoxic T cells[17].

During acute infection, most viral molecules are cleared rapidly from liver by cytokines released by innate immune system and later by HBV specific CD_8 cells[18]. By contrast, chronic infected persons exhibit infrequent weak T cell responses. The mononuclear cells in liver are non- antigenic specific[19, 20].

CD_8 cytotoxic T cells contribute to disease process by resulting in apoptosis of infected hepatocytes. CD_4 cells produce antiviral cytokines to neutralize antibody production and this neutralization helps in limiting intrahepatic spread of infection and thus prevents re-infection.

PHASES OF CHRONIC HBV INFECTION:

Four phases of HBV infection has been described

- Immune tolerance phase
- Immune clearance phase or Immune active phase
- Inactive carrier state
- Reactivation phase

Typically perinatally acquired infection or during infancy has three phases viz immune tolerance phase, immune clearance phase and inactive residual phase [21, 23]. In some of the inactive carriers, HBV may get reactivated and trigger immune mediating liver injuries. This phase can be viewed as a variant of the immune clearance phase. Whereas in infection acquired during adult period there is no immune tolerant phase [22].

IMMUNE TOLERANT PHASE:

This is the earliest phase to be recognized when the infection is acquired at birth or during infancy. This phase is characterized by the presence of serum HBeAg, high levels of HBsAg and HBV DNA levels ($\geq 10^7$ IU/ml) with normal

enzymes (ALT) and normal or minimal histological changes [24] with intrahepatic expression of core antigen (HBcAg) predominantly in the nuclei of hepatocytes.

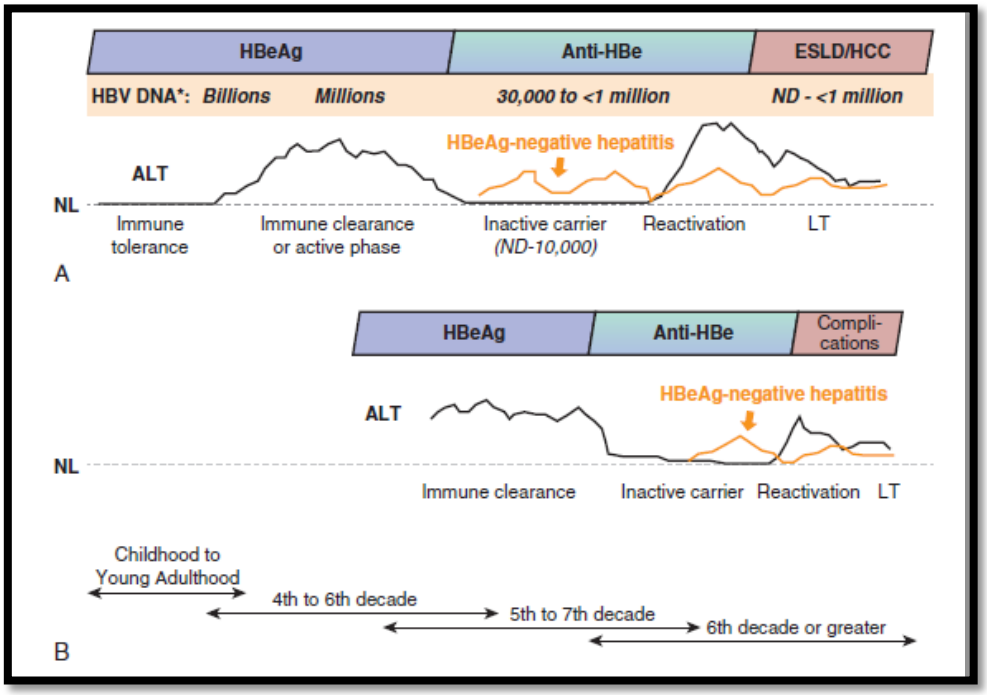


Fig 3: Natural history of Chronic HBV infection. A) Early life B) Adult life.

Normally HBV do not cross the placenta whereas the envelope antigen does. Experiments in mice showed that the transfer of HBeAg through placenta induces unresponsiveness of helper T cells in neonates resulting in ineffective cytotoxic T cell response to core antigen (HBcAg) [24, 25].

IMMUNE CLEARANCE OR IMMUNE ACTIVE PHASE:

The immune active phase usually begins in 2nd and 3rd decade, characterized by elevated serum aminotransferase (ALT), lower HBV DNA than immune tolerant phase with evidence of inflammatory changes in

histopathological examination. The mechanism is poorly understood but CD8 cytotoxic cells lyses the infected hepatocytes. This is accompanied by the transfer of intrahepatic distribution of core antigen from nucleus to cytoplasm[26]. The continued action of immune system leads to HBeAg seroconversion (loss of antigen with development of Anti Hbe). The annual rate of spontaneous seroconversion ranges from 8% to 15% [27].

HBeAg to anti-HBe seroconversion [28, 29, 30, 31, 32, 33]:

The immune clearance phase lasts for many years until HBeAg seroconvert which is noticed by marked reduction in HBV DNA levels detectable only by PCR with ALT normalization and resolution of liver necroinflammation [28, 29, 30]. The rate of seroconversion depends on various factors which include age, genotype, ALT levels and histological activity [31, 32]. The likelihood of seroconversion correlates with ALT levels : at 18 months follow up are 0%, 3-8%, 17%, 59-70% if ALT levels are <1, 1-2.5, 2.5 – 5, ≥5 elevated frombaseline respectively. Similarly HBeAg seroconversion is less than 25% and more than 65% in mild and active hepatitis respectively. Other factors like age >30 yrs, female gender and genotype B are independent for early seroconversion.

INACTIVE CARRIER STATE:

The hallmark of HBeAg seroconversion indicates transition from active phase to inactive carrier state [30]. This phase is characterized by absence of HBeAg, presence of anti-HBe, persistently normal ALT and low or undetectable serum DNA levels. Liver biopsy usually shows mild hepatitis and minimal fibrosis. These patients have a good prognosis evident by studies. A large nested case control study during a long term follow up of upto 13 years showed that maintenance of HBV DNA less than 4.39 $\log_{10}$ copies was associated with persistently normal ALT and poor progression of disease including hepatocellular carcinoma[33]. Hence Inactive carrier state was defined as HBeAg negative noncirrhotic HBsAg carrier with persistently normal ALT (PNALT) and HBV DNA < 20,000 IU/ml [34]. The incidence of cirrhosis and hepaocellular carcinoma was even lower [35] among those who underwent HBeAg seroconversion before the age of 30 years.

REACTIVATION PHASE:

A group of patients undergo spontaneous reactivation of the virus after HBeAg seroconversion causing replication and reappearance of HBV DNA. Reactivation is defined as the reappearance of high levels of HBV DNA (> 2×10^4 IU/ml) in serum with or without HBeAg seroconversion usually associated

with elevated transaminases. Only a small proportion of patients are associated with reappearance of HBeAg (HBe reversion). This suggests that reactivation is mostly due to HBV strain with procore or core promoter mutation that downregulates HBeAg production [28]. But HBV reactivation can also happen as a result of immunosuppressant or chemotherapy [36].

SPONTANEOUS HBsAg SEROCLEARANCE:

During inactive phase, the surface antigen level decrease gradually or even disappear from the serum spontaneously. Short term follow up studies showed that the rate of spontaneous HBsAg seroconversion was 1% to 2% per year. However a recent 11 year (mean) follow up study in 1965 asymptomatic patients with a median age of 34(16-6) years showed an annual HBsAg seroconversion of about 1.2%. The overall HBsAg seroclearance was 8% at 10 years, and then it increased disproportionately to 25% at 20 years and 45% at 25 years [37].

Various factors are associated significantly with HBsAg seroclearance viz age, viral load, genotype, and presence of cirrhosis or HCV superinfection. Age more than 50 years is an important predictor of HBsAg seroclearance. Genotype B patient seroconvert earlier compared to genotype C and low levels of HBsAg (<100IU/ml) and HBV DNA (<1000IU/ml) are associated with higher rates of seroclearance [38, 39]. It was demonstrated from a study that HBsAg < 200IU/ml combined with a ≥ 1 log10 IU/ml decline in the preceding 2 years may predict

HBsAg seroclearance in 3 years with high accuracy [40]. Interestingly a study from Taiwan showed that HBsAg carriers had spontaneous seroclearance with significantly higher BMI (Body Mass Index) and fatty liver than those without, suggesting BMI and fatty liver as an independent factor associated with HBsAg seroclearance [41].

The surface antigen seroclearance is followed by loss of serum markers of viral replication, including undetectable HBV DNA in 95% of cases with improved liver histology [42]. However intrahepatic viral particles in the form of cccDNA persist lifelong. Antibody to surface antigen (anti-HBs) may appear and increases to 59% at 51 months of follow up after seroclearance [43]. Patients with spontaneous surface antigen clearance has an excellent prognosis as none of the non cirrhotic patients without concurrent HCV or HDV superinfection developed sequelae during a median follow up period of 62(12 to 179) months[39, 41, 43].

HBeAg Negative Chronic Hepatitis:

The prevalence of HBeAg negative chronic hepatitis B infection varies among different geographical areas [44]. The prevalence of this group of patient is about 80% to 90% in Mediterranean region, 30% to 40% in Taiwan and less than 10% in United States and Northern Europe [45]. The incidence of

reactivation of hepatitis B in HBeAg negative patients also differs in various regions. A study was conducted in Taiwan which included 1241 patients, out of which 211patients (17%) developed HBeAg negative chronic hepatitis during a mean follow up period of 12 years with cumulative rate being 20% after 20 years. Reactivation of infection occurs more commonly during the first 5 to 10 years and rare after 20 years [46].

Patients with E negative chronic hepatitis tend to be old with low levels of HBV DNA, low inflammatory activity, fewer flares but have more advanced liver disease.

There are various factors [47, 48, 49] which predict the reactivation of HBV after HBeAg seroconversion. They are male gender, genotype C > B, genotype D > A, and a baseline HBV DNA levels of $>10^4$ copies or 2000IU/ml. These findings suggest that virus easily get reactivated if vigorous immune mediated hepatocytolysis or a prolonged immune clearance phase occurs.

DIAGNOSIS:

The clinical diagnosis of HBV infection depends on the presence of antigens viz surface antigen (HBsAg), envelop antigen (HbeAg) and antibodies of core, envelope and surface antigen, anti-HBc, anti-HBe and anti-HBs respectively. In addition quantitative measurement of HBV DNA helps in assessing the phase of disease and its activity.

Diagnostic Criteria [3]:

Chronic hepatitis B:

1. HBsAg-positive > 6 months
2. Serum HBV DNA > 20,000 IU/mL (105copies/mL), lower values 2,000 to 20,000 IU/mL (104-105 copies/mL) are often seen in HBeAg-negative chronic hepatitis B
3. Persistent or intermittent elevation in ALT/AST levels
4. Liver biopsy showing chronic hepatitis with moderate or severe necroinflammation

Inactive HBsAg carrier state:

1. HBsAg-positive > 6 months
2. HBeAg –, anti-HBe +
3. Serum HBV DNA < 2,000 IU/mL
4. Persistently normal ALT/AST levels
5. Liver biopsy confirms absence of significant hepatitis

Resolved hepatitis B:

1. Previous known history of acute or chronic hepatitis B or the presence of anti-HBc ±anti-HBs
2. HBsAg negative
3. Undetectable serum HBV DNA
4. Normal ALT levels

HBsAg can be detected in the serum within six days after exposure upto two weeks along with DNA before the onset of symptoms. In self limiting acute infection HBsAg tends to decrease and become undetectable after 4 to 6 months. Persistence of HBsAg for more than six months suggests chronic infection. The antibody to surface antigen develops several weeks after the disappearance of surface antigen and usually persists for lifelong providing immunity.

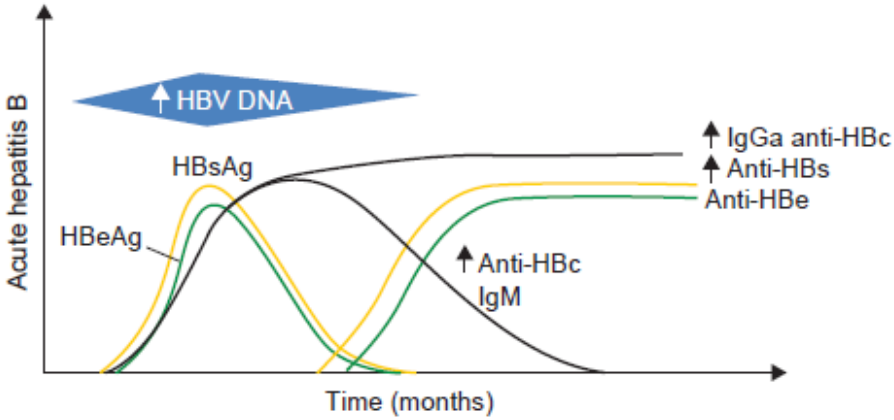


Fig 4: Serological Markers in Acute Hepatitis B infection

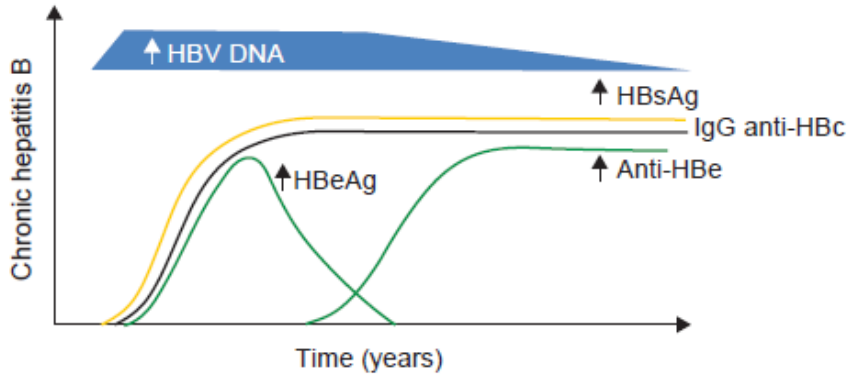


Fig 5: Serological marker in Chronic Hepatitis B infection

HBeAg is a viral protein that appears soon after the appearance of surface antigen and is usually associated with elevated aminotransferase. The presence of envelop antigen suggest active replication and the patient is highly infective during this period. The antigen tends to disappear soon after the elevation of liver enzymes but the presences of it for more than 3 months suggest development of chronicity.

The core antibody (Anti – HBc) is detectable both in acute and chronic infection. But during acute viral infection IgM class of anti-HBc is predominantly seen in serum and is useful in detecting acute viral infection and during window period. “Serological Window” is the period during early clearance of surface antigen and hence HBsAg and anti-HBs are undetectable with only detectable anti-HBc. Anti-HBc IgG is seen in chronic infection and after recovery from acute infection.

Hence anti-HBc IgM is useful in detecting

- acute infection with undetectable surface antigen or in window period,
- occult hepatitis B infection
- acute flare of chronic infection
- false positive result

HBV DNA measurement [50, 51, 52, 53] is important in diagnosis of occult infection and in assessing treatment response. It can be measured by qualitative and quantitative assays. Quantitative method is widely used as it helps in detecting the candidate for antiviral therapy and also to assess treatment response.

Molecular tests available for the purpose of measurement include

(1) Molecular hybridization and signal amplification

(2) Target amplification techniques

(3) qRT-PCR technology.

Molecular hybridization uses either oligonucleotide or RNA probe that hybridizes with HBV DNA to form a probe-DNA complex. This complex is detected along with multiple antibodies conjugated to alkaline phosphatise. When this complex is treated with chemical luminescent substrate, it can be measured by light spectrometer. This test is highly specific and detects HBV DNA levels of 350 IU/ml.

Target amplification relies on use of specific primer that attaches to a strand of targeted DNA. Using PCR amplification the DNA is multiplied many times resulting in detection with a lower limit of 50 IU/ml (25 copies/ml).

Quantitative Real Time PCR (qRT-PCR) has a higher dynamic range and is faster than conventional amplification technique. It detects HBV DNA as low as 10 copies/ml. The most commonly used product is Cobas Taqman from Roche Molecular Diagnostics. Qualitative PCR has been reported to be sensitive than quantitative PCR but has limited role in clinical decision making regarding follow up and treatment. HBsAg quantification has come up in recent days in assessing the treatment response. Clinical trials have demonstrated a decline in HBsAg levels with treatment than DNA levels. The high negative predictive value of this assay helps in predicting virological response at 12 weeks enabling the treating physician to continue or alter the treatment regimen [54, 55].

SEQUELAE OF CHRONIC HEPATITIS:

The sequela of chronic hepatitis B infection varies from inactive carrier state to chronic hepatitis, cirrhosis of liver, hepatic decompensation and hepatocellular carcinoma. It is estimated that cirrhosis develops in approximately 20% of patients with chronic hepatitis. The annual rate of progression from chronic hepatitis to cirrhosis has been estimated to be 2 - 5% in HBeAg positive and 3 - 10% in HBeAg negative individuals [56]. There are various factors that contribute to increased rate of progression viz older age, male gender, genotype (C>B and D>A), persistent HBeAg seropositivity and

high levels of DNA, recurrent flares, coinfection with HCV, HDV, HIV and associated risk factors like alcohol consumption, obesity and diabetes mellitus [56, 57]. These data suggest that the outcome of disease depends on the duration of immune clearance, the antigenic seroconversion and severity of liver damage. A large population based long term prospective cohort study (REVEAL – HBV Study) from Taiwan which followed 3600 untreated carriers over a period of 11 years showed that the risk of cirrhosis proportionately increased with high DNA levels at the time of presentation [58].

Hepatic decompensation and mortality [59]:

The annual rate of progression of disease from compensated to decompensated cirrhosis is estimated to be 3 – 5%. About 3 -5% of the compensated cirrhotic patients develop jaundice, ascities, variceal bleed or encephalopathy every year. The risk is four fold increased in e-antigen positive patients with detectable or high viral load. One of the important risk factor for decompensation is acute flare caused by reactivation or co-infection. The 5 year survival rate of compensated Child A cirrhosis is approximately 85%, where as in decompensated Child B or C the survival rate is 14 -35%. The lifetime risk of liver related death in patients who has acquired infection perinatally has been estimated to be 40 – 50% and 15% in men and women respectively [60].

Hepatitis B and Hepatocellular Carcinoma:

Hepatocellular carcinoma is the third most cause of cancer death. Hepatitis B accounts for 80% of HCC which occurs in high prevalence in Southeastern Asia and Sub-Saharan Africa. Long term follow up studies have shown a close correlation between HBsAg carriers and HCC. The annual rate of incidence of HCC is approximately 0.5 – 1.0% in noncirrhotics and about 2 -3% in cirrhotic patients.

One study from Taiwan showed that patients of HBsAg carriers were followed for nine years and the incidence of hepatocellular carcinoma was 495/1,00,000 per year for HBsAg positive and 5/1,00,000 per year for negative patients with a relative risk of 98. High risk population are the individuals who acquired infection in childhood by perinatal transmission with persistence carrier state face an increased lifetime risk of developing hepatocellular carcinoma than uninfected individuals.

There are various risk factors [56, 57] proposed for the development of hepatocellular carcinoma in patients infected with chronic hepatitis infection. They are male gender, older age and family history of HCC, history of reversion from anti-HBe to HBeAg, presence of cirrhosis, persistent high viral load (more than 20,000 IU/ml) in persons over 30 years and core promoter mutation. It has

been shown in several studies from Taiwan and Alaska that genotype C is more prone for developing carcinoma than other genotypes with the incidence being double than genotype B. Other factors which contribute to development of carcinoma are smoking, alcohol, co-infection with HCV, diabetes and exposure to aflatoxin.

Mechanisms of hepatocarcinogenesis [61, 62]:

The precise mechanism by which hepatitis B infection causes hepatocellular carcinoma is not known. However various mechanism are proposed that Hepatitis B can cause carcinoma directly by activating cellular oncogenes or by inactivating tumor suppressor genes or can cause indirectly by chronic liver injury, persistent inflammation and regeneration.

Possible direct carcinogenic effects include cis-activation of cellular genes as a result of viral integration, transcriptional activation of remote cellular genes by HBV-encoded proteins (particularly the X protein), changes in the DNA sequences flanking the integrated viral DNA and effects resulting from viral mutations. HBV DNA is seen integrated in neoplastic and non neoplastic liver cells of patients with HCC. Integration of viral DNA may activate cellular proto-oncogenes inducing carcinogenesis via transactivation. The HBV X protein has been shown to be a potent transactivator of carcinogenesis. This is

mediated by specific transcription factors, activation of mitogen activated protein (MAP) and Janus Kinase/Signal transducer (JAK/STAT) pathways which has an effect on apoptosis and modulation of DNA repair.

Indirect carcinogenetic effect is through induction of liver injury. Chronic inflammatory activity is characterized by increased rates of cellular DNA synthesis with impaired cell repair. This result in mutation and regeneration of the transformed cells generate mutagenic reactive oxygen species and eventually tumour formation.

HEPATIC FIBROSIS [63, 64, 65]:

Liver fibrosis is defined as the building up of excessive amount of exracellular matrix also known as scar tissue. Actually fibrogenesis is a process involved in the normal healing mechanism in response to any injury. This process includes recruitment of immune and /or inflammatory cells, secretion of extracellular matrix (ECM) proteins and regeneration of hepatic tissue. However chronic damage to the liver parenchyma leads to excess accumulation of fibrous tissue finally leading to cirrhosis.

The most important cell involved in the production of extracellular matrix is the Myofibroblast. There are a wide variety of cells that can be converted into fibrogenic myofibroblast but the predominant one are the quiescent Hepatic

Stellate Cells (HSC) also known as Ito cells or peri-sinusoidal cells. These cells are present in the space of Disse which gets activated by reactive oxygen species, proteases and lipid metabolites like prostaglandins and thromboxane produced as a result of injury and inflammatory process. Once the hepatic stellate cells get activated they express transforming growth factor (TGF-β) and platelet derived growth factor (PDGF). TGF is the central mediator of fibrogenesis and PDGF induces stellate cell proliferation. Activation of stellate cells gradually replaces extracellular matrix in space of dissie by the collagen fibres leading to formation of fibrous bands. In advanced fibrosis stage, liver contains six times more ECM components than normal liver.

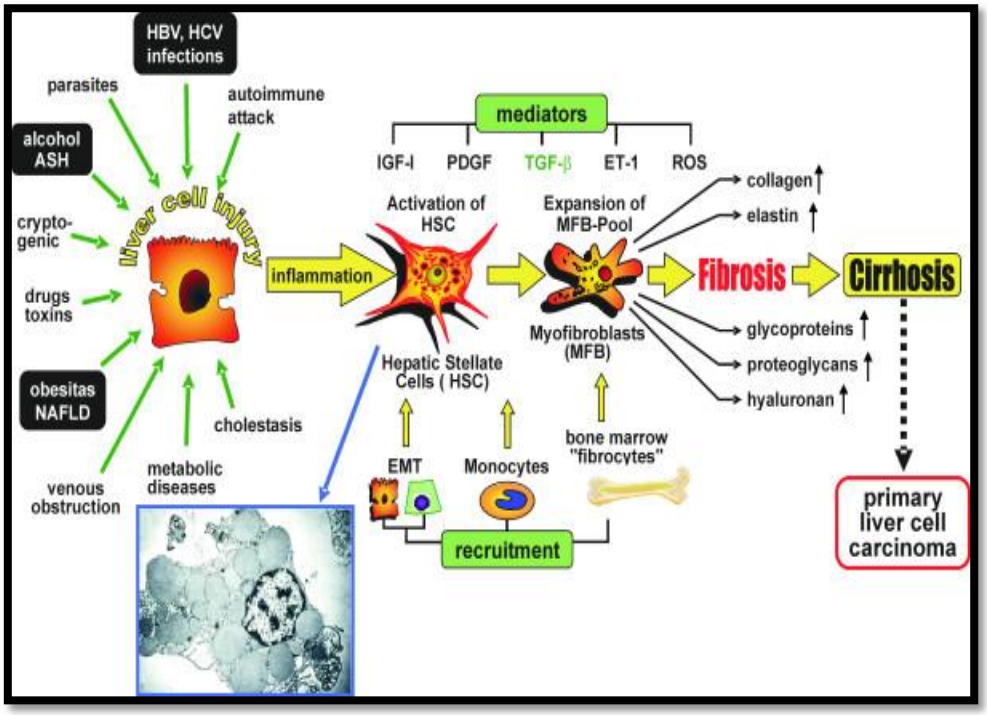


Fig 6: Pathogenic cascade of hepatic fibrosis

In chronic HBV infection, the mechanism of liver fibrosis is multifactorial which involves both viral and host specific factors like oxidative stress, steatosis, increased iron stores and increased rate of hepatocyte apoptosis with high viral replication and load.

METHODS TO DETECT AND ASSESS LIVER FIBROSIS [66, 67]:

The accurate assessment of liver fibrosis plays a major role in management of the disease. The major limitation is the lack of accurate, reproducible and easily applied methods for the assessment of hepatic fibrosis. But with the development of advanced medical science, various modalities are available for detecting liver fibrosis from serum markers, imaging to invasive techniques.

Various modalities to detect hepatic fibrosis can be broadly categorized as

Invasive method

- Liver Biopsy

Non-invasive method

- Serum markers
    - Direct markers
    - Indirect markers
- Proteomics studies
- Imaging modalities

INVASIVE METHOD TO DETECT HEPATIC FIBROSIS:

LIVER BIOPSY:

Liver biopsy is considered as the gold standard test in assessing and staging of liver fibrosis for the past 50 years. This technique allows diagnostic information not only about fibrosis but also on inflammation, necrosis, steatosis and elemental deposition in liver parenchyma.

Pre procedure requirements for liver biopsy:

- Informed consent
- Patients blood group, platelet count, bleeding and clotting time, prothrombin time.
- Anticoagulants should be stopped atleast 72hrs prior to and after procedure.
- NSAIDS and Salicylates to be withheld 1 week prior to biopsy.
- To be under local anaesthesia and ultrasound guidance.

Sampling of liver tissue:

Two types of liver biopsy needles are available to perform liver biopsy

a) Aspiration or Suction type needle:

eg: Jamsidi, Klatskin and Menghini needle.

The Menghini needle is the commonly used needle in India which is about 6 cm long with an oblique tip slightly convex towards the outside.

A retaining device is attached to the proximal end to collect the tissue. The diameter of the needle usually does not exceed 1.4 mm.

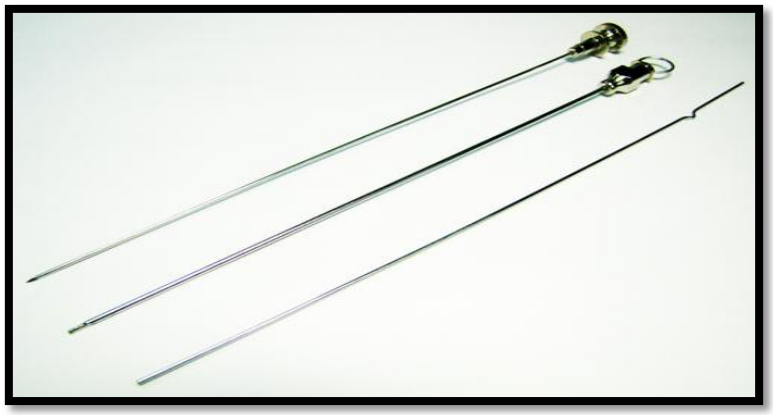


Fig 7: Menghini liver biopsy needle

b) Cutting type needles:

eg: Tru-Cut, Vim-Silverman and Spring loaded automatic devices.

Vim-Silverman needle consist of a long sharp needle, a stillete, prong which is longer than the needle without a guard. The prong has a cutting edge.

Fig 8: Vim-Silverman Needle

Tru-Cut biopsy needle is disposable modification of Vim-Silverman needle with a pointed end obturator and a cannula. Automatic spring loaded device has a trigger which is a rapid firing side notch.

According to the APASL guidelines the sample should be atleast 1.5cm in length and it should contain atleast ten portal tracts for assessment.

Histopathology:

The only histopatological feature of chronic hepatitis B infection is the presence of ground glass hepatocytes which can be seen by Victoria blue or aldeyde fuchin stain. This is due to the accumulation of HbsAg particles in endoplasmic reticulum. The core antigen HBcAg can be seen inside the hepatocyte nuclei by electron microscopic and immunofluroscence technique

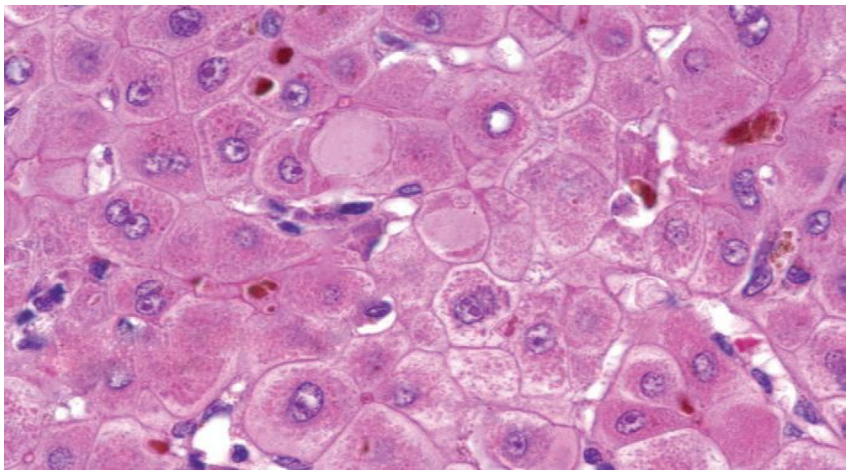


Fig 9: Histopathology of HBV infection

Quantification of liver fibrosis:

The history of liver biopsy staging system dates back 1981 when Knodell and his colleagues proposed the histological scoring system. Later this scoring is modified commonly referred as the Ishak System and the other system which is no regularly used is the Metavir Scoring System.

KNODELL OR HISTOLOGY ACTIVITY INDEX (HAI) [68]:

It is a complex and extensive scoring system but it is a better tool for defining the extent of liver inflammation and damage. The limitation of this system is the inter-observer variation.

It is composed of three components

i Periportal and/or Bridging necrosis scored from 0 to 10.

ii Interlobular degeneration and focal necrosis scored from 0 to 4

iii Portal inflammation scored from 0 to 4.

The combination of these three components indicates the amount of inflammation

0 = no inflammation

1 – 4 = minimal inflammation

5 – 8 = mild inflammation

9 – 12 = moderate inflammation

13 – 18 = marked inflammation

ISHAK or MODIFIED KNODELL SYSTEM [70]:

It is the modification of the Knodell system which lacks two stages of the original Knodell system and it scores from 0 to 6.

Description	Score	Fibrosis measurement
No fibrosis	0	1.9%
Fibrous expansion of some portal areas	1	3.0%
Fibrous expansion of most portal areas +/- short fibrous septa	2	3.6%
Fibrous expansion of most portal areas with occ portal to portal bridging	3	6.5%
Fibrous expansion with marked bridging of portal to portal as well as portal to central area	4	13.7%
Marked bridging(P-P,P-C) with occ nodules	5	24.3%
Cirrhosis, probable or definite	6	27.8%

METAVIR SCORING SYSTEM [69]:

The Metavir system was originally designed for patients with hepatitis C infection using a sum of ten experienced pathologist. The scoring system uses both grading and staging systems as it includes two scores one for necroinflammatory grading (A) and other for fibrosis (F). The main advantage of this score is its simplicity and its focus on necroinflammatory lesions.

The Grading (A) is based on the degree of inflammation

A0 – No activity

A1 – Mild activity

A2 – Moderate activity

A3 – Severe activity.

The Fibrosis stage (F) consists of

F0 – no fibrosis

F1 – Portal fibrosis without septa

F2 – Portal fibrosis with septa

F3 – Numerous septa without cirrhosis

F4 – Cirrhosis.

Limitations of liver biopsy are

- Invasive procedure
- Sampling error
- Intra and inter observer variation
- Complications like intraperitoneal bleeding, pain and hypotension.

NON INVASIVE METHODS TO DETECT LIVER FIBROSIS:

Understanding the pathogenesis of hepatic fibrosis at the molecular level has led to the identification of several serum markers. Non invasive markers are helpful in assessing the stage of fibrosis in patients with no clear indication for

liver biopsy such as Chronic Hepatitis B with persistently normal enzymes, chronic hepatitis C, patients who require follow up to assess treatment response. Either individually or in combination these serum marker have a great potential in detecting fibrosis. In addition, radiological methods like fibroscan and MRI can also assess fibrosis.

SERUM MARKERS:

Serum markers offer an attractive and alternate option to liver biopsy in assessing liver fibrosis. Serum markers refer to the measurement of one or more molecules in serum as a marker of liver fibrosis. An ideal serum marker [71] should be

- Liver specific
- Easy to perform
- Independent of metabolic alterations
- Reflective of fibrosis irrespective of cause
- Sensitive to delineate stages of fibrosis
- Correlate with changes in fibrogenesis.

Biomarkers of fibrosis are broadly divided into Direct and Indirect markers. Direct markers are fragments of liver matrix components produced by hepatic stellate cells during the process of remodelling. Indirect markers are

molecules that are released in the blood due to inflammation, molecules synthesized or disregulated by liver or due to liver impairment.

DIRECT MARKERS:

Procollagen type I carboxy terminal peptide (PICP) and Procollagen type III amino – terminal peptide (PIIINP):

In healthy individuals type I and III are seen abundantly in liver. Durin=g fibrogenesis the levels increase to eight fold. PICP gets elevated in 50% of patients with moderate fibrosis and cirrhosis. Whereas PIIICP are seen elevated in acute hepatitis where it correlates with aminotransferases and also in chronic pancreatitis, lung fibrosis and rheumatological disease making it a non specific marker.

Matrix Metalloproteinases (MMP):

MMP are a family of proteolytic enzymes that mediate degradation of ECM. Three types of MMP are known viz MMP -2, MMP- 3 and MMP-9. Of these types MMP-2 is secreted by stellate cells and is increased during fibrogenesis. MMP-9 is seen elevated in hepatocellular carcinoma.

Tissue inhibitor of matrix metalloproteinases (TIMP):

TIMP are secreted proteins that modulate the function of MMP. Two types of TIMP (1 &2) are present. TIMP-2 specifically inhibits MMP-2 thereby

promoting fibrosis. TIMP-2 is seen elevated in chronic liver disease thus prompting its use as an non-invasive marker of fibrosis.

Hyaluronic acid:

It is a glycosaminoglycan component of extracellular matrix synthesized by stellate cells. It was found to have an important role in detecting fibrosis in NAFLD patients. Since it has a high negative predictive value its main utility is to rule out advanced fibrosis and cirrhosis

Transforming growth factor – β 1(TGF):

TGF is a cytokine involved in tissue growth, ECM production and in immune response. Three isoforms (β1, β2, β3) are available of which TGF β1 is linked to fibrosis progression.

Other markers are YKL -40, Laminin, Paraoxonase 1 and microfibril associated glycoprotein.

Limitations of direct markers are

- Reflect rate of matrix turnover
- Not liver specific as can be elevated in other inflammatory conditions
- Dependent on their clearance rates as influenced by impaired biliary and renal excretion.

INDIRECT MARKERS:

AST/ALT Ratio:

The serum aminotransferases (AST and ALT) are released in the blood during injury to hepatocytes. The ratio has been used in both alcoholic and non-alcoholic liver disease, viral hepatitis, primary sclerosing cholangitis and primary biliary cirrhosis. In these diseases with chronic liver injury the ratio is 1 or less but it is greater than 2 in alcoholic liver disease and hence it lacks its specificity in diagnosing the severity of a particular disease.

AST to Platelet Ratio (APRI):

The APRI ratio was developed by Wai et al in 2003 and is measured by (AST/upper limit of normal)/Platelet (10^9/L) ×100. Studies have shown that ratio of more than 1 and 1.5 suggest fibrosis F3 and cirrhosis respectively. APRI is useful in viral hepatitis, co-infection (HIV/HCV) and in autoimmune hepatitis. Several studies have shown that APRI has high accuracy in detecting advanced fibrosis than in intermediate stage.

Forn's Index:

This index was described by Forns in 2002. It is calculated based on age of the patient, platelet, cholesterol and gamma glutamyl transferase. At a cut off value of 6.9 it helps in differentiating mild (F1) from severe fibrosis (F3-F4) but is less accurate in differentiating F2 from F4.

FIB-4 Score:

This score was first developed by Sterling et al to assess fibrosis in HIV/HCV co-infection. It uses age, platelet, AST and ALT for calculation. It showed AUC of 0.85 and 0.81 in detecting severe fibrosis in HCV and HBV monoinfection respectively. Its performance is better validated in NAFLD when compared to other non-invasive markers.

FIBRO test (Fibrosure):

It is the most validated test in detecting hepatic fibrosis. This test is conducted based on age, gender, serum haptoglobulin, α2 macroglobulin, apolipoprotein A1, gamma glutamyl transferase and bilirubin. The accuracy of the test has been assessed in chronic hepatitis B, C, and in alcoholic and NAFLD. Variable range of values are obtained, 0.75 – 1 and 0.73- 0.75 for F4 and F3 stage of fibrosis. Poynard et al showed its high accuracy in detecting steatohepatitis and during follow up to monitor progression of disease.

ACTI test:

ACTI test is a modification of Fibro test in which ALT is added to the other parameters. It reflects both necroinflammatory activity and fibrosis. Sebastiani et al showed that Acti test has more of negative predictive value for excluding significant fibrosis.

Fibro Index:

This index was formulated by Koda et al in 2007 which constitutes platelet count, AST and gamma globulin. A cut off value of 2.25 is associated with severe fibrosis with a negative predictive value of 90%.

Fibro Q test:

This test was proposed by Hesieh et al in 2009 and it uses age, AST, ALT, platelet count, prothrombin time in calculating the severity of the disease. Studies showed that FibroQ is superior to FIB-4, APRI and AST/ALT ratio in detecting significant fibrosis.

SHASTA Index:

The index is based on AST, albumin and hyaluronic acid. In a study of 95 patients index of 0.3 showed a sensitivity of >88% with a negative predictive value of 94% and a index of 0.8 showed specificity and positive predictive value of 100% in detecting F3 fibrosis.

Fibrospect II:

This combines three parameters – hyaluronic acid, TIMP and α 2 macroglobulin. It is validated in chronic hepatitis C patients and a cut off value of 42 differentiates mild F0-F1 from severe fibrosis F3.

Fibrometer:

This test was first described by Cales et al [72] in 2005. It is performed by combination of panel consisting of age, gender, platelet count, prothrombin time, AST, blood urea nitrogen, α 2 macroglobulin and hyaluronic acid. The test indicates the amount of fibrosis as a percentage of liver tissue fibrosis. This test was validated in chronic hepatitis and alcoholic liver disease and the study demonstrated a AUC of 0.8 and 0.96 for detecting F2-F4 fibrosis in chronic hepatitis B and C infection respectively. When compared to other non invasive marker test, this test has a higher AUC for detecting fibrosis.

Hepascore Model:

The Hepascore model was proposed by Adams et al in 2005. It combines age, gender, bilirubin, GGT, hyaluronic acid and α 2 macroglobulin. The cut off value of 0.5 showed a AUC of 0.9, 0.89 in detecting advanced fibrosis and cirrhosis.

European Liver fibrosis panel (ELF):

ELF calculation is based on age, hyaluronic acid, amino-terminal peptide of type III collagen (PIIINP) and TIMP I. The sensitivity of ELF in detecting stage 3 or 4 fibrosis is 90% with negative predictive value of 92%. It is useful in chronic viral hepatitis, autoimmune hepatitis, ALD and NAFLD.

^{13}C – methacetin breath test (MBT):

MBT and ^{13}C caffeine breath test (CBT) assess cytochrome P450 dependent hepaocellular function. ^{13}C methacetin is metabolized in healty liver into acetaminophen and CO_2. The increase in levels is measured using mass spectrometry or infrared spectroscopy.

PROTEOMICS:

Proteomics based tests assess patterns of protein or glycoprotein by mass spectroscopy. Recent studies have shown that these methods are of limited value in assessing liver fibrosis.

Advantages of Non invasive blood markers (NIBM):

- Non invasive
- Can be repeated for confirmation.
- No associated with complication

Limitations of NIBM:

- Not easily available in all laboratories
- Not useful as a diagnostic tool
- Not liver specific
- Not standardized
- Not informative regarding complications like varices

IMAGING TECHNIQUES:

ABDOMINAL ULTRASOUND:

Ultrasound is a simple technique in diagnosing liver pathology. It can assess liver size, echotexture of the liver, nodularity, portal vein, inerior vena cava, nodes around hepatic artery and spleen size. It is useful in diagnosing advanced disease but its efficacy in detecting fibrosis is doubtful. A new index named Fibrosis Index (FI) was obtained which is calculated using portal vein peak velocity (PVPV) and hepatic atery resistive index (HARI).

FI = HARI/PVPV × 100.

F1 is higher in cirrhotics and is helpful in differentiating it from chronic hepatitis with a cut off value of 3.6. Although ultrasound gives information regarding texture of the liver and portal vein it reliability in differentiating cirrhosis from mild fibrosis has not been proven.

FIBROSCAN:

Fibroscan (Transient Elastography) is a non-invasive technique developed in 2003 to assess the hardness of the liver. Liver stiffness is measured by the velocity of the vibration also called “Shear wave” generated on the skin. In this technique, a 50MHz wave is passed into the liver from a small transducer that can measure the velocity of shear wave in meters per second. Three types of probes/transducer are available viz the M probe or standard probe for most

patients, XL probe for obese patients with a BMI of more than 30kg/m^2 and S probe or paediatric probe for children or adults with narrow rib cage.

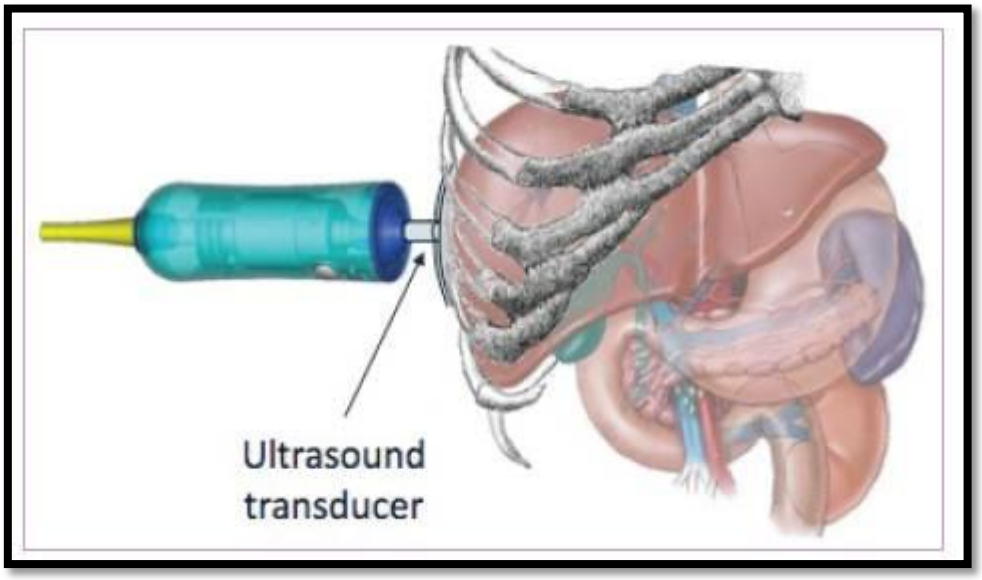


Fig 8: Transient Elastography with Probe

With the patient in lying position, the probe is placed on the skin over the liver area usually in the mid axillary line. Shear wave velocity is determined by measuring the time the vibration wave takes to travel to a particular depth inside the liver. The velocity of the shear wave is directly related to the stiffness of the liver parenchyma, the harder the tissue, the faster the wave propagates. This is then converted into liver stiffness and expressed in kilopascals (kPa).

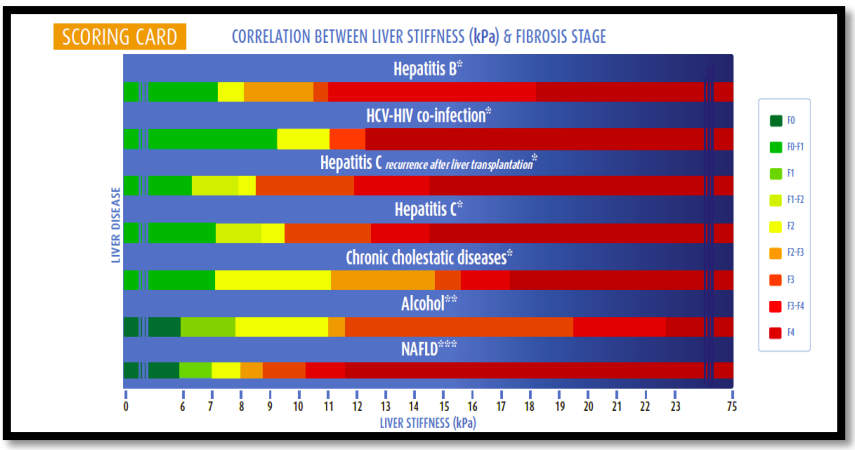


Fig 9: Correlation of liver stiffness with Metavir stage

To improve the efficacy and reliability minimum of 10 valid readings are taken and commuted. Transient elastography explores 1/500 of the total volume of liver parencymal mass at least 100 times the sample taken during liver biopsy.

The normal value of fibroscan ranges from 4.8 to 6.9 kilopascals (kPa). Transient elastography is highly reproducible with minimal inter observer variation. The absolute contraindication to TE is pregnancy and the presence of implantable devices like defibrillators.

ACOUSTIC RADIATION FORCE IMPULSE (ARFI):

ARFI is a type of ultrasound elastography which uses acoustic radiation force associated with the propagation of acoustic waves to generate images of the mechanical properties of the soft tissue. The pulse induce the compression of the tissue producing shear wave that propagates at a velocity proportional to

tissue stiffness which is detected by ultrasound probe. The tracking beams are conventional B mode ultrasound beams (A –lines) used to monitor underlying physiological motion followed by pushing pulse along the same line. The value of more than 2.6 m/sec suggests cirrhosis.

MR ELASTOGRAPHY (MRE):

Magentic resonance elastography is a dynamic elasticity imaging technique that uses mechanical waves to quantify the stiffness of the liver tissue. It is available as an up gradation of MRI scan. The three basic steps of MRE are

i An external driver induces a shear wave of 50-500Hz

ii MRI images these waves in the body

iii The data are quantified to generate images displaying the stiffness.

The liver stiffness directly correlates with the fibrosis stage and progression of the disease. Based on the ROC analysis, a cut off of 2.93 kPa was found to be optimal threshold for distinguishing healthy individuals from fibrotics with a sensitivity and specificity of 98% and 99% respectively.

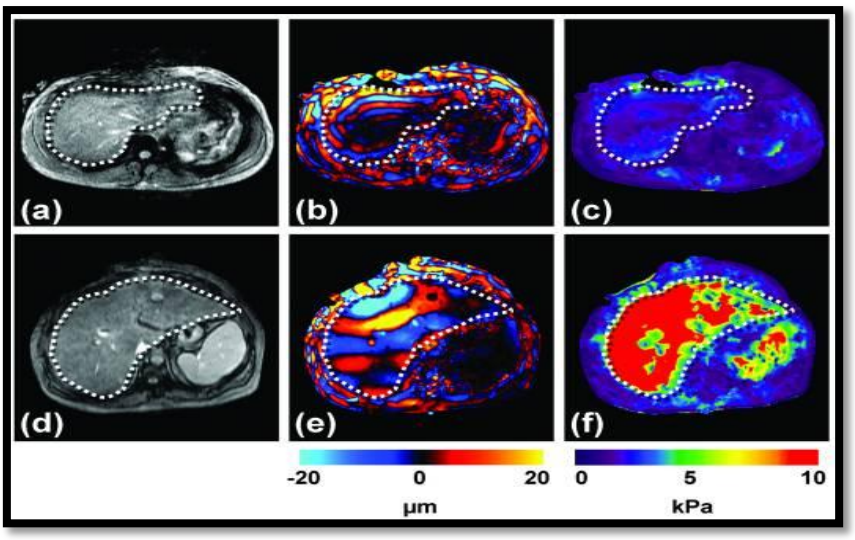


Fig 10: Magnetic Resonance Elastography

a,d : Conventional abdominal MR images of the two patients, showing no significant difference between the two livers. **b,e :**Wave images from the MRE acquisition at 60 Hz showing shear waves with a shorter wavelength in the first patient, and a substantially longer wavelength in the second patient. **c,f** :The corresponding elastograms indicating that the liver in top image was normal (1.7 kPa) and cirrhotic (18.83 kPa) in the bottom picture.

EXTRA-HEPATIC MANIFESTATIONS:

- ❖ Arthritis – Dermatitis
- ❖ Polyarteritis nodosa
- ❖ Glomerulonephritis
- ❖ Cryoglobulinemia
- ❖ Acrodermatitis

TREATMENT OF HEPATITIS B INFECTION:

The primary treatment goals for chronic hepatitis B infection are

- To prevent the progression of disease
- Prevent complications like cirrhosis, liver failure and hepatocellular carcinoma
- Increase the survival of the patient.

Definition of response:

Biochemical response: requires normalization of ALT

Virological response: requires sustained disappearance of HBV DNA from serum for atleast 6 months after treatment and seroconversion of HBeAg

Histological response: required a 2 point improvement in necroinflammatory score without worsening fibrosis.

Various Nucleoside and Nucleotide antivirals are available for the management of chronic Hepatitis B infection. Currently, seven therapeutic agents have been approved for the treatment of adults with chronic hepatitis B in the United States.

- ❖ Interferon (IFN) — conventional and pegylated.
- ❖ Five nucleos(t)ide analogues under three groups.

L –nucleosides – Lamivudine and Telbivudine;

Acyclic nucleoside – Adefovir dipivoxil

Tenofovir disoproxil fumarate; and

Deoxyguanosine analogues – Entecavir

The choice of antivirals are

Clinical Situation	First-Line Therapy	Second-Line Therapy	Comment
Treatment naïve	Entecavir or tenofovir	Telbivudine	Discontinue telbivudine if HBV DNA is still detected in serum at week 24
Prior lamivudine or telbivudine exposure	Switch to tenofovir	Entecavir	Entecavir resistance is facilitated by resistance to either lamivudine or telbivudine
Proved lamivudine resistance	Switch to tenofovir	Add adefovir*	Addition of adefovir late in the course may not control viral replication
Proved adefovir resistance	Switch to entecavir	Tenofovir	Slight reduction in drug susceptibility to tenofovir
Primary drug failure with both lamivudine and adefovir†	Switch to tenofovir or entecavir	Telbivudine	Suspect poor adherence if lamivudine had been used; see telbivudine precaution above
Proved entecavir resistance	Switch to tenofovir	Add adefovir*	—
Proved telbivudine resistance	Switch to tenofovir	Add adefovir*	—
Persistent low-level viremia during treatment with high-genetic-barrier drug	Continue tenofovir or entecavir or switch to the other	None	Nonadherence is possible; resistance testing may be needed
Treatment naïve, decrease in GFR (60-90 mL/min)	Entecavir	Telbivudine	Discontinue telbivudine if HBV DNA is still detected at week 24

AIM OF THE STUDY

AIM OF THE STUDY

1. To detect liver fibrosis in patients with chronic hepatitis B infection using non invasive markers – Fibroscan and Fibrometer test.

2. To compare its efficacy with the gold standard liver biopsy.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN:

This prospective study was conducted in our department, Department of Digestive Health and Diseases, Government Peripheral Hospital, Anna nagar, Chennai attached to Kilpauk Medical College over a period of six months. This study included 30 patients diagnosed to have hepatitis B infection who attended our outpatient department.

INCLUSION CRITERIA:

- Patients infected with chronic hepatitis B virus
- Age more than 18 years

EXCLUSION CRITERIA:

1. Acute hepatitis B infection
2. Patient in immune-tolerant phase
3. Diagnosed to have compensated or decompensated liver disease
4. Co-infection with HCV or HIV
5. Age less than 18 years
6. History of significant alcohol intake.
7. Patients who did not give consent for the study.

METHODS:

CLINICAL:

All the patients included in the study were informed about the study and consent obtained. Detailed history regarding how they were diagnosed to have infection, history of exposure, past history of jaundice or history suggestive of decompensation like ascities, upper GI bleed and encephalopathy, diabetes, hypertension, dyslipidemia were obtained. Patient's personal history regarding smoking, alcohol consumption, tattooing and sexual exposure was obtained.

Patient's general physical and Abdominal, Cardiovascular, Respiratory and Central nervous system were examined in detail to rule out underlying undiagnosed chronic liver disease.

LABORATORY:

Patients were subjected to investigations to evaluate the stage of infection and to assess the degree of fibrosis which included

- Complete blood count
- Blood Sugar, Urea and Serum Creatinine
- Liver function test
- Prothrombin time with INR
- Serology – HbsAg, Anti-HCV, HIV.
- HBeAg, HBV DNA Quantification
- Ultrasound Abdomen
- Fibroscan

- Fibrometer test
- Liver biopsy

FIBROSCAN:

Fibroscan or Transient Elastography (TE) was done at the Department of Hepatology, Madras Medical College and Hospital, Chennai. Patients were referred to the department to undergo scan and to review with results.

The instrument used was Fibroscan (ECHOSENS) 502 Touch model, Version 5. The scan was performed by experienced hepatologist and the results are commuted using Software C1.2 and 1.3. The probes used are M^+ probe or the standard probe which produces a central frequency of 3.5MHz which measures stiffness to a depth of 25 to 65mm. For obese individuals XL probe is used which produces a frequency of 2 MHz and can measure upto a depth of 75 mm. It can measure stiffen from 1.5kPa to a maximum of 75kPa (KiloPascal). A value of upto 6.5 kpa is normal. A value between 6.5 to 8 kpa correlates with Metavir F1/F2, 8 – 12.5 suggests F3 and value more than 12.5 suggest F4 (Cirrhosis).

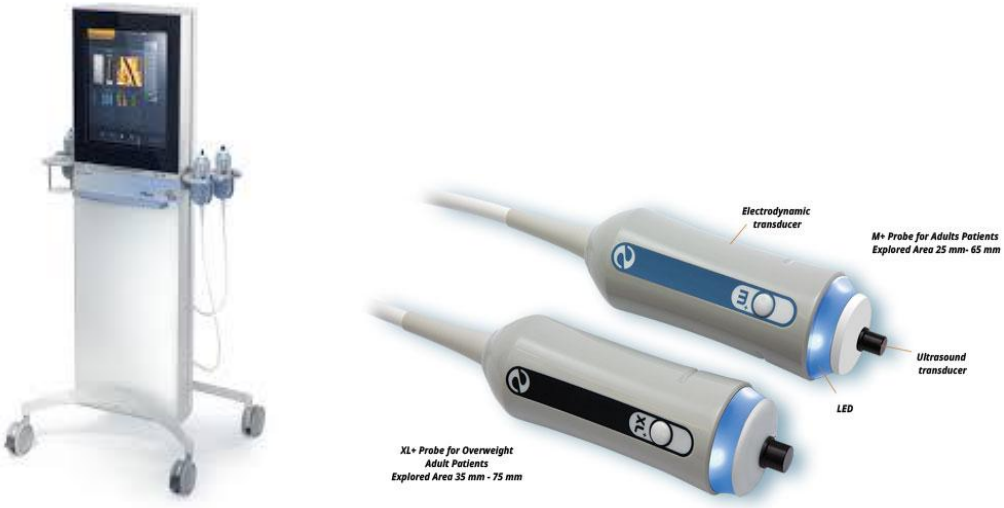


Fig 11: Fibroscan 502 Touch

FIBROMETER TEST:

Fibrometer is a panel of blood test intended as a surrogate marker of liver fibrosis, Cirrhosis and Necro-inflammatory activity.

It consists of various components

Age	Platelets
Gender	Prothrombin index (INR)
AST	Alpa 2 macroglobulin
ALT	Gamma glutamyl tramsferase
Urea	

An algorithm calculates and compares results of these parameters along with age and gender to provide scores (from 0 to 1) and corresponding fibrosis stage and activity grade according to the Metavir Scoring System (F1 – F4).

The test is done outside at SRL Diagnostics centre situated near to our hospital. Patients serum is sampled at the laboratory and results obtained within 3 days.

LIVER BIOPSY:

Liver biopsy was done in our department under ultrasound guidance. Patients were assessed / screened prior to biopsy. Patients were asked regarding history of analgesics, antiplatelets or anticoagulant intake and is so was advised to stop 5 days prior to the procedure. Their blood group, platelet, bleeding time, clotting time and prothrombin time was checked prior to biopsy.

Procedure:

Liver biopsy was done using Automatic Spring loaded gun available in the department. Patient was made lie in supine position and Ultrasound screening was done to mark the site of puncture for biopsy. The area is cleaned with betadine and spirit and drapped. Under strict aseptic precautions, using xylocaine as local anaesthesia liver biopsy was done using gun in patient's expiratory breath and sample obtained. Then the tissue is put in formalin bottle

to be sent for histopatological analysis. Patient was observed in the hospital for a day to look for complications related to biopsy and later discharged in the evening.

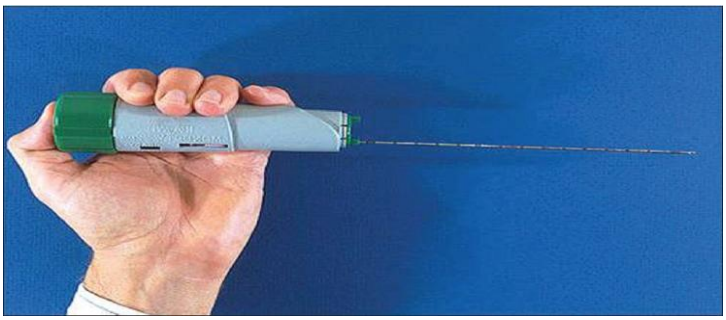


Fig 12: Liver biopsy Gun

STATISTICS:

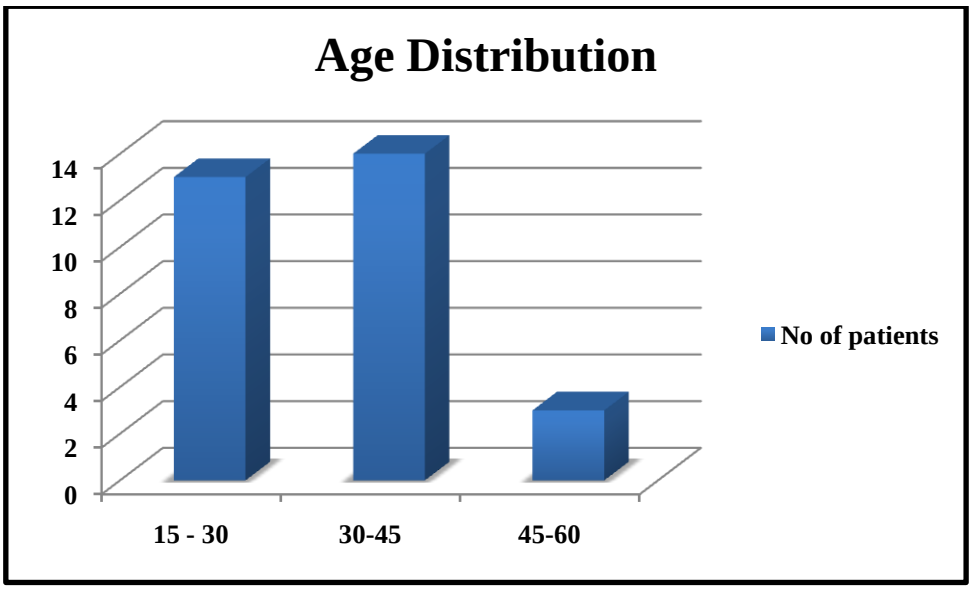
Statistical analysis of these data was done using SPSS version 22. Quantitative data was analysed using Chi square test.

Any statistical difference was considered significant at $P< 0.05$.

OBSERVATION AND RESULTS

AGE DISTRIBUTION OF STUDY POPULATION

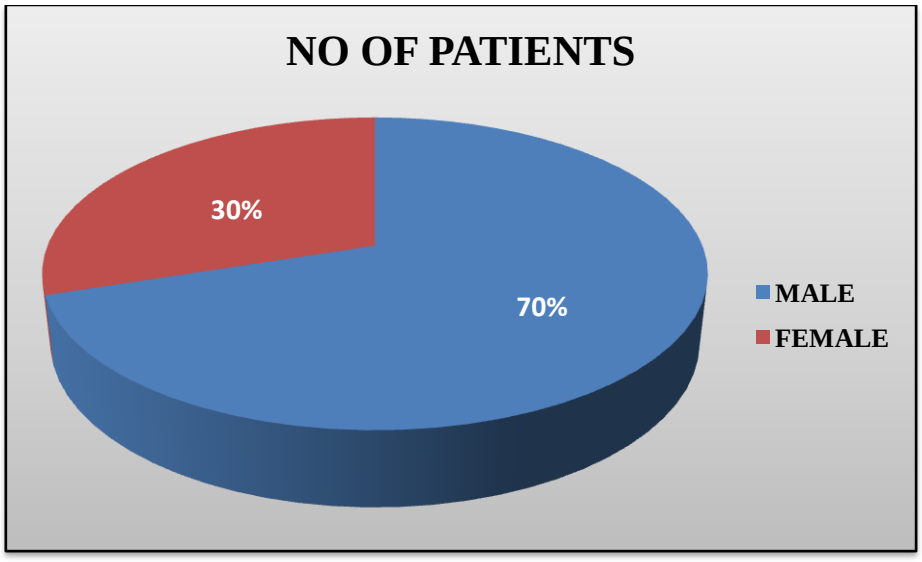
AGE OF PATIENT (Yrs)	NO OF PATIENT	PERCENTAGE
15 – 30	13	43.3%
30 – 45	14	46.7%
45 - 60	3	10%



In our study out of 30 patients 27 were below 45 years of age. Only 3 patients (10%) were above 45 years suggesting early diagnosis of the infection probably because of screening.

SEX DISTRIBUTION

SEX	NO OF PATIENTS	PERCENTAGE
MALE	21	70%
FEMALE	9	30%

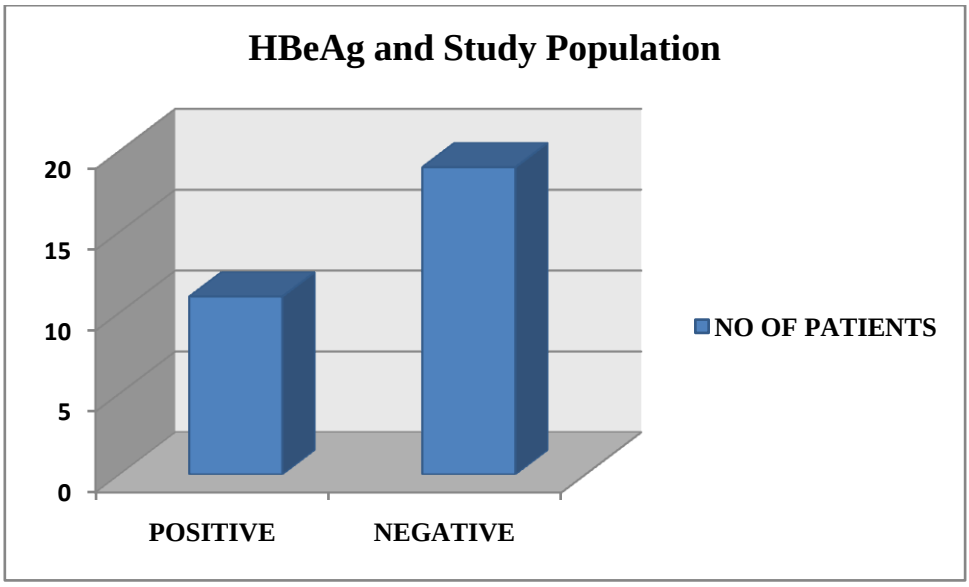


In our study 70% (21) were male patients and 30% were females.

This is probably because of incidence of hepatitis B infection more in males and effective vaccination of females during pregnancy rendering them immune against the infection.

HBeAg AND STUDY POPULATION

HBeAg	NO OF PATIENTS	PERCENTAGE
POSITIVE	11	37%
NEGATIVE	19	63%

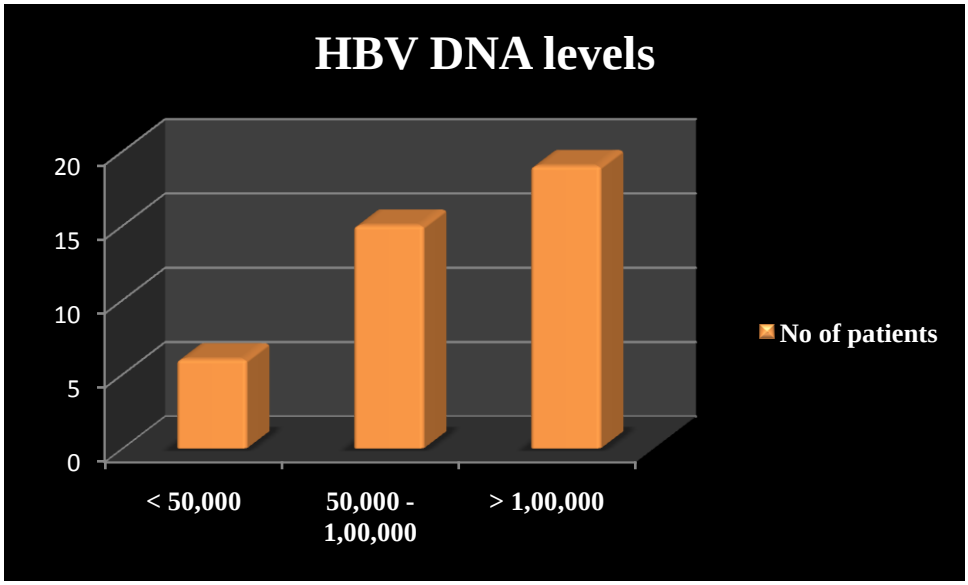


The number of HBeAg positive patients was 11 (37%) and HBeAg negative patient was 19 (63%).

In our study there was more number of HBeAg negative patients (63%) probably due to mutant virus infection or inactive carrier state. HBeAg negative chronic hepatitis B infections are more prone for reactivation in later stage progressing to develop complications.

HBV DNA LEVELS AND STUDY POPULATION

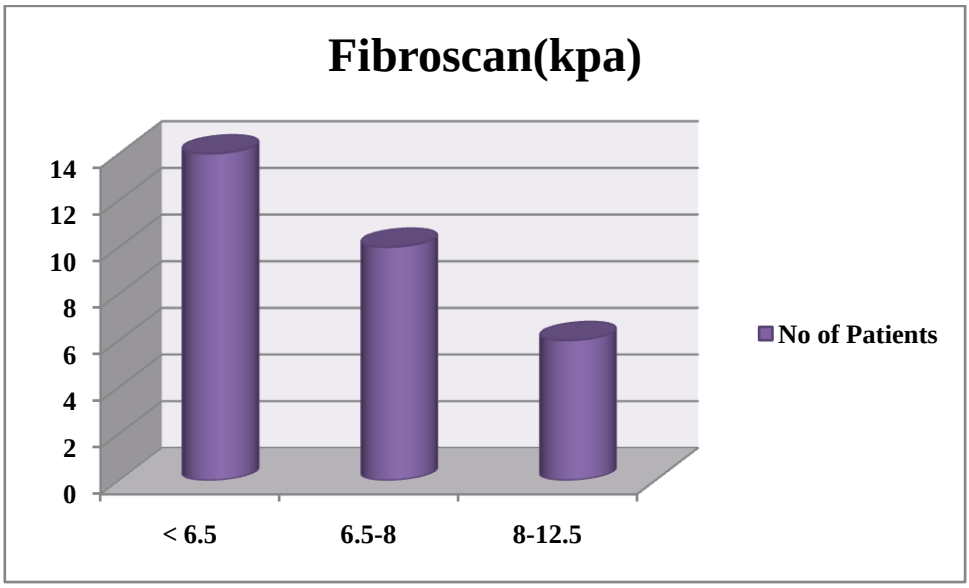
HBV DNA LEVELS (IU/ml)	NO OF PATIENTS	PERCENTAGE
< 50,000	6	20%
50,000 – 1,00,000	5	16.67%
>1,00,000	19	63.33%



About 19 patients (63.33%) of the total study population had viral load of more than one lakh suggesting that these patients were in the immune active phase or chronic active hepatitis. Out of the rest 11 patients 5 had DNA between 50,000 to one lakh and 6 patients had less than 50,000 IU/ml of viral particles.

FIBROSCAN AND STUDY POPULATION

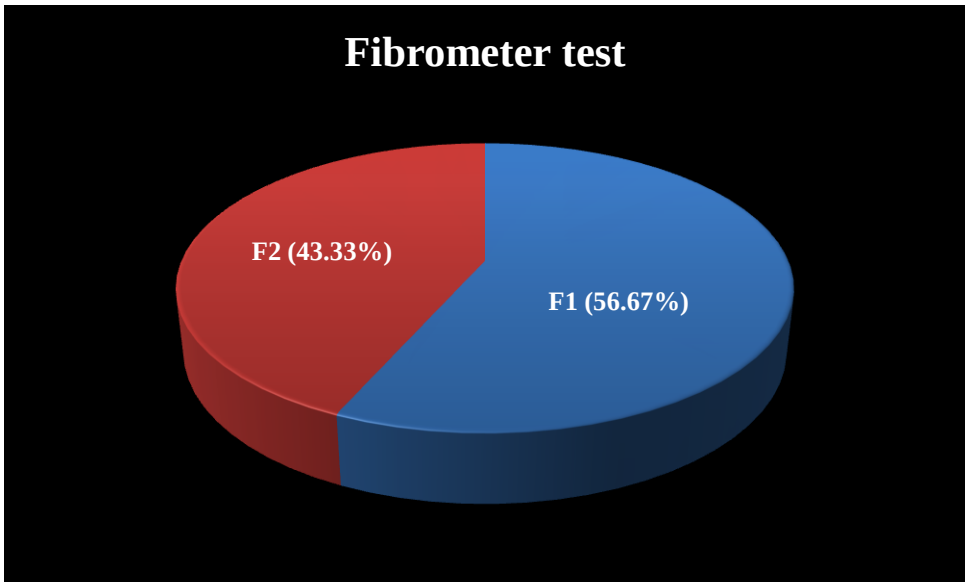
FIBROSCAN (kpa)	NO OF PATIENTS	PERCENTAGE
< 6.5	14	46.67%
6.5 – 8	10	33.33%
8 – 12.5	6	20%



Out of 30 patients 14 (46.67%) patients had value below 6.5 kPa suggesting no hepatitis fibrosis. 10 patients had value between 6.5 to 8 kPa suggesting mild fibrosis of Metavir F1/F2 and 6 had findings suggesting moderate fibrosis.

FIBROMETER AND STUDY POPULATION

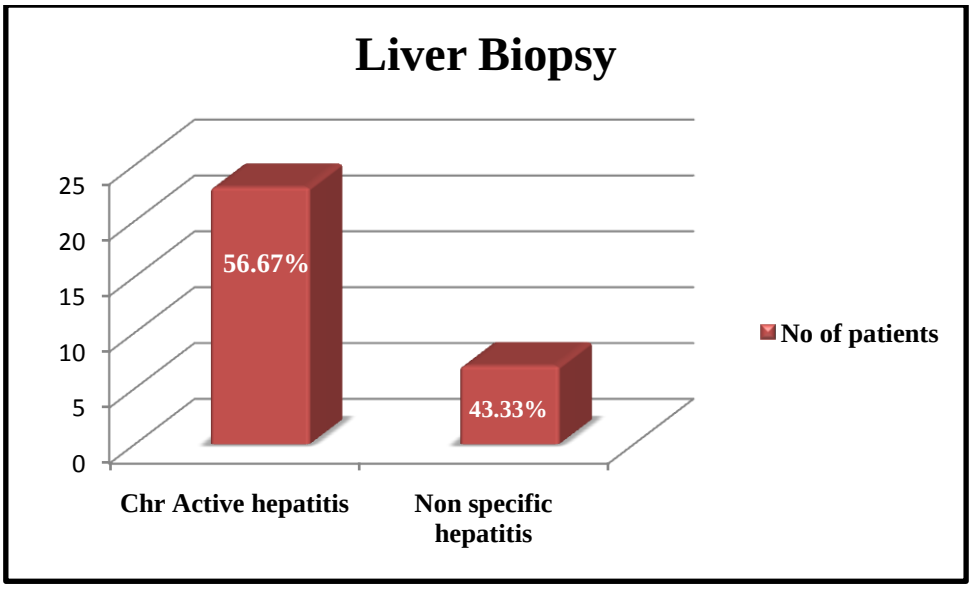
FIBROMETER	NO OF PATIENTS	PERCENTAGE
F1	17	56.67%
F2	13	43.33%



Among 30 patients in our study, 17 (56.67%) patients belonged to F1 stage of Metavir and 13 (43.33%) belonged to F2 stage. None of patients had a value suggesting severe fibrosis or cirrhosis.

LIVER BIOPSY AND STUDY POPULATION

LIVER BIOPSY	NO OF PATIENTS	PERCENTAGE
CHRONIC ACTIVE HEPATITIS	23	76.67%
NON SPECIFIC HEPATITIS	7	23.33%

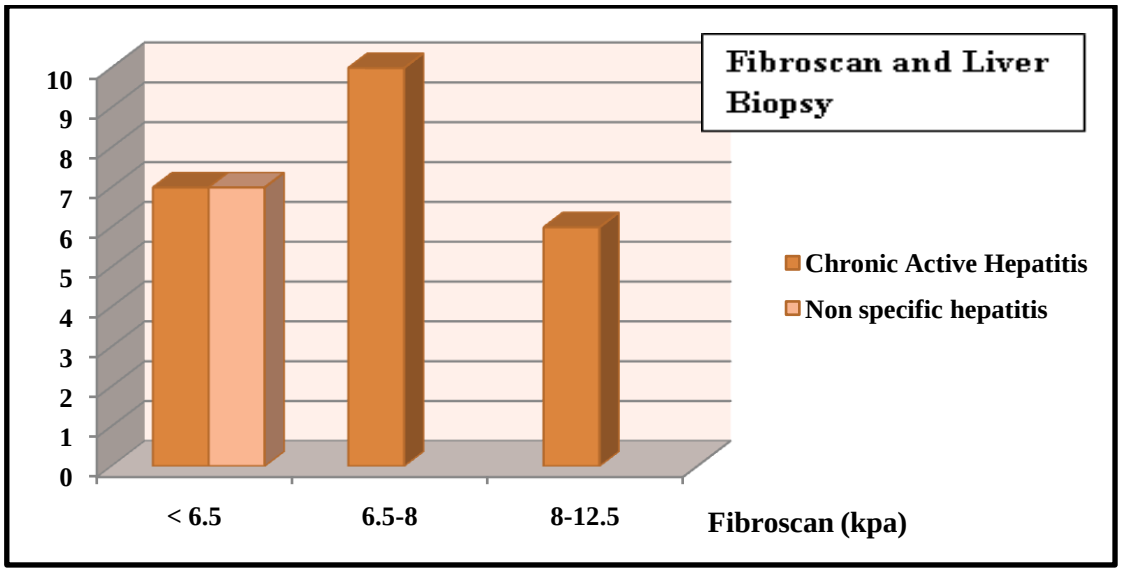


In our study, out of 30 patients 18 patients had histopathological finding suggesting Chronic Active Hepatitis, 3 had features of chronic hepatitis and one had interface hepatitis and mild active hepatitis each. Rest of the patients had only non specific findings not suggestive of active hepatitis. To avoid complexity during analysis patients were grouped under two categories as Chronic Active hepatitis and Non specific hepatitis.

CORRELATION OF PARAMETERS

FIBROSCAN AND LIVER BIOPSY

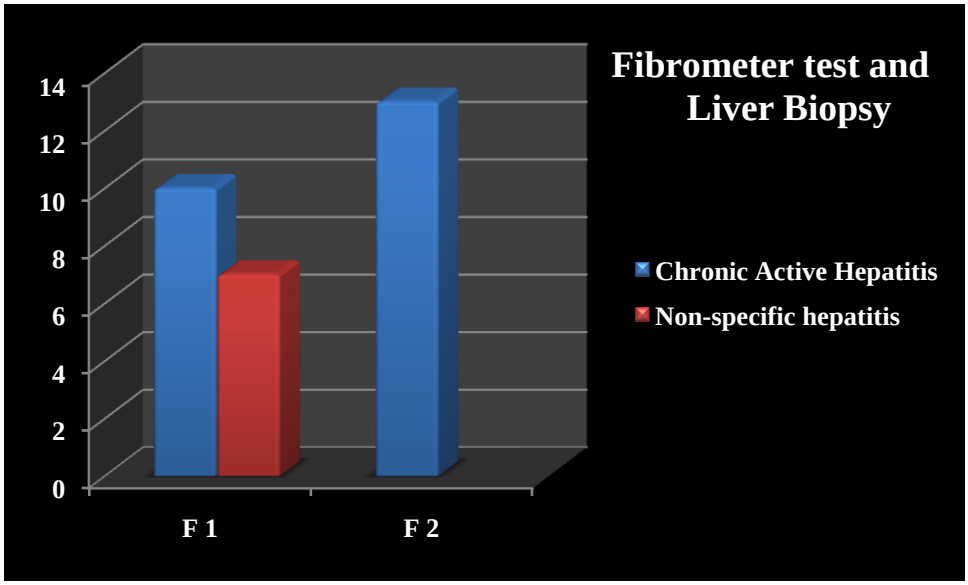
FIBROSCAN	NO OF PATIENTS	CHRONIC ACTIVE HEPATITIS	NON SPECIFIC HEPATITIS	P VALUE
< 6.5	14	7	7	
6.5 – 8	10	10	NIL	**0.005**
8 -12.5	6	6	NIL	



Out of 30 patients 14 patients who had normal fibroscan findings, 7 patients had evidence of chronic active hepatitis in liver biopsy and 7 had non-specific findings. But 10 and 6 patients who had mild and moderate hepatitis based on fibroscan had chronic active hepatitis in liver biopsy which was statistically significant with a P value of 0.005

FIBROMETER AND LIVER BIOPSY

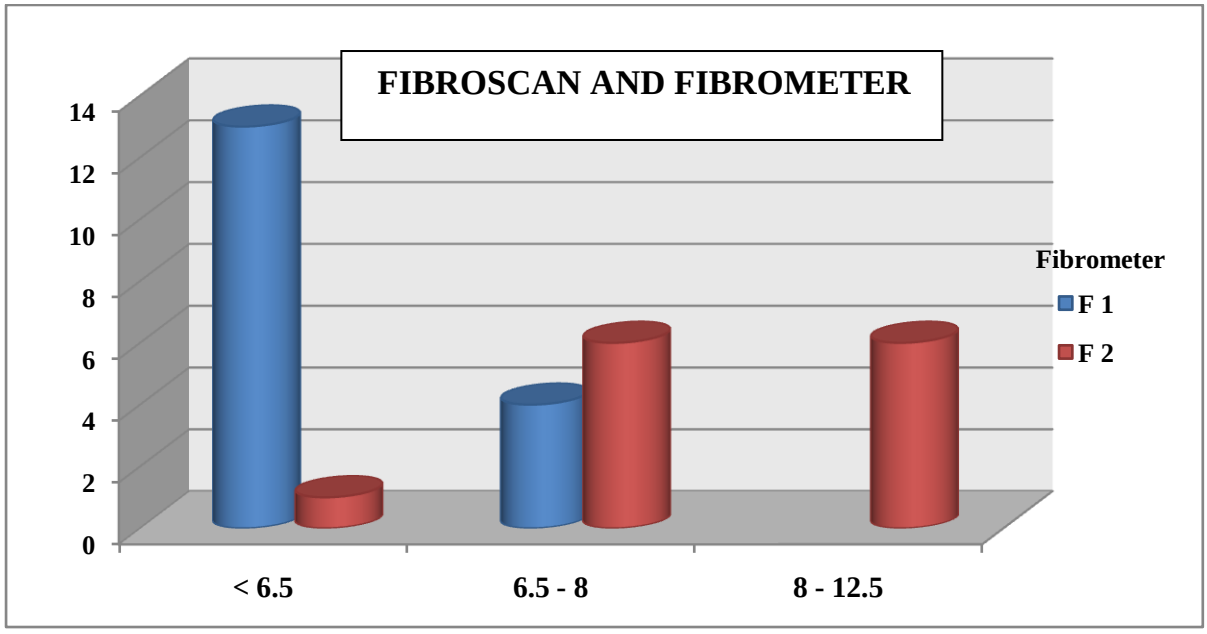
	FIBROMETER		CAH	NSH	P VALUE
NO OF PATIENTS	F1	17	10	7	0.004
	F2	13	13	NIL	



Out of 30 patients 17 patients had scores to suggest F1 and 13 had values suggestive of F2. However out of 17 patients who were categorized for F1 (minimal or nil fibrosis) on fibrometer test, 10 patients had chronic active hepatitis on histo-pathological examination suggesting the superiority of liver biopsy in detecting liver fibrosis than fibrometer test, but of the 13 patients who were categorized as F2 correlated with histopathological finding of active hepatitis which was statistically significant with a P value of 0.004.

FIBROSCAN AND FIBROMETER

FIBROSCAN	NO OF PATIENTS	FIBROMETER		P VALUE
		F1	F2	
< 6.5	14	13	1	
6. 5 – 8	10	4	6	0.001
8 – 12.5	6	NIL	6	



We correlated the efficacy of the two tests in detecting hepatic fibrosis. On correlating, 14 patients who were found to have normal sonography showed minimal or nil fibrosis in Fibrometer test. Same way 6 patients who had high fibroscan value had significant fibrosis (F2) in fibrometer test which was statistically significant with a P value of 0.001.

DISCUSSION

DISCUSSION

Our study was conducted in the Department of Digestive Health and Diseases, Anna nagar over a period of 6 months. Asymptomatic people who were diagnosed to have HBsAg positive on either routine or intentional screening were evaluated for the stage of the disease and evidence of complication ie hepatic fibrosis.

Chronic hepatitis B infection is a dynamic state involving interaction between virus, host hepatocyte and immune response. The clinical presentation of chronic hepatitis is complex and variable that the natural course of disease has changing phases. Only a small percentage of people with chronic hepatitis have past history of acute hepatitis. Rest of the carriers are diagnosed either during health check up or routine screening or for non specific constitutional symptoms. These asymptomatic patients would have acquired infection perinatally (90%) or during childhood (20%) or during adolescence (5%) suggesting that the risk of developing chronic infection is directly proportional to the age of infection, the early the age of infection more likely the chance of chronicity.

The likelihood of spontaneous viral clearance in chronic HBV infection is very low because of extrahepatic reservoir, the integration of HBV DNA into host genome (cccDNA). The overall annual rate or progression of chronic hepatitis to cirrhosis is estimated to be 2 -10%, as it may vary depending upon

the envelope antigen (HBeAg) status. HBeAg negative chronic hepatitis patients are more prone to develop complications, probably related to mutant virus or more advanced liver disease at the time of presentation. Hence these chronic hepatitis patients need to be evaluated for complications, as early intervention prevents progression of disease to cirrhosis and also can avoid long term complication like hepatocellular carcinoma.

The average age of the study population was 33 years with the youngest age being 18 years. Asymptomatic people were diagnosed to have hepatitis B infection probably detected during screening.

In our study group males were predominant (70%) probably due to selection bias or due to decreased incidence of infection among women because of effective vaccination schedule during pregnancy. Other factor which predisposes more prevalence in males is probably due to factors like tattooing, sexual promiscuity and aseptic techniques of injections. As HBV is stable in environment on inanimate surface for one week, transmission can occur even in the absence of visible blood [73].

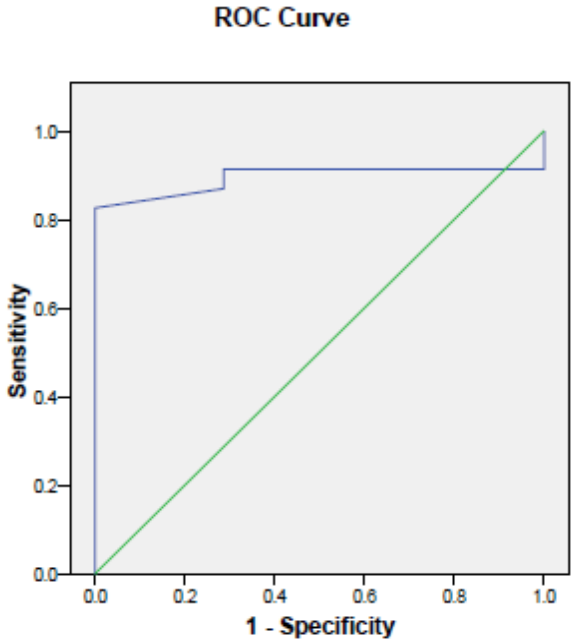
Our study showed a high prevalence of HBeAg negative patients in our group probably suggesting mutant virus infection. The prevalence of HBeAg negative chronic hepatitis varies from different geographical areas with a high proportion in Mediterranean region (80% - 90%). A study by Amarapurkar[74] in 2002 quotes that HBeAg chronic hepatitis is not a uncommon entity and these

patients progress to chronic liver disease which correlate our study of high prevalence of HBeAg negative patients.

In this study 63.33% (19 out of 30) of patients had very high viral load of more than one lakh suggesting that these patients are in immune active phase or reactivation phase. These patients need evaluation to rule out hepatic fibrosis as persistent high viral load progress to liver disease which is evident by Asian studies[75] that serum HBV DNA is the single best predictor of future progression to liver disease and later hepatocellular carcinoma.

Fibroscan is a non invasive method of assessing hepatic fibrosis. The values are graded with a range that correlates with the Metavir scoring system. The average value in our study was 6.4 with a min of 3.2 kpa and a maximum of 12.3 kpa. In our study 14 patients (46.67%) has a score less than 6.5 suggesting nil hepatic fibrosis. 33% of the study group had values suggesting mild fibrosis and 20% had severe fibrosis. Among these 14 patients, histopathological examination showed chronic active hepatitis in half of the patients and non-specific hepatitis suggesting that sensitivity of fibroscan in detecting early fibrosis is poor. However with a value greater than 6.5 kpa the efficacy of fibroscan correlates with the liver biopsy with statistically significant P value of 0.005. Our study showed that the fibroscan value of ≥ 8kpa had 100% specificity in detecting significant fibrosis with ROC value of 0.89 (95%

CF – 0.76 to 0.9). This correlates with the previous studies that, diagnosing significant fibrosis of F2/F3 by fibroscan should have a higher kpa.

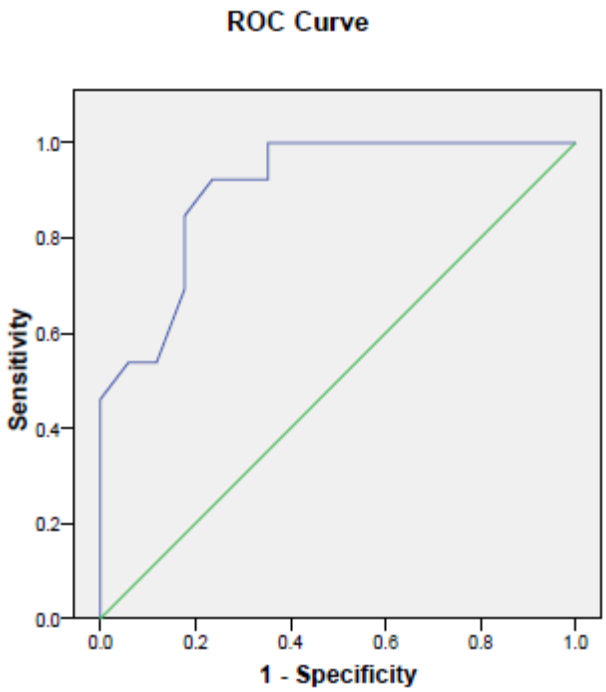


Ref.	Patients (*n*)	Cut-off (kPa)	Sn	Sp	LR⁻	LR⁺	AUROC (95%CI)
Oliveri *et al*[21]	188	7.5	93%	88%	0.07	8.2	0.96 (0.94-0.99)
Marcellin *et al*[89]	173	7.2	70%	83%	0.36	2.6	0.81 (0.73-0.86)
Chan *et al*[34]	161	8.4	84%	76%	0.20	3.5	0.87 (0.82-0.93)
Degos *et al*[90]	284	5.2	89%	38%	0.28	1.4	-
Viganò *et al*[25]	217	8.7	64%	92%	0.40	7.5	-
Verveer *et al*[27]	241	6.0	-	-	-	-	0.85
Cardoso *et al*[26]	202	7.2	74%	88%	0.30	6.2	0.86

Fibroscan has a good efficacy in detecting moderate to severe fibrosis and also has got good negative predictive value to rule out a disease. But in our study fibroscan was able to detect only severe fibrosis which correlated with gold standard liver biopsy. Marcellin P et al [76] showed that the utility of fibroscan in detecting stage four fibrosis (cirrhosis) has a sensitivity of 87% and

a specificity of 91%. These datas suggest that Fibroscan is a good non invasive method to detect liver fibrosis in moderate and severe stage, but its efficacy in detecting early fibrosis is questionable. However various studies have concluded that values of normal range can be used as a marker to rule out hepatic fibrosis in chronic hepatitis B infection.

Fibrometer is a panel of blood test to detect hepatic fibrosis. In our study 13 patients (43.33%) had significant fibrosis which as detected both by fibrometer and liver biopsy which was statistically significant with a P value of 0.004. The ROC value of fibrometer in detecting significant fibrosis is 0.905 with 95% (CF) confidence interval of 0.80 to 0.99. This correlates with the study that Fibrometer can be used to detect moderate and severe fibrosis.



Both fibrometer and fibroscan has good efficacy in detecting significant fibrosis. For a liver stiffness measurement of more than 6.5 kpa the sensitivity and specificity of detecting significant fibrosis increases, which correlates with the histopahological examination. The statistical data and ROC value also showed not much of difference between the two tests in detecting significant fibrosis. In our study the detection of fibrosis by fibroscan with a liver stiffness measurement value of > 8.5 and by fibrometer is 100% which correlated with the liver biopsy, however both these tests had a low sensitivity in detecting liver fibrosis with a value <6.5 and F1.

A study of liver stiffness measurement from Senegalese Chronic Hepatitis B [77] patients with normal ALT and high viral load showed that low values of fibroscan, fibrometer were not useful in detecting liver fibrosis.

CONCLUSION

CONCLUSION

1. Fibroscan is a good non invasive marker in detecting moderate to severe fibrosis.
2. Fibrometer test also detected hepatic fibrosis, but lower values suggesting F1 were not correlating with liver biopsy suggesting that fibrometer is a good test in predicting moderate fibrosis which correlated with histopatological study, but its efficacy in detecting early fibrosis is poor.
3. Both Fibroscan and Fibrometer has similar efficacy in detecting moderate to severe fibrosis.
4. Liver biopsy still is the gold standard test in detecting hepatic fibrosis, but can be considered in patients with low LSM and fibrometer values to detect early fibrosis.
5. Fibroscan with a Liver Stiffness Measurement value of more than 8 can be taken as a cut off to start anti-viral therapy.
6. Fibrometer is a good test and can be combined with fibroscan in assessing hepatic fibrosis in asymptomatic hepatitis B infected patients.

LIMITATION

LIMITATIONS

1. It is a pilot study.
2. Small study population.
3. Fibrometer is not universally available in all centres making it difficult to use as a fibrosis detection tool.
4. More studies with more study population is required to correlate the study.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Sleisenger and Fordtran's – Gastrointestinal and Liver Diseases – 10th edition.
2. Schiff's – Disease of the liver – 11th edition.
3. AASLD Guidelines 2009.
4. McQuillan GM, Coleman PJ, Kruszon-Moran D, Moyer LA, Lambert SB, Margolis HS. Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994. [see comments]. Am J Pub Health 1999;89(1):14-18.
5. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma.Cancer 1988; 61(10):1942-1956.
6. Fibroscan can avoid liver biopsy in Indian patients with chronic hepatitis B. Goyal R, Mallick SR, Mahanta M, Kedia S, Shalimar, Dhingra R, Sharma H, Das P, Datta Gupta S, Panda S, Acharya SK. J Gastroenterol Hepatol. 2013 Nov;28(11):1738-45.
7. FibroMeters: a family of blood tests for liver fibrosis.Calès P, Boursier J, Oberti F, Hubert I, Gallois Y, Rousselet MC, Dib N, Moal V, Macchi L, Chevailler A, Michalak S, Hunault G, Chaigneau J, Sawadogo A Lunel Gastroenterol Clin Biol. 2008 Sep;32(6 Suppl 1):40-51. doi: 10.1016/S0399-8320(08)73992-7
8. Ott JJ, Stevens GA, George J, Weisma ST. Global epidemiology of hepatitis B virus infection. New estimates of age specific HBsAg seroprevalence and endemicity. Vaccine 2012; 30:2212 – 2219.
9. Mason A, Wick M, White H, et al. Hepatitis B virus replication in diverse cell types during chronic hepatitis B virus infection. Hepatology 1993; 18:781-9.
10. WHO. Introduction of Hepatitis B vaccine into childhood immunization service Management Guidelines. Geneva WHO 2001.

11. Lok ASF, McMohan BJ. Chronic Hepatitis B. AASLD Practical guidelines. Hepatology 2007; 45:507-539.
12. Nowak MA, Bonhoeffer S, Hill AM, et al. Viral dynamics in hepatitis B virus infection. Proc Natl Acad Sci USA 1996; 93:4398-402.
13. Lin CL, Kao JH. The clinical implications of hepatitis B virus genotype: Recent advances. J Gastroenterol Hepatol 2011; 26(Suppl 1):123-30.
14. Chu CJ, Keeffe EB, Han SH, et al. Hepatitis B virus genotypes in the United States: Results of a nationwide study. Gastroenterology 2003; 125:444-51.
15. Schiodt FV, Davern TJ, Shakil AO, et al. Viral hepatitis related acute liver failure. Am J Gastroenterol 2003; 98:448-53.
16. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis 2005; 25(Suppl 1):3-8.
17. Rehermann B. Immune responses in hepatitis B virus infection. Semin Liver Dis 2003; 23:21-38.
18. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol 2001; 19:65-91.
19. Ferrari C, Missale G, Boni C, et al. Immunopathogenesis of hepatitis B. J Hepatol 2003; 39(Suppl 1):S36-42.
20. Curry MP, Koziel M. The dynamics of the immune response in acute hepatitis B: New lessons using new techniques. Hepatology 2000; 32:1177-9.
21. Liaw YF, Brunetto M, Hadziyannis S. The natural history of chronic HBV infection and geographical difference. Anivir Ther 2010; 15:S25-S33.
22. Liaw YF, Chu CM. Hepatitis B virus infection. Lancet 2009; 373:582 - 592.

23. Chu CM, Karayiannis P, Fowler MJ et al. Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. Hepatology 1985; 5: 431 -434.
24. Milich DR, Chen MK, Hughes JL, et al. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: A mechanism for persistence. J Immunol 1998; 160:2013-21.
25. Gonzalez S, Perrillo R. Immunologic understanding of chronic hepatitis B and the clinical events that shape its natural history. In: Gershwin E, Vierling JM, Manns MP, editors. The liver: Basic and clinical practices of immune mediated diseases. 3rd ed. New York: Springer; 2013.
26. Chu CM, Liaw. Intrahepatic distribution of hepatitis B surface antigen and core antigens in Chronic HBV infection, as a possible target for hepatolysis. Gastroenterology 1987; 92:220-225.
27. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: Special emphasis on disease progression and prognostic factors. J Hepatol 2008; 48:335-52.
28. Hsu YS, Chein RN, Yeh CT et al. Long term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002;35 :1522-1527.
29. Chen YC, Huang SF, Chu CM, Liaw YF. Serial HBV DNA levels in patients with persistently normal transaminases over 10yrs following spontaneous HBeAg seroconversion. J Viral Hepat 2011; 19:138-146.
30. Liaw YF, Lau GKK, KaoJH, Gane E. Hepatiis B e antigen seroconversion : a critical event in chronic hepatitis B infection. Dig Dis Sci 2010; 55: 2727-2734.
31. Chu CM, Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal sudy of HBeAg positive patients

with normal aminotransferase level at baseline. J.Hepatol 2005; 43:411-417.

32. Livingston SE, Simonetti JP, Bulkow LR, et al. Clearance of hepatitis e antigen in patients with chronic hepatitis B and genotypes A,B,C,D and F. Gastroenterology 2007; 133: 1452-1457.
33. Wu CF, Yu MW, Lin CL et al. Long term tracking of hepatitis B viral load and the relationship with risk of hepatocellular carcinoma in men. Carcinogenesis 2008; 29: 106 -112
34. Liaw YF,Kao JH, Piratvisuth T et al. Asian – Pacific Consensus statement on the management of chronic hepatitis B: 2012 update. Hepat Int 2012; 6:531- 561.
35. Chen YC, Chu CM, Liaw YF. Age specific prognosis following spontaneous hepatitis B e antigen seroconversion in chronic hepatitis B. Hepatology 2010; 51: 435-444.
36. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunological mediated liver disease. Gastroenterology 2001; 120: 1009-1022.
37. Chu CM, Law YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow up. Hepatology 2007; 45: 1187 -1192.
38. Chan HL, Wong GL, Tse CH et al. Viral determinants of hepatitis B surface antigen seroclearance in hepatitis e antigen negative chronic Hep B patients. J Infec Dis 2011; 204: 408-414.
39. Tseng TC, Liu CJ, Yang HC e al. Determinants of spontaneous surface antigen loss in HBeAg negative patients with a low viral load. Hepatology 2012; 55: 68-76.
40. Chen YC, Jeng WJ, Chu CM, Liaw YF. Decreasing levels of HBsAg predict HBsAg seroclearance in patients with inactive chronic Hep B virus infection. Clin Gastroenterol Hepatol 2012; 10: 297-302.

41. Liu J, Yang HI, Lee M et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community based follow up study. Gastroenterology 2010; 139: 474-482.
42. Chu CM, Liaw YF. Prevalence of and risk factors for hepatitis B viremia after spontaneous hepatitis B surface antigen seroclearance in hepatitis B carrier. Clin Infect Dis 2012; 54: 88-90.
43. Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther 2010; 15: 133-143.
44. Liaw YF, Brunetto M, Hadziyannis. The natural history o chronic BV infection and geographical differences. Antivir Ther 2010; 15: S25 –S33.
45. Funk ML, Rosenberg DM, Lok AS.Worldwide epidemiology of HBeAg negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat 2002; 9: 52-61.
46. Chu CM et al. Spontaneous relapse of hepatitis in inactive HBsAg carrier. Hepatol Int 2007; 1: 311-315.
47. Yang , Hung HL, Lee MH et al. Incidence and determinants of spontaneous seroclearance of HBe anigen and DNA in patients with chronic hepatitis B. Clin Gastroenterol Hepatol 2012; 10: 527-534.
48. Chu CM et al. Predictive factors for reactivation of hepatitis B following HBeAg seroconversion in Chronic hepatitis B. Gastroenterology 2007; 133: 1458-1465.
49. Zacharakis G, Koskinas J, Kotsiau S et al. The role of serial measurement of serum HBV DNA levels in patients with chronic HBeAg negative hepatitis B infection: association with liver disease progression.A Prospective cohort study. J Hepatol 2008; 49: 884-891.
50. Alexandra Valsamakis. Department of Pathology, Division of Clinical Microbiology, The Johns Hopkins Medical Institutions, Baltimore, Maryland. Molecular Testing in the Diagnosis and Management of

Chronic Hepatitis B. Clinical microbiology reviews, July 2007, Vol 20 No.3, P: 426–439

51. Hui CK, Bowden S, Zhang HY, Wong A, Lewin S, Rousseau F, Mommeja-Marin H, Lee NP, Luk JM, Locarnini S, Leung N, Naoumov NV, Lau GK. Comparison of real-time PCR assays for monitoring serum hepatitis B virus DNA levels during antiviral therapy.J Clin Microbiol. 2006 Aug; 44(8):2983-7
52. Shyamala V, Arcangel P, Cottrell J, Coit D, Medina-Selby A, McCoin C, Madriaga D, Chien D, Phelps B. Assessment of the target-capture PCR hepatitis B virus (HBV) DNA quantitative assay and comparison with commercial HBV DNA quantitative assays. J Clin Microbiol. 2004 Nov; 42(11):5199-204.
53. Hochberger S, Althof D, Gallegos de Schrott R, Nachbaur N, Röck H, Leying H. Fully automated quantitation of hepatitis B virus (HBV) DNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan system.J Clin Virol.2006 Apr; 35(4):373-80
54. Moucari R, Mackiewicz V, Lada O, et al. Early serum HBsAg drop: A strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009; 49:1151-7.
55. Chan HL, Thompson A, Martinot-Peignoux M, et al. Hepatitis B surface antigen quantification: Why and how to use it in 2011. A core group report. J Hepatol 2011; 55:1121-31.
56. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008; 48:335–52.
57. Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: What we knew in 1981 and what we know in 2005. Hepatology 2006; 43:S173–81.

58. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295:65-73.
59. Chu CM, Liaw YF. Hepatitis B virus related cirrhosis: Natural history and treatment. Semin Liver Dis 2006; 26:142-152.
60. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988; 61:1942–56.
61. Brechot C. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. Gastroenterology 2004; 127:S56–61.
62. Koike K. Hepatitis B virus X gene is implicated in liver carcinogenesis. Cancer Lett 2009; 286:60–8.
63. Malhi H, Guicciardi ME, Gregory J, Gores GJ: Hepatocyte death: a clear and present danger. Physiol Rev 2010, 90:1165-1194.
64. Roberts RA, Ganey PE, Ju C, Kamendulis LM, Rusyn I, Klaunig JE: Role of the Kupffer Cell in Mediating Hepatic Toxicity and Carcinogenesis. Toxicological Sciences 2006, 96(1):2-7, Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev. 2008, 88(1): 125-172.
65. Jiao J, Friedman SL, Aloman C: Hepatic fibrosis. Curr Opin Gastroenterol 2009, 25(3):223-229.
66. Baranora et al. Non invasive Markers of hepatic fibrosis. BMC Gastroenterology 2011. 11:91
67. SK Sarin et al. Liver Fibrosis: Consensus recommendation of the Asian Pacific Association for the Sudy of Liver (APASL). Hepatol Int 2009. 3: 323- 333.
68. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–435.

69. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996; 24:289–293.
70. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22:696–699.
71. Kelleher TB, Afdhal N. Noninvasive Assessment of Liver Fibrosis. Clinics in Liver Disease 2005; 9: 667-683.
72. P. Cal`es, F. Oberti, S. Michalak et al., "A novel panel of blood markers to assess the degree of liver fibrosis," Hepatology, vol. 42, no. 6, pp. 1373–1381, 2005.
73. Lok ASF, Macmohan BJ. Chronic Hepatitis B: AASLD practice guidelines. Hepatology 2007; 45: 507-539.
74. Amarapurkar DN et al. Profile of hepatitis B e antigen negative chronic hepatitis B infection. Indian J Gastroenterol 2002. June; 21(3): 99-101.
75. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295:65-73.
76. Marcellin P et al. Diagnosis of fibrosis and cirrhosis by transient elastography in HIV/HCV coinfected patients. J Acquir Immune Def Synd 2006; 41: 175 – 179.
77. Papa Saliou Mbaye et al. Liver Stiffness Measurement and Biochemical Markers in Senegalese Chronic Hepatitis B Patients with Normal ALT and High Viral Load. July 2011; Volume 6: Issue 7.

APPENDIX

Class Homepage

This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.

Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations

	Info	Dates	Similarity	
TNMGRMU EXAMINATIONS	ⓘ	Start 01-Sep-2014 11:27AM Due 15-Aug-2015 11:59PM Post 15-Aug-2015 12:00AM	24%	Resubmit View

PROFORMA

Name: Age/Sex: IP No:

Complaints:

Past history:

DM HTN TB DYSLIPIDEMIA

Family history:

Personal history: Alcohol: Smoking:

Others:

Examination:

GPE: pallor /icterus/cyanosis/clubbing/edema/lymphnodes

Pulse : B.P

Abdomen :

C.V.S: R.S:

CNS:

Investigations:

Hb%: TC: ESR: Plt:

LFT: TB: DB/IB: OT/PT:

SAP: Protein/albumin/globulin:

RBS : FBS/PPBS:

PT/INR:

Blood urea: S. Creatinine:

HBsAg: HBeAg/antiHBeAg:

Anti HCV: HBV DNA:

HIV:

USG abdomen:

Chest X-ray:

OGD:

FIBROMETER VIRUS:

FIBROSCAN:

LIVER BIOPSY:

SI NO	NAME	AGE	SEX	PAST HISTORY		SURGERY	BLOOD TRF	TATTOO	SMOKING	ALCOHOL	GEN EXAM	OTHER SYS	PER ABD
				DM/HTN	DYSLIPID								
1	JOTHILAKSHMI	51	F	NO	NO	PS	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
2	SASIRAJA	33	M	NO	NO	NIL	NO	NO	NO	YES*	NORMAL	NORMAL	NORMAL
3	JEEVARATHINAM	33	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
4	DHANASEKAR	39	M	1 Yr/NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
5	KUPPAN	36	M	NO	NO	NIL	NO	YES	NO	YES*	NORMAL	NORMAL	NORMAL
6	RANJITH	34	M	NO	NO	NIL	NO	NO	YES	NO	NORMAL	NORMAL	NORMAL
7	BHOOPATHI	59	M	2Yr/3Yr	NO	NIL	NO	NO	NO	OCC	NORMAL	NORMAL	NORMAL
8	GOPINATH	23	M	NO	NO	YES*	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
9	ARUTSELVAN	29	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
10	PALANIAMMAL	34	F	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
11	MANIGANDAN	34	M	NO	NO	NIL	NO	NO	YES	YES*	NORMAL	NORMAL	NORMAL
12	KIRUBAKARAN	18	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
13	JHANSI RANI	19	F	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
14	PERUMAL	32	M	NO	NO	NIL	NO	NO	NO	YES*	NORMAL	NORMAL	NORMAL
15	VIJAYALAKSHMI	30	F	NO	NO	NIL	NO	NO	9	NO	NORMAL	NORMAL	NORMAL
16	RAJESWARI	35	F	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
17	PARVATHY	30	F	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
18	CHANDRA	40	F	NO/YES	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
19	CHANDRU	29	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
20	MARKANDAIYAN	43	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
21	KEVIN	47	M	NO/YES	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
22	DALPATTU SAMY	26	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
23	SABARIPRASANNA	37	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
24	ANAND	34	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
25	KRISHNAVENI	23	F	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
26	KUMARAVEL	28	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
27	PUNITHA	20	F	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
28	KARUNAKARAN	28	M	NO	NO	NIL	NO	NO	NO	YES*	NORMAL	NORMAL	NORMAL
29	SIVA	22	M	NO	NO	NIL	NO	NO	NO	NO	NORMAL	NORMAL	NORMAL
30	MANNAR	45	M	NO	NO	NIL	NO	NO	YES	NO	NORMAL	NORMAL	NORMAL

SI NO	NAME	HB(%)	TC/DC(P/L)	ESR(1hr)	PLATELET	TB/DB	ALT/AST	SAP	PROT/ ALB	SUGAR	UREA	CREAT	PT/INR
1	JOTHILAKSHMI	13.5	6500/64/28	28	167,000	0.8/0.4	39/42	100	6.9/4.5	104	28	1	12/1.0
2	SASIRAJA	13.8	9100/72/22	10	297,000	0.6/0.3	21/22	110	7.7/4.4	79	30	0.9	13.O/1
3	JEEVARATHINAM	11.4	7400/66/34	8	146,000	0.8/0.5	35/32	116	7.8/4.9	112	28	0.8	13.0/1.02
4	DHANASEKAR	10.4	8400/58/38	26	210,000	1.4/0.8	32/17	146	6.2/4.3	185	30	1.1	14/1.2
5	KUPPAN	12.8	8100/67/30	15	240,000	0.5/0.3	14/30	42	6.6/3.8	124	30	0.9	12.3/0.8
6	RANJITH	11.8	7100/67/33	20	210,000	1.4/0.8	30/47	89	6.8/4.2	124	32	1	10.8/0.97
7	BHOOPATHI	11.4	9200/54/46	10	267,000	1.0/0.5	30/43	127	5.8/3.5	146	32	1.1	13.1/0.94
8	GOPINATH	11.6	7600/60/36	20	197,000	1.2/0.8	40/97	136	5.6/3.2	108	32	1.2	13.1/1.09
9	ARUTSELVAN	12.1	7800/76/23	24	230,000	1.2/0.7	47/42	57	7.2/3.7	114	28	0.9	14.1/1.2
10	PALANIAMMAL	10.8	6400/54/43	16	176,000	1.0/0.6	14/22	84	7.3/3.8	103	26	1	15.6/1.16
11	MANIGANDAN	11.4	6700/62/30	16	236,000	1.6/1.0	56/38	55	7.6/4.4	118	25	0.9	13.2/1.17
12	KIRUBAKARAN	11.8	7800/72/28	10	214,000	2.3/1.8	21/46	92	7.2/4.5	96	26	1	12.8/1.0
13	JHANSI RANI	10.8	5600/60/24	12	136,000	0.7/0.4	27/20	141	7.1/4.6	84	23	0.8	12.6/1.0
14	PERUMAL	12.8	7200/63/34	20	219,000	1.4/0.9	40/36	184	6.0/3.6	112	32	1	13.6/1.0
15	VIJAYALAKSHMI	11.4	5200/75/23	20	105,000	1.4/0.8	25/37	125	5.9/3.1	97	30	1	14.6/1.13
16	RAJESWARI	11.8	8500/68/22	24	220,000	0.9/0.6	35/47	67	6.5/3.8	93	0	0.7	13.1/1
17	PARVATHY	9.7	5600/58/36	12	136,000	0.9/0.5	37/20	69	8.5/4.6	148	17	0.5	10.7/0.75
18	CHANDRA	10.8	6000/56/37	27	226,000	1.5/0.9	26/16	68	6.8/4.0	134	34	1.1	13.1/1.15
19	CHANDRU	14.1	4800/56/33	18	170,000	0.9/0.5	49/56	63	7.7/4.2	88	19	1	11.8/0.9
20	MARKANDAIYAN	12.6	9700/75/15	16	324,000	0.5/0.3	21/40	74	6.7/3.7	136	32	1	16.3/1.22
21	KEVIN	10.6	8800/69/31	6	208,000	101/0.6	23/30	124	6.2/4.0	104	24	0.9	12/1.0
22	DALPATTU SAMY	11	6300/56/40	18	196,000	1.2/0.6	20/22	104	5.6/3.3	96	18	0.8	11.2/0.9
23	SABARIPRASANNA	12.1	5900/50/42	14	145,000	0.85/0.5	16/28	104	5.4/3.1	98	28	1.2	12.3/1.0
24	ANAND	11.2	5800/60/28	12	164,000	1.2/0.7	36/28	108	6.7/4.0	108	32	1.3	12.8/1.1
25	KRISHNAVENI	10.4	6100/59/38	6	210,000	1.1/0.7	26/34	96	6.0/3.4	94	24	0.8	0.9
26	KUMARAVEL	11.6	8400/70/28	8	210,000	1.0/0.7	38/42	112	6.8/4.0	112	26	0.9	12.4/1.0
27	PUNITHA	10	6400/62/26	8	224,000	1.1/0.6	24/44	96	6.4/3.9	90	20	0.9	13.0/1.1
28	KARUNAKARAN	11	7300/70/26	12	180,000	1.2/0.8	42/38	112	7.0/4.6	104	26	1	1.09
29	SIVA	12.1	8200/68/31	18	210,000	0.8/0.4	42/38	96	6.8/4.0	96	24	1.2	13.0/1.1
30	MANNAR	12.8	9200/76/22	20	131,000	1.4/1.0	52/34	96	7.0/4.0	110	32	0.9	11.8/0.9

SI NO	NAME	HBeAg	HBV DNA IU/MI	Anti HCV	USG ABD	VOGD	FIBROSCAN kPa	FIBROMETER	LIVER BIOPSY
1	JOTHILAKSHMI	NEG	75780	NEG	FATTY LIVER	NORMAL	9.3	F2(0,5)	CHR ACTIVE HEPATITIS
2	SASIRAJA	NEG	62000	NEG	NORMAL	NORMAL	7.2	F2(0,38)	CHR ACTIVE HEPATITIS
3	JEEVARATHINAM	REACTIVE	491,258	NEG	NORMAL	NORMAL	4.8	F1(0,17)	MILD NON SPECIFIC HEPATITIS
4	DHANASEKAR	REACTIVE	530,206	NEG	NORMAL	NORMAL	12.3	F2(0,48)	CHR ACTIVE HEPATITIS
5	KUPPAN	NEG	140,000	NEG	FATTY LIVER	NORMAL	3.4	F1(0,21)	NSH WITH FATTY CHANGE
6	RANJITH	REACTIVE	156,190	NEG	NORMAL	NORMAL	11	F2(0,48)	CHR ACTIVE HEPATITIS
7	BHOOPATHI	NEG	236,000	NEG	FATTY LIVER	NORMAL	7.8	F2(0,42)	CHRONIC HEPATITIS
8	GOPINATH	REACTIVE	1,100,000	NEG	NORMAL	NORMAL	7.6	F1(0,17)	CHR ACTIVE HEPATITIS
9	ARUTSELVAN	REACTIVE	32,657	NEG	NORMAL	NORMAL	3.8	F1(0,2)	MILD NON SPECIFIC HEPATITIS
10	PALANIAMMAL	NEG	36,124	NEG	FATTY LIVER	NORMAL	6.8	F2(0,38)	INTERFACE HEPATITIS
11	MANIGANDAN	REACTIVE	64,824	NEG	NORMAL	ESOPHAGITIS	3.2	F1(0,15)	CHR ACTIVE HEPATITIS
12	KIRUBAKARAN	REACTIVE	110,000	NEG	NORMAL	NORMAL	7.2	F2(0,38)	CHR ACTIVE HEPATITIS
13	JHANSI RANI	NEG	21,165	NEG	NORMAL	NORMAL	3.6	F1(0,18)	MILD NON SPECIFIC HEPATITIS
14	PERUMAL	NEG	11,000,000	NEG	NORMAL	NORMAL	7.4	F2(0,4)	CHR ACTIVE HEPATITIS
15	VIJAYALAKSHMI	NEG	11,000,000	NEG	NORMAL	ESOPHAGITIS	7.4	F2(0,39)	CHR ACTIVE HEPATITIS
16	RAJESWARI	REACTIVE	156,180	NEG	FATTY LIVER	NORMAL	3.4	F1(0,21)	NON SPECIFIC HEPATITIS
17	PARVATHY	NEG	162,000	NEG	NORMAL	ESOPHAGITIS	4.8	F1(0,15)	MILD HEPATITIS
18	CHANDRA	NEG	97,234	NEG	FATTY LIVER	NORMAL	5.6	F1(0,27)	CHR ACTIVE HEPATITIS
19	CHANDRU	REACTIVE	137,000	NEG	NORMAL	NORMAL	8.7	F2(0,42)	CHR ACTIVE HEPATITIS
20	MARKANDAIYAN	REACTIVE	243,768	NEG	NORMAL	NORMAL	5.1	F2(0,4)	MILD ACTIVE HEPATITIS
21	KEVIN	NEG	36,000	NEG	NORMAL	NORMAL	3.8	F1(0,1)	MILD NON SPECIFIC HEPATITIS
22	DALPATTU SAMY	NEG	46,000	NEG	NORMAL	NORMAL	3.2	F1(0,12)	CHR ACTIVE HEPATITIS
23	SABARIPRASANNA	NEG	176,000	NEG	NORMAL	NORMAL	4.5	F1(0,19)	CHR ACTIVE HEPATITIS
24	ANAND	NEG	187,650	NEG	NORMAL	NORMAL	5.4	F1(0,17)	CHRONIC HEPATITIS
25	KRISHNAVENI	REACTIVE	46,284	NEG	NORMAL	NORMAL	4.8	F1(0,17)	CHRONIC HEPATITIS
26	KUMARAVEL	NEG	214,850	NEG	NORMAL	NORMAL	6.8	F1(0,32)	CHR ACTIVE HEPATITIS
27	PUNITHA	NEG	167,000	NEG	NORMAL	NORMAL	9.4	F2(0,2)	CHR ACTIVE HEPATITIS
28	KARUNAKARAN	NEG	124,000	NEG	NORMAL	NORMAL	8.4	F2(0,38)	CHR ACTIVE HEPATITIS
29	SIVA	NEG	1,970,000	NEG	NORMAL	NORMAL	7.8	F1(0,2)	CHR ACTIVE HEPATITIS
30	MANNAR	NEG	96,719	NEG	NORMAL	NORMAL	7.4	F1(0,2)	CHR ACTIVE HEPATITIS