DIAGNOSTIC ACCURACY OF ACOUSTIC RADIATION FORCE IMPULSE (ARFI) ELASTOGRAPHY OF THE LIVER AND SPLEEN TO IDENTIFY NONCIRRHOTIC PORTAL FIBROSIS (NCPF) FROM CIRRHOSIS WITH PORTAL HYPERTENSION AND COMPARISON WITH LIVER BIOPSY

A dissertation submitted in partial fulfilment of MD Radiodiagnosis (Branch VIII) examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2014.

CERTIFICATE:

This is to certify that the dissertation entitled “Diagnostic accuracy of Acoustic Radiation Force Impulse (ARFI) Elastography of liver and spleen to identify Noncirrhotic portal fibrosis (NCPF) from cirrhosis with portal hypertension and comparison with liver biopsy” is a bonafide original work of Dr. Rachel Gandi submitted in partial fulfilment of the requirement for MD Radiodiagnosis (Branch VIII) Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2014.

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This is to certify that the dissertation entitled “Diagnostic accuracy of Acoustic Radiation Force Impulse (ARFI) Elastography of liver and spleen to identify Noncirrhotic portal fibrosis (NCPF) from cirrhosis with portal hypertension and comparison with liver biopsy” is a bonafide original work of Dr. Rachel Gandi submitted in partial fulfilment of the requirement for MD Radiodiagnosis (Branch VIII) Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2014.

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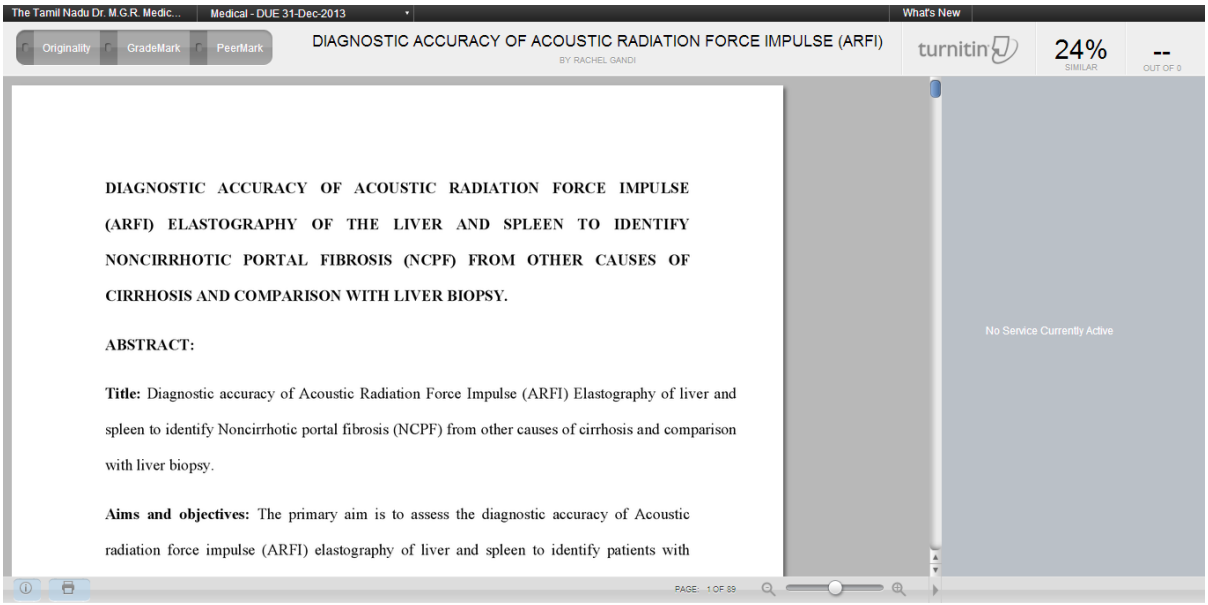

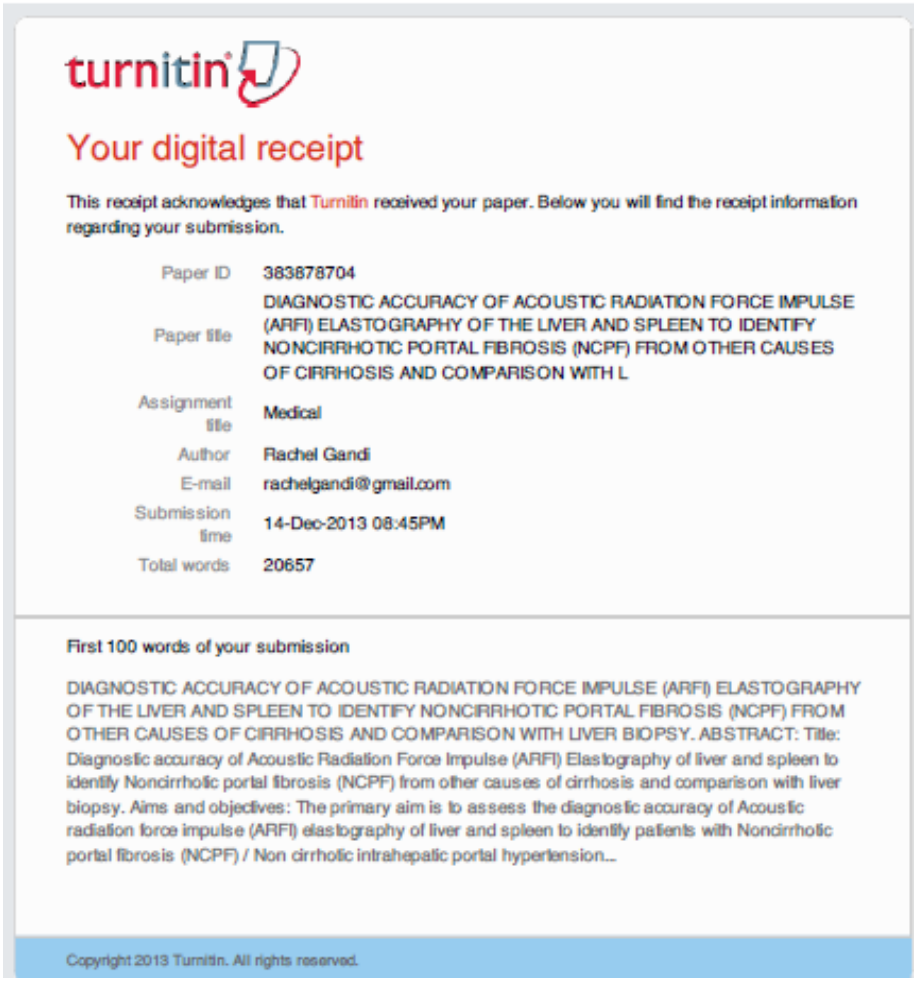

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November 16, 2012

Dr. Rachel Gandi
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Dear Dr. Rachel Gandi,

Sub: **FLUID Research grant project NEW PROPOSAL:**
Acoustic Radiation Forced Impulse (ARFI) elastography of liver and Spleen in non cirrhotic portal fibrosis (NCPF).
Dr. Rachel Gandi, Radiology. Dr. Anu Eapen. Dr. Anuradha Chandramohan, Dr. C.E. Eapen, Hepatology.

Ref: IRB Min. No. 7987 dated 08.09.2012

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.

A sum of ₹ 37,500/- (Rupees Thirty seven thousand five hundred only) can be sanctioned for 12 months.

Yours sincerely

Dr. Nihal Thomas
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Above all, I thank GOD for his abundant grace.

ABBREVIATIONS:

ALT: Alanine aminotransferase

APASL: Asian Pacific Association for Study of Liver

APRI: Aspartate aminotransferase (AST)-to-platelet ratio index

ARFI: Acoustic radiation force impulse

AST: Aspartate aminotransferase

AUROC: Area under receiver operator curve

BMI: Body mass index

BRTO: Balloon-occluded retrograde transvenous obliteration

CLD: Chronic liver disease

CPT: Complete portal tracts

CSPH: Clinically significant portal hypertension

DIA: digital-image analysis

EGD: Endoscopy

EVL: Endoscopic variceal ligation

EV: Esophageal varices

FHVP: Free hepatic venous pressure

FIB-4: Fibrosis 4 index

GOV: Gastroesophageal varices

HAI: Histology activity index

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

HVPG: Hepatic venous pressure gradient

IGV: Isolated gastric varices

IPH: Idiopathic portal hypertension

INR: International normalized ratio

LR: Likelihood ratio

LS: Liver stiffness

MELD: Model for End- Stage Liver Disease score

NAFLD: Non alcoholic fatty liver disease

NCPF: Noncirrhotic portal fibrosis

NCIPH: Noncirrhotic intrahepatic portal hypertension

NPV: Negative predictive value

PTO: Percutaneous transhepatic obliteration

PH: Portal hypertension

PPG: Portal pressure gradient

PPV: Positive predictive value

ROI: Region of interest

SS: Spleen stiffness

SWV: Shear wave velocity

TE: Transient elastography

TJLB: Transjugular liver biopsy

TIPS: Transjugular intrahepatic porto-systemic shunt

VTQ: Virtual Touch Tissue Quantification

WHVP: Wedged hepatic venous pressure

INDEX:

ABSTRACT:

Title: Diagnostic accuracy of Acoustic Radiation Force Impulse (ARFI) Elastography of liver and spleen to identify Noncirrhotic portal fibrosis (NCPF) from cirrhosis with portal hypertension and comparison with liver biopsy.

Aims and objectives: The primary aim is to assess the diagnostic accuracy of ARFI elastography of liver and spleen to identify patients with NCPF / Non cirrhotic intrahepatic portal hypertension (NCIPH) from cirrhosis with portal hypertension as compared to liver biopsy.

To assess the diagnostic accuracy of ARFI elastography of liver and spleen to differentiate significant liver fibrosis from non-significant liver fibrosis in patients with portal hypertension and to predict clinically significant portal hypertension (CSPH) as compared to Hepatic venous pressure gradient (HVPG) and the presence high risk esophageal varices (EV) on endoscopy.

Methods:

Prospective study approved by IRB. Total 58 patients were studied. Patients with pre-biopsy preliminary diagnosis of cryptogenic cirrhosis were included and underwent ARFI elastography of the liver and the spleen. The liver stiffness (LS), spleen stiffness (SS), spleen stiffness to liver stiffness ratio (SS/LS) velocity measurements were correlated with the grades of liver fibrosis on biopsy, clinical and biochemical parameters, HVPG and the presence of high risk varices on endoscopy. Patients with other causes of portal hypertension were also studied who did not undergo liver biopsy.

Results:

48 cases with preliminary diagnosis of cryptogenic cirrhosis were studied. 39 cases had liver biopsy. 11 cases (28%) were proven to be NCPF on biopsy. The rest of the 28 cases were autoimmune chronic liver disease, cryptogenic cirrhosis, Wilson's disease, drug induced hepatitis, Non alcoholic fatty liver disease, Thalassemia and HBV related chronic liver disease. 9 cases were clinically suspected to have NCPF who did not undergo liver biopsy were also studied.

58 cases of portal hypertension of any etiology were studied to assess the correlation of ARFI of the liver and the spleen with the presence of high risk esophageal varices on endoscopy. This group includes 39 liver biopsy cases, 9 suspected NCPF cases without liver biopsy and 10 cases of portal hypertension due to other known etiologies.

LS had 36% sensitivity, 93% specificity, PPV of 90%, NPV of 78.8% and kappa of 0.28 and p value of 0.04 to identify NCPF from other liver cirrhosis which was statistically significant. SS had 70% sensitivity, 20% specificity, PPV of 26.9% and NPV of 62.5%, kappa of -0.63 and p value of 0.5 which was not statistically significant. The SS/ LS ratio had 70% sensitivity, 79.2% specificity, PPV of 58.3%, NPV of 86.4% with kappa of 0.46 and p value of 0.06 which was statistically not significant. The combination of either LS, SS or SS/ LS had sensitivity of 82%, specificity of 21.4 %, PPV of 29%, NPV of 75% and kappa of 0.02 (p=0.8).The best ARFI elastography parameter to identify NCPF from other causes of cirrhosis was found to be SS/LS with good sensitivity, specificity, negative predictive value and good kappa correlation.

LS, product of LS and SS and ratio of SS/ LS were found to statistically significant differentiate between significant from non-significant liver fibrosis. Spearman correlation

(rho) of the liver stiffness (LS) with the fibrosis grades on liver biopsy was 0.54 with p value of < 0.001 which was statistically significant.

Both LS and SS were found to have low sensitivity with low kappa agreement and were statistically not significant to identify clinically significant portal hypertension as compared to HVPG.

Conclusions: ARFI elastography of the liver and the spleen in combination have good sensitivity to identify NCPF from cirrhosis with portal hypertension as compared to liver biopsy. The best elastography parameter was the spleen stiffness to liver stiffness ratio. Liver and spleen stiffness independently are not adequate. LS and the SS/LS have good sensitivity to identify significant liver fibrosis as compared to liver biopsy. There is good correlation with liver stiffness velocities and grades of liver fibrosis. ARFI of the liver and spleen have low sensitivity and low specificity to identify CSPH as compared to HVPG and the presence of high risk EV on endoscopy.

AIMS AND OBJECTIVES:

- Primary aim is to assess the diagnostic accuracy of Acoustic radiation force impulse (ARFI) elastography of liver and spleen to identify Noncirrhotic portal fibrosis (NCPF)/ Noncirrhotic portal hypertension (NCIPH) from cirrhosis with portal hypertension as compared to liver biopsy in patients with portal hypertension.
- To assess the diagnostic accuracy of ARFI elastography of liver and spleen to differentiate significant liver fibrosis from non-significant liver fibrosis.
- To assess the diagnostic accuracy of ARFI elastography of liver and spleen to predict clinically significant portal hypertension (CSPH) as compared to Hepatic venous pressure gradient (HVPG) and the presence high risk esophageal varices on endoscopy.

JUSTIFICATION OF THE STUDY:

Patients with portal hypertension, in whom no cause for liver disease is found, are often labelled as cryptogenic cirrhosis. Occult hepatitis B infection, end-stage non alcoholic fatty liver disease and autoimmune liver disease are potential causes of cryptogenic cirrhosis in India.

Noncirrhotic portal fibrosis (NCPF) / Noncirrhotic intrahepatic portal hypertension (NCIPH) is a disease which is seen more commonly in Indians and Japanese as compared to the west. It is difficult to diagnose NCPF on routine USG and Doppler as imaging characteristics mimic cryptogenic cirrhosis and therefore is commonly misdiagnosed as cirrhosis on imaging. However, these patients do not have features of cirrhosis on histopathology and majority do not progress into liver cell failure. The main morbidity in NCPF portal hypertension causing variceal bleed, which when adequately treated, most of these patients have an excellent prognosis. Hence, there is a need to accurately diagnose NCPF from other causes of cryptogenic cirrhosis and monitor the progression of disease in these patients.

The gold standard method for detecting the presence of liver fibrosis and the degree of fibrosis is by liver biopsy which is an invasive procedure with known complications and limitations. In the recent past, ARFI elastography of the liver has emerged as a novel ultrasound based modality to non-invasively predict fibrosis of liver and spleen. There are studies which have used ARFI elastography of the liver and the spleen for viral hepatitis and have shown good correlation of elastography velocity score with the grades of liver fibrosis on biopsy. Studies have shown that upto 40% of patients with a preliminary diagnosis of cryptogenic cirrhosis who undergo liver biopsy are NCPF.

ARFI elastography has been studied in patients with portal hypertension. To our knowledge after literature review, only one published study is reported in literature about the use of ARFI elastography in of liver and spleen to diagnose NCPF. There are no Indian studies regarding the use of ARFI elastography to predict NCPF. Hence this study aims at assessing the diagnostic accuracy of ARFI elastography of liver and spleen to identify NCPF in Indian population from patients with other causes of cirrhosis as compared to liver biopsy which is the gold standard.

In future, we hope ARFI elastography can be used as a non invasive predictor of NCPF, and is included as one of the diagnostic criterion for non-invasive diagnosis and follow up of NCPF and thereby reduce the need for liver biopsy.

Clinically significant portal hypertension (CSPH) is the main cause of morbidity in patients with chronic liver disease. The gold standard to diagnose CSPH is hepatic venous pressure gradient (HVPG) which is an invasive procedure. This study also aims at studying the diagnostic accuracy of ARFI elastography of the liver and spleen to predict CSPH as compared to HVPG and the presence of high risk esophageal varices on endoscopy.

LITERATURE REVIEW:

- Introduction to portal hypertension
- Natural history of portal hypertension
- Pathophysiology of portal hypertension
- Classification of portal hypertension
- Incidence of portal hypertension
- Noncirrhotic portal fibrosis (NCPF) / Noncirrhotic intrahepatic portal hypertension / Idiopathic portal hypertension (IPH)
- Non invasive methods to assess liver fibrosis by ultrasound elastography
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- Acoustic Radiation Force Impulse (ARFI) elastography
    - Principle and protocol for ARFI elastography of liver
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    - Factors influencing ARFI elastography measurements
    - Limitations and contraindications of ARFI elastography

- ARFI elastography of liver in correlation with Transient elastography and advantages of ARFI elastography of liver over Transient elastography
- ARFI elastography in NCPF / NCIPH
- Diagnostic accuracy of ARFI elastography of the liver and spleen to predict portal hypertension
- Diagnostic accuracy of ARFI elastography of spleen to predict liver cirrhosis
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- Other imaging modalities to assess portal hypertension
- Gray scale ultrasound for assessing chronic liver disease and portal hypertension
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- Invasive methods to assess portal hypertension

  (i). Hepatic venous pressure gradient (HVPG)

  (ii). Endoscopy for varices

  (iii). Liver biopsy
- Scoring system of liver fibrosis on histopathology

INTRODUCTION TO PORTAL HYPERTENSION:

Portal hypertension is a common and unavoidable sequel of cirrhosis in patients with chronic liver disease. The relevance of portal hypertension is due to the frequency and severity of its complications which is the main cause for hospital admission, mortality and liver transplantation. Portal hypertension (PH) is due to sustained increase in pressure in the portal venous system. The normal portal pressure is expressed as portal venous pressure gradient. It is the difference between the absolute pressure in the portal venous system and the intra-abdominal systemic venous pressure (IVC) and represents the perfusion pressure of the liver with the portal blood. Elevated portal pressure leads to splenomegaly, development of porto-systemic collaterals which shunt the portal blood bypassing the liver into the systemic circulation and a hyperkinetic circulation. Normal portal pressure gradient (PPG) is 1-5 mm of Hg. Clinically significant portal hypertension which is associated with various complications is when the PPG exceeds 10 mm of Hg. Subclinical portal hypertension is when the PPG is between 5-9 mm of Hg.(1)

NATURAL HISTORY OF PORTAL HYPERTENSION:

Among asymptomatic patients with cirrhosis 80- 90% have elevated PPG which is clinically measured as elevated hepatic venous pressure gradient (HVPG) and 40 % of these patients have esophageal varices on endoscopy. The esophageal varices develop at the rate of 6% per year among the patient's asymptomatic patients without varices and about 10% per year in patients with HVPG> 10 mm of Hg. 10- 30% of patients with untreated esophageal varices bleed within 2 years. The incidence of variceal bleed increases with the size of varices, presence of red colour signs on endoscopy and the degree of liver cell failure. These patients have 6- 20% mortality during the episode of variceal bleed and two thirds of the patients can rebleed within 2 years. Current medical treatments reduced the risk of first variceal bleed and rebleed rate by 50% (2).

PATHOPHYSIOLOGY OF PORTAL HYPERTENSION:

The portal venous system is unique unlike other venous systems in the body. It is the main vascular supply to the liver, making the liver the organ to receive maximum blood flow. Changes in hepatic resistance results in marked circulatory changes in the rest of the body. Portal hypertension is initiated by an increased resistance to portal blood flow and is exacerbated by and increased portal collateral blood flow (3). The most common cause of increased portal blood flow resistance is chronic liver disease, the next common being hepatic schistosomiasis. Other less common causes are extra hepatic portal vein occlusion and idiopathic non cirrhotic portal hypertension which account for approximately 10 % (2). There are two main mechanisms causing increase in hepatic vascular resistance; the structural and the dynamic component. The structural component is due to distortion of liver architecture by fibrosis, nodule formation, angiogenesis and vascular occlusion. The dynamic component is due to increased vascular tone by the activated hepatic stellate cells and myofibroblasts around the sinusoids, in the fibrous septa and in the smooth muscle cells in the hepatic vasculature. The dynamic component represents liver vascular dysfunction accounting for 30% of the increase in hepatic resistance. There is also increase in the vasoconstrictors and decrease in vasodilators. In the advanced stage of portal hypertension, there is increase splanchnic blood flow in the portal collaterals. This is due to splanchnic arteriolar vasodilatation and neoangiogenesis due to increase in vasodilators like VGEF, nitric oxide. Due to splanchnic vasodilatation there is systemic hypotension, vascular under filling, activation of endogenous vasoactive system, plasma volume expansion and increase in cardiac index, leading to hyperkinetic syndrome which leads to ascites and renal dysfunction in cirrhosis. Formation of porto-systemic collaterals and varices are due to opening of pre-existing communicating vessels between the portal and the systemic circulation and angiogenesis (4).

CLASSIFICATION OF PORTAL HYPERTENSION:

Any disease which causes interruption of blood flow between the spleen and the right atrium can cause portal hypertension. Portal hypertension is divided into prehepatic, hepatic and post hepatic portal hypertension.

The prehepatic portal hypertension group involves the spleno-portal mesenteric axis and have normal wedged hepatic venous pressure (WHVP), free hepatic venous pressure (FHVP) and normal hepatic venous pressure gradient (HVPG). The common causes are caused portal vein thrombosis, splenic vein thrombosis, congestive splenomegaly (Banti's syndrome) and arterio-venous fistulae. The intrahepatic portal hypertension group have increased wedged hepatic venous pressure (WHVP), normal free hepatic venous pressure (FHVP) and increased hepatic venous pressure gradient (HVPG). They are further subdivided into presinisoidal, sinusoidal and post sinusoidal causes of hepatic portal hypertension. The presinisoidal causes are Non-cirrhotic portal fibrosis (NCPF), schistosomiasis, and congenital hepatic fibrosis. The sinusoidal portal hypertension is seen in cirrhosis due to various etiologies, Alcoholic hepatitis, nodular regenerative hyperplasia and polycystic liver disease. The post sinusoidal causes are sinusoidal obstructive syndrome and Budd- Chiari syndrome. The post hepatic portal hypertension group have increased wedged hepatic venous pressure (WHVP) and free hepatic venous pressure (FHVP) and normal hepatic venous pressure gradient (HVPG). Common causes of post hepatic portal hypertension are Budd Chiari syndrome, cardiac causes like congestive cardiac failure, constrictive pericarditis and restrictive cardiomyopathy and pulmonary hypertension (5).

INCIDENCE PORTAL HYPERTENSION:

The most common cause of portal hypertension in the western countries is cirrhosis. Non cirrhotic portal hypertension accounts for 10 % of the cases of portal hypertension (2). Studies in various other countries have found that hepatitis C and alcohol related liver disease are the main causes of portal hypertension, however the scenario in India differs from the rest. In India, few studies are reported in literature studying the various etiologies of portal hypertension. One of the largest studies is by Goel et al. who studied the spectrum of various etiologies of portal hypertension among adults at a tertiary care centre in South India. They have studied a total of 583 patients with portal hypertension. Based on non-invasive methods, they have found that the commonest cause of portal hypertension as cryptogenic cirrhosis(35%), the next common causes being chronic liver disease due to alcohol consumption (29%), chronic viral hepatitis like hepatitis B(17%) and hepatitis C as (9%). Of the patients with cryptogenic cirrhosis who underwent liver biopsy for further evaluation, 41% were found to be non-cirrhotic portal hypertension (NCIPH), and 5 patients had non alcoholic fatty liver disease. The vascular disorders causing portal hypertension were found to be 10%.

In the future, due to increase in sedentary lifestyle, better standards of living and metabolic syndrome, there in an expected increase in the patients with non-alcoholic fatty liver disease (NAFLD) and cirrhosis related to alcohol consumption. With increase in various awareness programmes, there seems to be a decline in the incidence of chronic viral hepatitis causing portal hypertension. In India, among children, for reasons yet unclear, the vascular causes like extra-hepatic portal vein obstruction is the commonest cause of portal hypertension, the others being non- cirrhotic portal hypertension and Wilson’s disease (6).

NONCIRRHOTIC PORTAL FIBROSIS (NCPF) / NONCIRRHOTIC INTRAHEPATIC PORTAL HYPERTENSION (NCIPH):

Noncirrhotic portal fibrosis (NCPF) /Non cirrhotic intrahepatic portal hypertension (NCIPH) is a group of disorder characterized by elevated portal pressure due to intrahepatic or prehepatic pathologies in the absence of cirrhosis. Non cirrhotic portal fibrosis (NCPF) is one of the important causes of non cirrhotic portal hypertension among Asians. Other terminologies for the same condition are non cirrhotic intrahepatic portal hypertension (7), Idiopathic non cirrhotic portal hypertension and hepato-portal sclerosis. The Asian Pacific Association for Study of Liver (APASL), working party on portal hypertension defined as, "NCPF/ IPH is a disease of uncertain etiology characterized by periportal fibrosis and involvement of small and medium branches of the portal vein, resulting in the development of portal hypertension. The liver functions and structure primarily remain normal.

EPIDEMIOLOGY OF NCPF:

NCPF is more common in the developing countries rather than the developed countries. It is more common among people from low socio- economic strata. In India initial studies in 1980s showed the incidence of 23%; however recent studies show a decline in the incidence. NCPF is more commonly seen in young adults in the third and the fourth decade of life and has no sex predilection (8).

ETIOPATHOGENESIS OF NCPF:

There is limited insight regarding the etiopathogenesis of NCPF and has various proposed hypothesis to explain this heterogenous group of disease. There is varied degree of injury of predominantly presinisoidal region of the portal venous system. The factors which have been associated to cause the portal venous injury are infections, xenobiotics exposure and various

immunological abnormalities. Repeated intestinal infection causes intramural thrombus formation and activation of stellate cells which leads to presinisoidal fibrosis. Arsenic induced hepatic fibrosis was found to be related to IL-6 and TNF- alpha which cause hepatic oxidative stress. Abnormalities of T- cells and adhesion molecules have been found, but the exact role in the pathogenesis is not clearly understood. Other association of NCPF are portal or umbilical pyemia, autoimmune disorders, prothrombotic states, toxins like chronic vinyl alcohol exposure, copper sulphate, prolonged treatment with methotrexate, 6-mercaptopurine, azathioprine and hypervitaminosis A. In majority of the cases, the exact cause remains unknown (8).

CLINICAL FEATURES OF NCPF:

Among patients with portal hypertension NCPF is an important cause of upper gastrointestinal bleed, constituting about 15% of cases. The variceal bleed is well tolerated in these patients. They commonly present with left upper quadrant mass (splenomegaly) or iron deficiency anaemia. Jaundice, ascites and edema are uncommon. The signs of liver cell failure like palmar erythema, parotid enlargement, spider angioma, testicular atrophy and gynaecomastia are rare (8).

LAB TESTS IN NCPF:

Liver function tests are usually normal in NCPF. Hypersplenism can cause pancytopenia with normochromic normocytic anaemia, leucopenia and thrombocytopenia. Microcytic hypochromic anaemia in these patients is due to gastrointestinal blood loss. Bone marrow examination shows hypercellular marrow. NCPF patients can have coagulation abnormalities with significantly increased international normalized ratio (INR), decrease in fibrinogen and platelet aggregation due to endotoxemia or porto-systemic collaterals. Esophageal varices are seen in majority of patients (85-90%) and are of often high grade at the time of diagnosis.

Gastric varices are seen in 25% of patients and portal hypertensive gastropathy is uncommon in NCPF as compared to cirrhotic patients. Anorectal varices are commonly seen in NCPF. Since the site of resistance within the liver is presinisoidal, the hepatic venous pressure gradient is normal or near normal (8).

HISTOPATHOLOGY OF NCPF:

The gross examination of liver in NCPF may be normal, enlarged or even shrunken. The liver surface may be smooth or nodular like cirrhosis with thickening of the capsule; however the deeper parenchyma is grossly normal. There is sclerosis of small and medium sized portal vein branches with increase in portal collagenous connective tissue and obliteration of small branches of portal vein. Hence histological hallmark of NCPF is termed as 'Obliterative portal venopathy'. Occasionally recanalized thrombi are seen. Liver needle biopsy shows small portal vein obliteration, aberrant vasculature, portal tract fibrosis and absence of regenerative nodules or features of cirrhosis (8).

DIAGNOSIS OF NCPF:

The diagnosis is relatively easy; however it is important to differentiate NCPF from Child A cirrhosis and extra-hepatic portal vein obstruction. The diagnostic features of NCPF include moderate to massive splenomegaly, portal hypertension represented as varices and or collaterals, absence of signs of chronic liver cell failure, patent spleno-portal axis and hepatic veins on ultrasound and doppler, normal or near normal liver function tests, absence of serum markers for hepatitis B and C infection, no known etiology for liver disease, normal or near normal hepatic venous pressure gradient (HVPG), no evidence of cirrhosis or parenchymal injury on histopathology, no decompensation after variceal bleed except transient ascites and on imaging dilated and thickened portal vein with peripheral pruning and periportal hyperechoic areas (3%) (8).

NATURAL HISTORY AND PROGNOSIS OF NCPF:

Unlike cirrhosis, NCPF is usually non-progressive and has good prognosis especially if the portal pressures are reduced by shunt surgery or other procedures. Recently, due to increase in orthotopic liver transplantation, it has been shown that native explants livers which were initially labelled as cryptogenic cirrhosis was subsequently diagnosed as NCPF. Hence NCPF can progress to end stage liver disease requiring liver transplantation (9). NCPF has excellent prognosis. The major cause of mortality is acute gastrointestinal bleed which is significantly lower than cirrhotic patients. Hence after successful treatment of esophagogastric varices, the survival is excellent. Selective shunt surgery can be done in NCPF; however the morbidity can be increased due to shunt occlusion or post shunt encephalopathy (8).

MANAGEMENT OF NCPF:

The management of patients with NCPF is to prevent active bleeding and to provide primary and secondary prophylaxis. Acute variceal bleed is controlled endoscopic therapy with band ligation and vasoactive drugs to decrease portal pressure. If the endoscopic therapy fails after 2 procedures then the alternative treatment options include surgery or transjugular intrahepatic porto-systemic shunt (TIPS). Primary prophylaxis for variceal bleed includes endoscopic variceal band ligation (EVL) or beta blockers. Shunt surgery in NCPF patients for primary prophylaxis of large esophageal varices can be done if the patient has large splenomegaly with very low platelet count (<20,000), stays far away from a medical centre where a upper gastrointestinal bleed can be managed and has a rare blood group. The secondary prophylaxis includes EVL or decompressive surgery, the former is preferred. Newer therapies like image guided interventions can be used to prevent variceal bleed. These include partial spleen embolization, Balloon-occluded retrograde transvenous obliteration (BORTO), percutaneous transhepatic obliteration (PTO) and TIPS (8).

NON INVASIVE METHODS TO ASSESS LIVER FIBROSIS:

The gold standard to asses liver fibrosis is liver biopsy with histopathological examination. However, liver biopsy has many limitations. It is an invasive procedure with known complications and mortality. Sampling error can occur with liver biopsy as the liver fibrosis is not uniform in the liver and only 1/ 50,000 th part of the liver is examined. Thus, liver biopsy can underestimate the degree and extent of fibrosis. There are known interobserver variations in reporting liver fibrosis. Liver biopsy specimen can get fragmented or sample may be inadequate. Hence, due to the limitations of liver biopsy, there is an increased need to non invasively diagnose, monitor patients with chronic disease with various degrees of liver fibrosis and also assess the treatment response. The non invasive methods reduce the need for liver biopsy, can predict the complications of portal hypertension and thereby are cost effective and reduce the need for invasive methods and the morbidity associated with those procedures. The various non invasive tests to assess liver fibrosis are elastography and serum fibrosis markers. They have been mostly studied in patients with chronic hepatitis C and hepatitis B.

ULTRASOUND ELASTOGRAPHY -NON INVASIVE TOOL TO ASSESS FIBROSIS:

Since ancient times one of the key method of detecting and characterizing pathologies is by assessment of tissue stiffness by palpation. In the recent times, ultrasound elastography has evolved as an imaging tool to assess tissue stiffness or elasticity which is clinically assessed by palpation. Elastography is the imaging technique used to assess tissue elasticity. It induces shear stress in the tissues by creating low frequency vibrations, analyses the resultant stress and quantifies tissue stiffness. Tissue stiffness is measured by Young's modulus which quantifies tissue stiffness and is expressed as kilopascals (kPa). Young's modulus or Elasticity (E) is defined as the ratio between applied stress and induced strain. E=s/e, where

E- elasticity, s-externally applied stress and e- induced strain. The normal elastography values of various tissues in the body have been defined and theses values differ in various pathological states. They are two types of externally applied mechanically induced waves by ultrasound. They are compression or bulk waves and shear waves. Compression waves traverse at a high velocity through the tissues (1500 cm/ sec) by compressing tissue layers and the reflected compressional wave echoes are analysed. Shear waves traverse slower (1 to 10 cm/ sec) though the tissues and propagate by tangential or sliding force between the tissue layers.

TYPES OF ELASTOGRAPHY:

They are three types of elastography; static, dynamic and shear wave based elastography. In static elastography, uniform compression of the tissue is obtained by external compression by the user on the body surface, and the deformation of the tissues is calculated and represented as an elastogram by the ultrasound machine. The tissues which are less deformed as represented as darker areas on elastogram. It is not a quantitative method to assess tissue stiffness, as the amount of externally applied force is unknown and hence Young's modulus cannot be reconstructed. Dynamic elastography uses continuous monochromatic vibrations to assess tissue elasticity. It is a quantitative method used in MR systems. Shear wave elastography is a quantitative method of assessing tissue stiffness where the external compression force is produced by the ultrasound transducer. The acoustic radiation force induced ultrasound beam pushes the tissues in the direction of propagation and the displaced mechanical waves in the human tissues is measured. Elasticity image is represented as a colour coded image superimposed on B mode gray scale image. Colour scale is quantitative as stiffer tissues are represented as red and softer tissues are represented as blue which correspond to the scale as kPa. The image resolution is 1mm and the elastography image is

refreshed in real time. The imaging frame rate corresponds to the acoustic output as per international standards.

TRANSIENT ELASTOGRAPHY (TE-FibroScan):

Transient elastography (FibroScan) is a non-invasive, rapid and reproducible ultrasound based method for assessing liver stiffness. It was developed by Echosens, based on the principle of Hooke's law, which demonstrates the material strain response to external stress. FibroScan has an ultrasound transducer probe which is mounted on the axis of a vibrator. This transmits low frequency vibrations which produce elastic shear waves in the liver. Pulse-echo ultrasound acquisition is used which measures the wave propagation velocity which is proportional to the tissue stiffness. The stiffer tissues have faster wave propagation velocities.

Liver fibrosis using transient elastography has been well studied as a non invasive tool to assess fibrosis. In TE the liver stiffness measurements are performed from the right lobe of the liver with the patient in the dorsal decubitus position with maximum abduction of the right arm. Using A- mode ultrasound images a portion of liver is selected between 25 mm and 65 mm below the skin surface free of liver vasculature. Ten measurements are obtained and for reliable measurements the success rate of 60% and interquartile range of less than 30% of the median liver stiffness measurement is suggested. The median value is taken as representative and is expressed as kPa. (10). The cut off value to differentiate liver fibrosis and cirrhosis is13 kPa and to differentiate between fibrosis and no significant fibrosis is 7.6 kPa has been suggested (11).

Nierhoff et al, compared meta-analysis of transient elastography TE and found that the overall diagnostic accuracies of 0.94 for the diagnosis of liver cirrhosis, 0.89 for the diagnosis of severe fibrosis and 0.84 for the diagnosis of significant fibrosis, respectively (12).

ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY (ARFI):

PRINCIPLE:

Acoustic radiation force impulse (ARFI) elastography is a radiation force based imaging method combined with conventional B mode ultrasound (Acuson S 2000, SEIMENS Medical Solutions). The 'Virtual Touch Tissue Quantification' (VTQ) provided by SEIMENS quantifies the tissue stiffness by shear wave velocity of acoustic radiation force impulse displacement within the human tissues. It is represented as m/sec and is proportional to the square root of the tissue elasticity (13).

To obtain a baseline signal, an initial ultrasonic pulse at diagnostic intensity level is transmitted by the transducer. Subsequently, a short duration (0.3 s) high intensity acoustic 'pushing pulse' is applied by the transducer, followed by a series of diagnostic intensity pulses. The diagnostic pulses are used to track the displacement of the tissues caused by the pushing pulse. The response of the tissues to the radiation force is assessed by conventional B-mode imaging pulses, and are represented shear wave velocity measurements as m/ sec.

PROTOCOL FOR ARFI ELASTOGRAPHY OF LIVER:

ARFI elastography Virtual Touch Tissue Quantification imaging is performed with curved array 4 MHz, B-mode ultrasound transducer. The anatomic region of interest is analysed using a 'region of interest' (ROI) cursor measuring 10 by 5 mm simultaneously with real time B- mode imaging. The patient lies in the dorsal decubitus position with the right arm maximally abducted. The right lobe of the liver is assessed through the intercostal approach. The other approach is the abdominal approach; the intercostal approach has been found to be superior to the abdominal approach (13).

PLACEMENT OF REGION OF INTEREST (ROI) IN THE LIVER USING ARFI ELASTOGRAPHY:

According to Goertz et al, who evaluated 57 patients, the best ARFI measurements with lowest rate of invalid measurements were using the intercostal approach to segments VII/ VIII of the liver (14). In a study done by Bota et al. among 83 patients in segments V and VIII of liver, there was no significant statistical difference between the mean liver stiffness between the two segments. They also found that the correlation between ARFI and fibrosis was similar in segments 5 and 8 of liver (r=0.836 vs. r=0.784) (p=0.33). There was no significant difference between the mean liver stiffness values between right and left lobe of the liver (2.06±0.1 vs.2.08±0.98 m/s, p=0.89) (15). The analysis of the right lobe was found to produce consistent values. The left lobe measurements have been found to have outliers, probably due to the interference of the shear wave by the cardiac pulsations. The ROI is placed 2-3 cm from the surface of the liver for accurate assessment, not exceeding a depth of 5 cm (13). There is no definite consensus on the depth of the ROI from the liver surface.

Short duration acoustic pulses with a fixed transmitted frequency of 2.6 MHz is used to mechanically excite and cause localised displacement of the tissues within the ROI. The tissue displacements create a shear wave propagation which is away from the region of excitation. The ultrasound beam tracking laterally to the single push beam is estimated as the maximum displacement. The shear wave speed can be reconstructed by measuring the time to peak displacement at each lateral location (16). The propagation velocity within the tissue is proportional to the square root of tissue elasticity. The result is expressed in meters per second (range, 0.5–4.4 m/s with±20 % accuracy over the range).

While taking the ARFI measurements, the patient is asked to stop breathing momentarily. Minimum of 10 measurements are taken from the liver and the median value is calculated. A good quality assessment includes IQR less than 30 % and success rate greater than 60% (17).

DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY IN STAGING LIVER FIBROSIS:

Nierhoff et al, performed systematic literature review of 36 articles and 3,951 patients evaluating the diagnostic accuracy of ARFI in staging liver fibrosis. Meta-analysis of the area under the receiver operating characteristic (ROC) curve (AUROC) and the diagnostic odds ratio (DOR) were performed. The inclusion criteria were all the studies which evaluated the efficacy of ARFI elastography of liver with liver biopsy as reference standard, METAVIR scoring system for liver fibrosis staging on biopsy, assessment of AUROC for fibrosis stage F>_2, F>_ 3, F=4 according to METAVIR or comparable liver fibrosis scoring system, and all the studies which studied the sensitivity, specificity, positive predictive value, negative predictive value according to the various ARFI shear wave velocity cut off values for various stage of liver fibrosis on biopsy.

A diagnostic tool is defined as perfect if the AUROC is 1, excellent if the AUROC is greater than 0.9 and good if the AUROC is greater than 0.8. The mean diagnostic accuracy of ARFI expressed as the AUROC was 0.84 for the diagnosis of significant fibrosis (F≥2), 0.89 for the diagnosis of severe fibrosis (F≥3) and 0.91 for the diagnosis of liver cirrhosis (F=4).

The studies which included HBV infected patients, the overall AUROC 0.87 (95 % CI, 0.85–0.90) and for studies without HBV-infected patients it was 0.92 (95 % CI, 0.89–0.95). There was significant influence on AUROC for F>_2 by mean BMI. It was found that with increase in BMI, there was significant decrease in the AUROC (P=0.0062) Hence, ARFI elastography of the liver has a good diagnostic accuracy for detecting significant liver fibrosis (F >_2, F>_3) and excellent for liver cirrhosis (F=4) (18).

REPRODUCIBILITY OF ARFI:

Since ARFI is real time ultrasound based quantitative measurement of tissue stiffness, interobserver variability has been studied. According to Boursier et al, the interobserver variability of ARFI is excellent with interclass correlation of 0.91 (19). Friedich Rust et al, found that there was 87% agreement between two observers regarding ARFI derived elastography stages (20).

ARFI ELASTOGRAPHY SHEAR WAVE VELOCITY (SWV) VALUES OF LIVER IN NORMAL HEALTHY SUBJECTS AND ITS CORRELATION WITH DIFFERENT STAGES OF FIBROSIS ON LIVER BIOPSY:

Several studies have been done by among healthy volunteers to determine the normal liver stiffness values by ARFI elastography.

Study done by Popescu et al. found the mean shear wave velocity of liver as 1.15 ± 0.21 m/s. Hoster et al. studied 68 healthy volunteers and have found the mean wave velocity of liver as 1.19 m/ sec. In another study done by Kim et al. among 133 healthy volunteers, that the mean shear wave velocity to be 1.08 ± 0.15 m/s (10).

A large study done by Kirches at al. in which the normal values and the shear wave velocity values of significant fibrosis and cirrhosis of liver was obtained with ARFI elastography using transient elastography (FibroScan- FS) as reference standard. The study enrolled six hundred and sixty six patients and sixty eight patients underwent liver biopsy. In this study they have found that the there was significant success rate of ARFI of liver as compared to FS [604/606 (99.7%) vs 482/606 (79.5%), $P < 0.001$]. There was significant correlation between ARFI-SWV and FibroScan liver stiffness (FS-LS) ($r = 0.920$, $P < 0.001$). As the stage of fibrosis increased, there was significant increase in the ARFI-SWV. For patients with no significant liver fibrosis, ARFI-SWV was found to be 1.09 ± 0.13 m/s and with FS-LS <

7.6 kPa); for patients with significant liver fibrosis as 1.46 ± 0.27 m/s (FS-LS ≤ 13.0 kPa); and for patients with liver cirrhosis as 2.55 ± 0.77 m/s (FS-LS > 13.0 kPa). Thus, according to Kirches at al. ARFI-SWV cut-off values of liver with no significant fibrosis was found to be (1.29 m/s; sensitivity, 91.4% and specificity 92.6%) and for liver cirrhosis (1.60 m/s; sensitivity, 92.3% and specificity 96.5%). The optimal cut-off ARFI shear wave velocity measurement for predicting liver fibrosis (F ≥ 2) was 1.32 m/s (sensitivity 87.0% and specificity 80.0%) and for liver cirrhosis (F4) 1.62 m/s (sensitivity 100% and specificity 85.7%), among the patients who underwent liver biopsy. There was excellent inter-and intraobserver reproducibility for ARFI-SWV determinations (10).

According to a large meta-analysis by Nierhoff et al, the ARFI shear wave velocity cut-off values of liver are 1.35 m/s for the diagnosis of significant fibrosis, 1.61 m/s for the diagnosis of severe fibrosis and 1.87 m/s for the diagnosis of liver cirrhosis (18).

FACTORS INFLUENCING ARFI ELASTOGRAPHY MEASUREMENTS:

The most important factor affecting the ARFI elastography score is the liver stiffness which is a surrogate marker for the extent of liver fibrosis. However, they are few factors which have been noted which affect the shear wave velocity measurements.

According to Rifai et al. the presence of liver inflammation causes increased shear wave velocity as opposed to those with no significant inflammation. They also found a positive correlation between the liver and the spleen size and the ARFI measurement (21). Takahashi et al. reported a positive correlation with increased ARFI measurements and elevated serum aspartate aminotransferase, alanine aminotransferase levels and liver pathological inflammation (22). Pathological liver steatosis has been found to be a significant factor which can affect liver ARFI measurements. Yoneda et al. suggested that ARFI measurements in liver steatosis are slower than normal individuals. Near normal ARFI values were seen in

patients with NAFLD and mild liver fibrosis. It was suggested that the presence of steatosis made the liver softer and thus causing low ARFI measurements (23). Patients with BMI greater than 40 kg/ m2 require XL ultrasound probe for ARFI measurements. However the standard cut-off values in this subset of patients has not yet been standardised.

LIMITATIONS AND CONTRAINDICATIONS OF ARFI ELASTOGRAPHY:

There are no significant contraindications or limitation to ARFI elastography evaluation of the liver. According to Japanese Society of Ultrasonics in Medicine, ARFI should be avoided after administration of few contrast enhanced ultrasound agents as it carries an increased risk of cavitation. The limitations of obesity, narrow intercostal space and ascites which were encountered by transient elastography, which do not apply for ARFI elastography. Palmeri et al, found that ARFI success rate of only 58% in patients with BMI> 40 kg/ m2 (13).

ARFI ELASTOGRAPHY OF LIVER IN CORRELATION WITH TRANSIENT ELASTOGRAPHY (TE FibroScan-FS):

Kirches et al, performed TE measurements of liver in six hundred and sixty six patients and compared them to ARFI and sixty eight patients and compared them with the fibrosis stage on liver biopsy. The overall success rate for TE was 77.8% ± 28.5% as compared to ARFI with a success rate of 93.3% ± 9.87% ($P < 0.001$). The success rate of liver stiffness measurement of 100% was observed in 262 (43.2%) patients by TE compared to 373 patients (61.6%, $P < 0.001$) by ARFI. Significant correlation was found between TE and ARFI ($p< 0.001$). Mean ARFI shear wave velocity measurements significantly increased with the stage of fibrosis. For patients with no significant fibrosis, the median ARFI value was 1.09 ± 0.13 m/s and (FS-LS < 7.6 kPa), for patients with significant fibrosis the median ARFI value was 1.44 ± 0.26 m/s (7.6 < FS-LS ≤ 13.0 kPa); and 2.55± 0.77 m/s for patients with liver cirrhosis

(13.0 < FS-LS). Cut-off values for patients with no significant fibrosis and patients with liver cirrhosis were taken in order to have maximum sensitivity and specificity. A cut-off value for ARFI shear wave velocity of 1.29 m/s was had sensitivity of 91.4% and specificity of 92.6% for patients with FS-LS < 7.6 kPa and a cut off value of 1.60 m/s for patients with FS-LS > 13.0 kPa with a sensitivity of 92.3% and specificity of 96.5%. Both the methods have high diagnostic accuracy for no significant fibrosis or liver cirrhosis (10).

ADVANTAGES OF ARFI ELASTOGRAPHY OVER TRANSIENT ELASTOGRAPHY:

ARFI as compared with TE has the advantage that it is integrated into a conventional ultrasound system. This enables measuring elastography scores of the liver and at the same time allows screening of focal liver lesion in patients with suspected chronic liver disease with the same ultrasound machine and the probe. In addition, the site of measurement ARFI can be visualised with B-mode ultrasound which allows more exact measurement of liver tissue elasticity by excluding small non-parenchymatous areas like the gall bladder, portal and biliary radicals within the ROI measurement site.

ARFI can be performed in both the lobes of the liver, which may enable a better overall estimation and distribution of liver fibrosis. Multiple biopsies would be required from different segments of the liver to assess accurate comparison with ARFI imaging. An advantage of TE over ARFI elastography is the larger measurement area of 4 cm in length, as compared to ROI of 1 cm in ARFI. This shortcoming is overcome by as ARFI elastography includes multiple measurements from the liver (18). The success rate of ARFI is better than transient elastography. Transient elastography is limited in patients with ascites and body mass index > 28 kg/ m2 (10).

ARFI ELASTOGRAPHY IN NONCIRRHOTIC PORTAL FIBROSIS (NCPF) OR IDIOPATHIC PORTAL HYPERTENSION (IPH):

Noncirrhotic portal fibrosis (NCPF) / Idiopathic portal hypertension (IPH) is a condition where in there is sclerosis of the peripheral portal veins, thin fibrous septa with absence of cirrhosis, resulting in portal hypertension. On imaging, this entity mimics cirrhosis and is difficult to distinguish it from cirrhosis which has a different clinical course and poorer prognosis. It is assumed that the stiffer the tissues , greater is the shear wave propagation velocity, hence in patients with NCPF/ IPH due to absence of bridging fibrosis, the ARFI shear wave velocities are likely to be lesser than liver cirrhosis. Clinically it manifests as anaemia, splenomegaly, esophagogastric varices, portal hypertensive gastropathy, ascites, and hepatic encephalopathy. Unlike other chronic hepatopathies, it usually does not lead to liver cirrhosis and does not develop into hepatocellular carcinoma. The main morbidity in these patients is variceal bleeding, which can be easily controlled unlike liver cirrhotic patients.

Liver biopsy is the essential to differentiate NCPF from liver cirrhosis. In order to avoid misdiagnosing these patients there is a need for a non invasive test. Seijo et al. reported that the liver stiffness measured by transient elastography in NCPH/ IPH is lower than liver cirrhosis. However the difference in the liver stiffness between NCPF/ IPH as compared to chronic hepatitis was not clear (24).

Spleen undergoes architectural changes like pulp hyperplasia, congestion due to increased blood flow and fibrosis in portal hypertension. Tawalkar et al. suggested that splenic stiffness increases with hepatic fibrosis and is independent of age, sex and body mass index. Splenic tissue enlargement due to passive spleen congestion and fibrogenesis is likely to cause increased splenic stiffness.

Yoshihiro Furuichi et al. studied 82 subjects in four groups of patients, namely 17 patients of NCPF/ IPH, 20 liver cirrhosis (LC) patients, 20 chronic hepatitis (CH) patients which were biopsy proven and 20 normal controls (NC). They have studied the median ARFI measurements of liver and splenic stiffness, splenic / liver stiffness ratio in these four groups. The spleen size was measured as the cross sectional splenic area which is given by the formula: Cross sectional area of spleen (cm2) = longest diameter (in cm) x shortest diameter (in cm). Splenomegaly was considered if the cross sectional area was greater than 40 cm2. They studied the correlation between the spleen size and splenic stiffness.

They found that the median ARFI elastography values (m/sec) of the liver in NCPF/ IPH group was 1.56 (0.98-2.37), LC 2.44 (1.08–3.83), CH 1.81 (1.03–2.36), NC group 1.13 (0.86–1.53). It was found that the median liver stiffness of the IPH group was lower than the liver cirrhosis group (p=0.00077), and was similar to the chronic hepatitis group (p=0.79) and was higher than the normal controls (p=0.0022) (25). The median splenic stiffness in (m/sec) of IPH was 3.88 (2.69–4.79), LC was 3.18 (2.06–4.52), CH was 2.27 (1.89–2.77), and NC was 1.99 (1.10–2.55). The splenic stiffness values of IPH group was higher than LC group (p=0.003) and CH group (p=0.00001). The spleen/ liver stiffness ratio was studied. The median values (m/ sec) of IPH was 2.47 (1.71–3.34), LC group was1.3 (0.78–2.64), CH was 1.32 (0.86–2.15), and NC was 1.78 (0.72–2.30). They suggested that the splenic stiffness is higher in IPH patients as compared the liver cirrhosis patients (25). The spleen/ liver stiffness cut-off value was taken as 1.53 and the AUROC was 0.920, with sensitivity of 1.000, specificity of 0.720, PPV of 0.708 and NPV of 1.00 indicating that spleen/ liver stiffness ratio is useful to differentiate IPH from liver cirrhosis.

The cut-off value for spleen stiffness was taken as 2.69 m/s to differentiate between IPH and liver cirrhosis. AUROC was 0.997, sensitivity 1.000 (0.895–1.000), specificity 0.950 (0.850–0.950), PPV0.944 (0.833–0.944), NPV 1.000 (0.895–1.000), and accuracy 0.973 (0.865–

0.973). The above findings in their study concluded that the splenic stiffness and the spleen/ liver stiffness ratio are important to specifically diagnose IPH/ NCPF and to differentiate from liver cirrhosis and chronic hepatitis (25).

In the study done by Yoshihiro Furuichi et al. the spleen size was the largest in the NCPF/ IPH group (102.5 cm2), followed by liver cirrhosis group (44cm2), chronic hepatitis group (28.9 cm2) and the normal subjects (22.4 cm2). There was no correlation between spleen size and spleen stiffness in each of the groups (rs = -0.081, 0.091, 0.300, 0.071, respectively, p = 0.742, 0.656, 0.190, 0.757). Hence the spleen size was not the cause of the increase in spleen stiffness in IPH. In this study, the liver functions, esophageal varices and radiological findings were similar in the IPH patients and the liver cirrhosis group. The size of the spleen was larger and stiffer in IPH as compared with liver cirrhosis group. The liver stiffness was marked in the liver cirrhosis group as compared with the IPH group. They have suggested that it is possible to non-invasively diagnose IPH by splenic stiffness and spleen/ liver stiffness ratio by ARFI elastography (25).

DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO PREDICT PORTAL HYPERTENSION:

There are published studies which have assessed the diagnostic accuracy of ARFI elastography in predicting portal hypertension which show varying results.

In the first study done by Sirli et al. 157 cirrhotic patients were studied using ARFI and also had a recent gastroscopy. The mean ARFI measurement of liver with significant esophageal varices (greater than grade 2) was not significantly different from the patients with no or small esophageal varices: 2.73±0.71 vs. 2.8±0.71m/s (p=0.49). There was significant difference in the mean ARFI values in patients with variceal bleeding and the without history of variceal bleeding: 2.78±0.81 vs.2.77±0.7m/s (p=0.99) (26).

The first study which has assessed the correlation of ARFI elastography with HVPG measurements was by Salzl et al. They have evaluated 36 patients with cirrhosis and 12 non cirrhotic patients and have obtained good correlation between the liver stiffness measurements by ARFI and HVPG (r=0.709). The AUROC for predicting clinically significant portal hypertension was 0.874 (26).

Riafai et al. evaluated both the liver and the splenic stiffness in 125 subjects (30 cirrhotics with portal hypertension, 70 chronic hepatopathies without portal hypertension and 25 healthy controls). They have found that the liver stiffness is a better predictor of portal hypertension than splenic stiffness (AUROC 0.90 vs 0.68). The liver stiffness cut-off for predicting portal hypertension in this study was 1.67m/sec which was lower than the other (54). The splenic stiffness cut-off for predicting portal hypertension was 3.29 m/ sec with 47% sensitivity and 73% specificity.

Gao et al. studied the correlation of liver stiffness and splenic stiffness by ARFI and HVPG measurements among 10 patients before and after transjugular intrahepatic porto-systemic shunt (TPIS) placement. There mean splenic stiffness values before shunt placement were higher than post stenting (3.65±0.32 m/s vs. 3.27±0.30 m/s, $p<0.001$) (27). There was no significant difference in the liver stiffness pre and post TIPS.

Mori et al. published the first study analysing the diagnostic accuracy of liver stiffness and the spleen stiffness by ARFI elastography as indicators of portal hypertension to predict ascites or esophageal varices in chronic hepatitis C patients. They found that the spleen stiffness and not the liver stiffness was associated with the presence of ascites ($p<0.05$). The

AUROC for splenic stiffness to predict ascites was 0.80.There was no association between the splenic stiffness and the presence of esophageal varices.

In advanced cirrhosis, they are extra-hepatic factors like hyperdynamic circulation; splanchnic vasodilatation and resistance to the portal flow by porto- systemic collaterals causes the raise in portal pressure. Thus, the liver stiffness may not represent complex changes in impending portal hypertension. The portal hypertension leads to pulp hyperplasia resulting in elevated splenic stiffness (28).

Another study was published by Xiao-ping et al. who studied 264 subjects, out of which 60 were healthy volunteers, 66 with chronic hepatitis who had undergone liver biopsy and 138 with hepatitis B related cirrhosis. They found significant linear correlations between liver (Spearman $\rho = 0.87$; $p < .001$) and spleen (Spearman $\rho = 0.76$; $p < .001$) stiffness and the fibrosis stage. Liver and spleen stiffness values increased as the fibrosis stage increased; however there was overlap in liver stiffness were detected in stages 0 and 1 and 1 and 2, and splenic stiffness between stages 0 and 1, 1 and 2, and 2 and 3. Liver stiffness cut off values were 1.69 m/s for predicting stage 3 or greater (AUROC = 0.99) and 1.88 m/s for stage 4 (AUROC = 0.97). The spleen stiffness cut off value was 2.72 m/s for stage 4 (AUROC = 0.96). The liver stiffness was not correlated with the grade of esophageal varices, whereas a significant linear correlation (Spearman $\rho = 0.65$; $P < .001$) was found between spleen stiffness and the grade of esophageal varices. Spleen stiffness cut off value for predicting varices was 3.16 m/s (AUROC = 0.83).They suggested that splenic stiffness by ARFI elastography can be used to determine the presence and severity of esophageal varices (29).

DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF SPLEEN TO PREDICT CIRRHOSIS:

Gallotti et al. were the first to study the splenic stiffness (SS) in 35 healthy volunteers. The mean SS value in healthy volunteers was 2.44 m/ sec. Bota et al. studied 82 subjects to determine the splenic stiffness (SS) in healthy volunteers and to assess the predictive of SS for liver cirrhosis and presence and severity of esophageal varices. The mean ARFI SS values (m/s) in healthy subjects was 2.04±0.28 and 3.10±0.55 in cirrhotic patients ($p<0.001$).They found that the best ARFI SS cut-off value for predicting cirrhosis was 2.51 m/s (AUROC 0.91, $p<0.0001$, with 85.2% Se,91.7% Sp, 95.8% PPV, 73.3% NPV and 87.1% accuracy). The accuracy increased when liver stiffness (LS) and SS were combined. There was no significant difference in SS between patients with esophageal varices (EV) and without EV and among those with and without a history of variceal bleed (30).

DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF SPLEEN TO PREDICT ESOPHAGEAL VARCIES:

Takuma Y et al. studied ARFI elastography of the spleen in 340 patients to predict esophageal varices among cirrhotic patients. They found that the patients with cirrhosis had higher LS and SS values as compared to controls ($p< o.0001$). The SS values were higher among patients with esophageal varices than controls. Higher SS values were noted in patients with high risk esophageal varices (EV). SS was found to have highest diagnostic accuracy than other non-invasive parameters and was independent of the etiology of cirrhosis. SS was also found to have a high NPV and sensitivity regardless of the severity and etiology of cirrhosis. The SS cut off value for indentifying patients with esophageal varices was 3.18 m/ sec with 98.4% negative predictive value, 98.5% sensitivity, 75.0% accuracy, and 0.025 negative likelihood ratio. The SS cut off value to identify cirrhotic patients with high risk esophageal varices was 3.30m/s with a 99.4% negative predictive value, 98.9%

sensitivity, 72.1% accuracy, and 0.018 negative likelihood ratio (31). The study proposed that SS values less than 3.3 m/s ruled out the presence of high-risk varices in patients with compensated or decompensated cirrhosis. The AUROC of liver stiffness (LS) to predict EV was 0.746 and is therefore useful but not excellent. With increase in portal hypertension, there is increase in intrahepatic vascular resistance from accumulation of fibrosis in the extracellular matrix. However in this study further increase in LS did not reflect late complications of portal hypertension (31).

Vizzutti et al. showed association between LS and hepatic venous pressure gradient (HVPG). The LS correlated well with HVPG when HVPG is less than 10 mm of Hg than when it is greater than 10 mm of Hg. Splenomegaly in portal hypertension is due to portal congestion and tissue hyperplasia. Some studies showed no difference in portal pressures in patients with cirrhosis with or without splenomegaly. Few studies showed that in chronic liver disease, there is increase in portal pressure with increase in the spleen size and presence of esophageal varices. In this study, 48 patients with normal spleen size had high SS values and 48% had EV. Among these patients there was significant difference in the LS and the platelet count between patients with and without EV. They found that platelet count, LS, SS and spleen diameter are independent parameters associated with presence of esophageal varices. SS had significantly better AUROC than spleen diameter to predict esophageal varices. It is proposed that there is increase in diffuse fibrosis of the spleen trabeculae rather than congestion of red pulp and tissue hyperplasia that causes increase in SS with normal spleen size. The limitation of this study was that there was no correlation with SS by ARFI elastography and HVPG. The failure rate to perform ARFI was 0.8% for LS and 4.5% for SS. They suggested that splenic stiffness by ARFI elastography can rule out the presence of esophageal varices, indentify the patients with high risk esophageal varices. It can be used as an initial non invasive screening tool which is more cost effective than screening endoscopy (31).

SYSTEMATIC REVIEW AND META-ANALYSIS OF THE DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF SPLEEN TO PPREDICT ESOPHGEAL VARICES IN CHRONIC LIVER DISEASE:

In a recent publication by Singh et al. a systematic review and meta-analysis of 12 studies was done that compared the diagnostic accuracy of splenic stiffness (SS) by ARFI elastography with that of endoscopy (EGD) in detecting esophageal varices (EV) in patients with chronic liver disease.

The study showed that SS detected with the presence of EV with 78% sensitivity (95% confidence interval [CI], 75%–81%), 76% specificity (95% CI, 72%–79%), a positive likelihood ratio (LR) of 3.4 (95% CI, 2.3–4.9), a negative LR of 0.2 (95% CI, 0.1–0.4), and a diagnostic odds ratio of 19.3 (95% CI, 7.5–49.8).

In a meta-analysis of 9 studies, SS detected the presence of clinically significant EV with 81% sensitivity (95% CI, 76%–86%), 66% specificity (95% CI, 61%–69%), a positive LR of 2.5 (95% CI, 1.7–3.9), a negative LR of 0.2 (95% CI, 0.1–0.5), and a diagnostic odds ratio of 12.6 (95% CI, 5.5–28.7).

Due to the different elastography techniques and study locations there was significant heterogeneity among the studies. They proposed that the current techniques of measuring splenic stiffness are limited to detect esophageal varices (32).

NON INVASIVE METHODS TO ASSESS LIVER FIBROSIS: SERUM FIBROSIS MARKERS WITH SERUM FIBROSIS SCORES:

SERUM FIBROSIS MARKERS:

The serum fibrosis markers have advantage over liver biopsy as they are less expensive, less dependence on professional expertise and can be frequently repeated. They can reflect the degree of liver fibrosis and can predict clinically relevant outcomes. They are divided into indirect and direct markers of fibrosis.

INDIRECT MARKERS OF LIVER FIBROSIS (Class II- simple lab tests): The diagnostic performance of these tests as 'surrogate markers' of liver fibrosis have been studied extensively. These include AST, ALT which are markers of cytolysis, gamma GT, bilirubin which indicates cholestasis, INR, cholesterol, Apo A1, haptoglobulin and N-glycans which indicate hepatocyte function and platelet count which indicate hypersplenism due to portal hypertension. These are also surrogate markers for liver inflammation and steatosis and can predict progression of fibrosis (33). They are about 20 indices or scores which are multiparameteric reported in literature based on routine lab tests. The serum tests which are biomarkers for liver fibrosis are Aspartate aminotransferase (AST)-to-platelet ratio index (APRI), FIB-4, Forns index, Fibroindex, Fibrotest, Fibrometer and Hepascore. The advantage of APRI, the Forns index and FIB-4 are that they are based on routine blood tests and thus are less expensive and more frequently used than others. Of these tests, APRI and Fibrotest have been proposed for clinical application. AST- to platelet ratio (APRI) test has been found to be a reliable marker to non- invasively predict significant fibrosis and cirrhosis in patients with chronic hepatitis C AUC of 0.80 (34). The FibroTest which is a combination of bilirubin, alpha 2 macroglobulin, gamma GT, Apo A1 and haptoglobulin is the most validated test for estimating liver fibrosis.

In a meta- analysis of 14 studies of various indirect serum fibrosis markers including APRI and FibroTest showed that they cannot reliably differentiae different grade of fibrosis as they can be influenced by multiple factors including ongoing drug therapies (35).

For portal hypertension Child- Pugh score correlates with HVPG and the prevalence of varices in the cirrhotic patients. Platelet count has been found to be independently correlates with the grade of esophageal varices in many studies.

DIRECT MARKERS OF LIVER FIBROSIS (Class 1):

The direct markers are biochemical markers assessed in peripheral blood which assess the changes in the content and composition of the extracellular matrix proteins, matrix modifying enzymes and their inhibitors in the liver. They are expensive tests and have been proposed to be used in various algorithms. They provide good discrimination between patients with and without liver fibrosis and have promising approach in future (33).

SERUM FIBROSIS SCORES:

Serum fibrosis scores that are based only on indirect fibrosis markers show poor discrimination between different stages of fibrosis than the scores based on both direct and indirect fibrosis markers. Most of these fibrosis scores have been primarily validated in HCV, HIV/ HCV coinfected patients and HBV infections. The advantage of APRI, the Forns index and FIB-4 are that they are based on routine blood tests and thus are less expensive and more frequently used than others (36). Lin ZH et al. in a meta-analysis of 40 studies assessed the performance of APRI as non invasive tool as an alternate to liver biopsy in HCV monoinfected and HIV and HCV coinfected patients. The summary AUROC of the APRI for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis were 0.77, 0.80, and 0.83, respectively. For significant fibrosis, an APRI threshold of 0.7 was 77% sensitive and 72% specific. For severe fibrosis, a threshold of 1.0 was 61% sensitive and 64% specific. For

cirrhosis, a threshold of 1.0 was 76% sensitive and 72% specific. Hence, it was found that APRI can identify hepatitis C-related fibrosis with a moderate degree of accuracy (37). Jin et al. assessed in a meta-analysis of 9 studies which included 1798 patients, the efficacy of APRI as alternative for liver biopsy for predicting significant fibrosis and cirrhosis in patients with hepatitis B. AUROC of the APRI for significant fibrosis and cirrhosis were 0.79 and 0.75, respectively. APRI cut off for significant fibrosis was 0.5 was 84% sensitive and 41% specific. At the cut off of 1.5, the summary sensitivity and specificity were 49% and 84%, respectively. APRI threshold for cirrhosis was 1.0-1.5 was 54% sensitive and 78% specific. At the cut off of 2.0, the summary sensitivity and specificity were 28% and 87%, respectively. Hence in patients with hepatitis B related liver disease, APRI showed limited value in to assess significant fibrosis and cirrhosis (38).

OTHER IMAGING MODLAITIES TO ASSESS PORTAL HYPERTENSION:

- Ultrasound
- Doppler
- CT
- MRI with MR elastography (MRE)

GRAY SCALE ULTRASOUND IMAGING IN CHRONIC LIVER DISEASE:

Ultrasound is the most commonly available, non invasive imaging modality used for diagnosis and surveillance of patients with chronic liver disease. For the diagnosis of cirrhosis on conventional ultrasound there are changes in liver morphology and signs of portal hypertension. The liver morphological changes include nodular surface, coarse echotexture, left lobe to right lobe ratio greater than 1.3, caudate lobe hypertrophy indicated by ratio of caudate lobe to right lobe ratio greater than 0.65 and reduction in the medial

segment of the left lobe of the liver. Of these signs, liver surface nodularity has been founder to have higher accuracy. Combination of liver surface nodularity and portal vein velocity more than 12 cm per second is 80 % accurate to differentiate between significant fibrosis and cirrhosis in patients with chronic hepatitis (39). There are studies in the literature which have looked at the diagnostic accuracy of gray scale ultrasound for early fibrosis, significant fibrosis, severe fibrosis and cirrhosis of liver. Few scoring systems have also been proposed. Choong et al used three liver morphologic parameters on ultrasound to grade liver in patients with CLD. They are liver echotexture, liver surface and liver edge. These three parameters were compared with the liver histopathological score of fibrosis. They have found that ultrasound has low sensitivity to identify or rule out early fibrosis. Surface nodularity on ultrasound best predicts significant liver fibrosis on histopathology. Combined evaluation of liver surface, texture and edge has highest sensitivity (63%) than each of the individual parameters to identify significant fibrosis on ultrasound. Liver edge has least sensitivity but has high specificity which would help to rule out significant fibrosis and cirrhosis (40).

Afzal et al. used six morphological parameters on gray scale ultrasound to obtain an ultrasound score to differentiate between mild/ no fibrosis and significant fibrosis. They have used liver echotexture, liver edge, liver surface, liver size, portal vein size and splenic size. Liver parenchymal echotexture if homogenous was scored as 0, coarse as 1 and highly inhomogeneous as 2. Smooth liver surface was scored as 0, irregular surface as 1 and nodular surface as 2. A sharp liver edge was scored as 0, blunted as 1 and rounded edge as 2. Normal liver size was scored as 0, enlarged liver (> 15 cm measured in the mid clavicular line) as 1 and shrunken liver (< 10 cm measured in the mid clavicular line) as 2. Portal vein diameter less than 13 cm was scored as 0 and greater than 13 cm was scored as 1 and spleen size less than 13 cm was scored as 0 and greater than 13 cm was scored as 1.They have noted that the liver surface evaluation had highest specificity (86%) and reproducibility for staging liver

fibrosis. Liver edge was also found to have good sensitivity (84%) for liver fibrosis which was not found in the other studies. The presence of steatosis affects the assessment of liver echotexture which can be seen in patients with chronic viral hepatitis (41).

SECOND LINE IMAGING METHODS FOR LIVER CIRRHOSIS AND PORTAL HYPERTENSION:

Computed tomography (CT) and magnetic resonance imaging (MRI) provide accurate visualization of the liver parenchyma and the portal system. CT is a reliable indicator for large esophageal varices with a specificity of 90- 100% and sensitivity of 84- 100%, however is a poor indicator of small varices. Blood flow in the portal vein and the azygos vein can be studied using dynamic contrast enhanced CT and MRI and phase contrast MR angiography. Blood flow in the azygos vein which is measured by MRI correlates with the presence of esophageal varices and increased risk of variceal bleeding. Estimation of portal fraction of liver perfusion and mean transit time at MRI has been shown to correlate with HVPG (42).

MR ELASTOGRAPHY (MRE):

MRE is a novel technique to assess liver fibrosis. The measurements in MRE are obtained using motion sensitive MR sequences with application of acoustic waves. Preliminary tests have shown that is can predict the stage of liver fibrosis in chronic liver disease. Splenic stiffness measured by MRE has been found to correlate well with the liver stiffness and might better predict portal pressure. MRE as compared with FibroScan does not require acoustic window, freely- oriented field of view and does not depend on patient body habitus. It is expensive and time consuming and is used for patients who are undergoing MRI for other reasons (42).

INVASIVE METHODS TO ASSESS PORTAL HYPERTENSION:

(i). Hepatic venous pressure gradient (HVPG).

(ii). Endoscopy for esophageal varices.

(iii). Liver biopsy.

HEPATIC VENOUS PRESSURE GRADIENT (HVPG):

HVPG is considered the gold standard for measurement of portal pressure. It is a safe and a reproducible technique used to measure portal pressure gradient (PPG) in cirrhosis (sinusoidal portal hypertension). The portal pressure gradient (PPG) is the pressure difference between the portal vein and the IVC and it represents the portal perfusion pressure. The portal pressure is measured via the transhepatic or transvenous catheterization of the portal vein and the IVC pressure is measured to obtain the portal pressure gradient (PPG).

Hepatic venous pressure gradient (HVPG) is defined as the difference between wedge hepatic venous pressure (WHVP) and free hepatic venous pressure (FHVP). Wedge hepatic pressure is based on the concept that is the hepatic vein outflow is blocked by a ‘wedged’ catheter, the static column of blood transmits the pressure from the hepatic sinusoids. Hence WHVP represents the hepatic sinusoidal pressure and not the portal pressure. As the intersinusoidal communications are lost in cirrhosis due to fibrosis, septa and regenerative nodule formation, the measurement of sinusoidal pressure will reflect the portal pressure. WHVP has been found to adequately reflect portal pressures in alcoholic liver disease, hepatitis C and B related chronic liver disease (43). The use of balloon tipped catheters has been recommended over wedged catheter as it senses a larger volume of liver circulation, thereby increasing the reliability and accuracy of measurement.

USE OF HVPG IN THE CLASSIFICATION OF PORTAL HYPERTENSION:

HVPG is a strong independent prognostic indicator in patients with compensated and decompensated cirrhosis. Among patients with an unknown cause for portal hypertension, elevated WHVP and HVPG are due to increase in sinusoidal pressure, of which the most common cause is cirrhosis. Normal HVPG and WHVP indicate presinisoidal portal hypertension, and in post sinusoidal portal hypertension both the FHVP and WHVP are increased.

HVPG IN COMPENSATED CIRRHOSIS:

Subclinical portal hypertension is when the HVPG is 6-9 mm of Hg with a negligible risk of developing complications related to portal hypertension. Clinically significant portal hypertension (CSPH) is seen when the HVPG is greater than 10 mm of Hg. At these portal pressures the patients with compensated cirrhosis are at an increased risk for decompensation and can develop complications like ascites, varices and hepatorenal syndrome and hepatocellular carcinoma (44). Increase risk of variceal bleeding can occur when the HVPG exceeds 12 mm of Hg. The mortality of patients increases if the HVPG is greater than 16 mm of Hg (45). HVPG when measured within 48 hours of admission following an acute variceal bleeding predicts the failure of haemostasis and poor 1 year survival (16).

HVPG IN DECOMPENSATED CIRRHOSIS:

HVPG greater than 16 mm of Hg is associated with increased risk of variceal bleeding and mortality. There is increased mortality in patients with alcoholic cirrhosis with acute alcoholic hepatitis if the HVPG is greater than 22 mm Hg(46). The risk of spontaneous bacterial peritonitis increases when the HVPG is greater than 30 mm of Hg (47). In patients with decompensated cirrhosis for liver transplantation, HVPG is an independent prognostic indicator from Model for End- Stage Liver Disease score (MELD) (48). HVPG greater than

10 mm of Hg is a contraindication for liver transplantation as these patients have high incidence of hepatic decompensation within 3 months (16, 17).

HVPG FOR ASSESSMENT OF THE HEMODYNAMIC RESPONSE TO PHARMACOLOGICAL THERAPY FOR PORTAL HYPERTENSION:

Good hemodynamic response is when the HVPG has reduced to 12 mm Hg or 20 % of baseline measurements due to pharmacological therapy or improvement in liver disease. By achieving the target portal pressure, there is a reduction in the risk of complications related to portal hypertension. Failure to attain the target portal pressure is an independent risk factor for variceal bleed (50).

HVPG IN EVALUATION OF PROGRESSION AND REGRESSION OF CHRONIC LIVER DISEASE:

Studies have shown that HVPG increases with increase in histological grade of liver fibrosis and decreases in response to antiviral therapy in patients with chronic viral hepatitis (51).

Hence serial measurements can be used to monitoring progression and regression in response to antiviral therapy in patients with hepatitis C related chronic liver disease (19).

HVPG IN POST LIVER TRANSPLANTATION:

Among patients undergoing liver transplantation, HVPG is an excellent tool for assessment of highest risk patients for HCV recurrence and post transplant hepatic decompensation. HVPG > 6 mm of Hg shows highest risk of post transplant HCV recurrence and cirrhosis which was found to be more reliable than liver biopsy (1). HVPG also demonstrates the changes to antiviral therapy in HCV recurrence post liver transplant and is correlates well with liver histology (52).

RELATIVE CONTRAINDICATION OF HVPG:

The relative contraindications for HVPG are platelet count less than 20, 000 or prothrombin time less than 30%. The procedure can be performed with fresh frozen plasma replacement. Allergy to iodinated contrast medium is also a relative contraindication as HVPG can be performed using carbon dioxide. Previous episode of cardiac arrhythmias when the catheter was removed from right atrium is a relative contraindication (2).

LIMITATIONS OF HVPG:

In conditions where the increased resistance is predominantly presinisoidal, HVPG does not accurately represent portal pressure gradient.

Communications between the hepatic veins may interfere with wedge hepatic pressure gradient measurements (2).

ENDOSCOPY:

Upper GI endoscopy is considered as gold standard for evaluation and endoscopic management of varices (53). Endoscopy identifies esophageal, gastric and rarely ectopic duodenal varices. The risk of bleeding from esophageal varices (EV) is proportional to the size of the varices and presence of 'high risk signs'. Size of esophageal varices is graded into small, medium and large. Small esophageal varices are veins which are minimally elevated but they do not disappear on insufflation. Medium varices are tortuous veins which occupy less than one third of the esophageal lumen. Large varices are tortuous veins occupying greater than one third if esophageal lumen (54). Esophageal varices is simplified and classified as small and large based on size less than or greater than 5 mm (53).

The presence of red wales and red spots indicate high risk of variceal bleed and require prompt primary prophylaxis even in the presence of small varices (54). Patients with advanced cirrhosis (Child-Pugh B and C) are also at increased risk of variceal bleed.

Gastroesophageal varices (GOV) account for 5-10% of GI bleed. They are an extension of esophageal varices and are of 2 types, GOV1 which are along the lesser curvature of the stomach which are more common, and GOV2 along the fundus. Isolated gastric varices (IGV) are of two types; IGV1 are located at the fundus and IGV2 are located in the body, antrum and the pylorus. Splenic vein thrombosis should be ruled out in type 1 isolated gastric varices (2). GOV1 and isolated gastric varices attain are larger in size and attain greater wall tension at lower portal pressure than esophageal varices (HVPG 10-12 mm of Hg).

Portal hypertensive gastropathy (PHG) is very common in patients with cirrhosis, the prevalence ranges from 11 to 80% (2).

Esophageal varices are seen in 30- 40% of patients with compensated cirrhosis and 60% in decompensated cirrhosis. Hence endoscopy is mandatory in newly diagnosed patient with cirrhosis to screen for esophageal varices. Among the patients without esophageal varices, 5-10% develops them every year; hence endoscopy should be done every 2-3 years in compensated cirrhosis (2). Patients with poor liver function and HVPG greater than 10 mm of Hg are at a higher risk of variceal bleed and must undergo endoscopy at shorter intervals. Small varices increase at the rate of 10-15 % per year and endoscopy should be repeated 1-2 yearly (53).

Variceal pressure can be measured endoscopically by direct puncture of the varices or by using endoscopic pressure sensitive gauge. The second method is more sensitive to assess pharmacological response for portal hypertension. However it requires specific training and use predominantly for research purposes (55).

LIVER BIOPSY:

Liver biopsy is a gold standard for assessing cirrhosis. It is an important tool in assessing and grading liver fibrosis and for making therapeutic decisions (56). Liver biopsy has an important role in the diagnosis of intrahepatic portal hypertension and has limited role in the prehepatic and post hepatic causes of portal hypertension. In patients with portal vein thrombosis, liver biopsy is indicated when there is cirrhosis or nodular regenerative hyperplasia. In Budd-Chari syndrome, it is indicated when the clinical suspicion is high but the imaging fails to demonstrate large hepatic veins or IVC obstruction (57). Liver biopsy can assess the severity of portal hypertension in cirrhosis. Higher HVPG values and clinical decompensation are associated with small size nodules with increased septal thickness on histology (58).

Newer technology like quantitative computer-assisted digital-image analysis (DIA) of histological liver sections has recently been validated for assessing liver fibrosis (as fibrosis area). Studies have shown that DIA is better than conventional scoring systems for liver fibrosis (eg: Ishak score) and should be used to validate studies assessing non invasive markers of liver fibrosis and liver stiffness. According to Calvaruso et al. the fibrosis staging using DIA correlated well with the HVPG measurements in patients with post transplant HCV infection (59). DIA has also been validated with HVPG measurements in patients with cirrhosis (60). The routes of liver biopsy are **p**ercutaneous biopsy, Transjugular biopsy (TJLB), laparoscopic biopsy, Biopsy or fine needle aspiration under ultrasonography or computed tomography and Peroperative biopsy.

TRANSJUGULAR LIVER BIOPSY (TJLB):

TJLB is indicated in situations where percutaneous liver biopsy is contraindicated due to ascites, coagulopathy, acute liver failure and liver transplant recipients. It also allows measurement of hepatic venous pressure gradient in the same sitting which has a prognostic value for survival and also assesses response to pharmacologic treatment of portal hypertension.

ADEQUATE LIVER BIOPSY SPECIMEN:

For histological diagnosis of chronic liver disease, liver specimen should be atleast 15 mm with greater than 6 complete portal tracts (CPTs). Ideally, the biopsy specimen should be 20-25 mm in length with greater than 11 CPTs to reliably stage and grade chronic liver disease (61).

LIMITATIONS OF LIVER BIOPSY:

It is an invasive procedure which requires inpatient care and post procedural monitoring for 24 hours. Sampling error can occur as liver fibrosis is not uniform throughout the liver. Liver biopsy samples only 1/ 50,000 Th part of the liver parenchyma; hence it may underestimate the degree and extent of liver fibrosis. Liver biopsy specimen may be inadequate or have fragmented specimens. Inter and intra-observer variability in interpretation especially in macronodular cirrhosis. Complications rate of 7% including hemorrhage, biliary leak or fistula.

SCORING SYSTEMS OF LIVER FIBROSIS ON HISTOPATHOLOGY:

They are several systems which have been proposed to classify the fibrosis into various stages based on the amount of collagen staining on liver biopsy specimen. The staging systems used are the Histology activity index (HAI: Knodell score), Ishak score modification of the HAI sore and the METAVIR score.

The METAVIR score is a semiquantitiative classification system which scores the necroinflamamtion and the degree of liver fibrosis. It recognises subtler variations in the degree of liver fibrosis as compared to the Knodell fibrosis score. It is a well validated scoring system with good inter and intra-observer reproducibility. Fibrosis is assessed on a scale of 0 to 4. Clinically significant fibrosis is defined as fibrosis greater than F2.

F0- no fibrosis

F1- portal fibrosis without septae

F2- portal fibrosis with few septae

F3- numerous septae without cirrhosis

F4- cirrhosis (62)

The necroinflamamtion on liver biopsy specimen is graded as A0-A3.

A0- no activity

A1- mild activity

A2- moderate activity

A3- severe activity (62)

MATERIALS AND METHODS:

STUDY DESIGN: Test of diagnostic accuracy**.** Prospective study approved by Institutional Research Board (IRB). IRB study number: 22X945

SETTING: Christian Medical College (CMC) Vellore is a tertiary care centre in northern Tamil Nadu. The institution was established in 1900 and is now a 2700 bedded hospital. The annual outpatient visits is around 1.9 million with inpatient admissions of ~ 120,000. The Department of Radiology in CMC, Vellore was established in 1936. Digitalization of the system and introduction of PACS (Picture Archival and Communication System) was done in the year 2000. The Department functions independently with around 70 radiologists. The radiological investigations routinely performed are radiographs, IVU, barium studies, ultrasonography and Doppler studies, mammograms, CT and MRI.

METHODOLOGY:

SAMPLE SIZE:

The total sample size calculated was 44. 22 NCPF patients and 22 patients with other causes of cirrhosis were required for the study.

INCLUSION CRITERIA OF THE STUDY POPULATION:

All the patients who are admitted in CMC for liver biopsy with a clinical diagnosis of cryptogenic cirrhosis for percutaneous ultrasound guided liver biopsy, percutaneous blind liver biopsy or trans-jugular liver biopsy from September 2012 to October 2013. Patients with who had liver biopsy in the past were also studied. 39 patients with a liver biopsy were included and ARFI elastography of the liver and the spleen was performed. 9 patients who were clinically suspected to have NCPF were also studied.

58 cases of portal hypertension of any etiology were studied to assess the correlation of ARFI elastography of the liver and the spleen with the presence of high risk esophageal varices on endoscopy. This group includes 39 liver biopsy cases, 9 suspected NCPF cases without liver biopsy and 10 cases of portal hypertension due to other known etiologies.

EXCLUSION CRITERIA:

Patients who cannot co-operate for breath hold during the ARFI elastography study, children less than 5 years of age and patients with BMI greater than 40 kg/ m2 were excluded from the study.

SAMPLING AND CONSENT:

The prospective study patients were admitted for liver biopsy under the department of Hepatology. All patients who fulfilled the inclusion criteria were included in the study. Personal data, clinical, biochemical, ultrasound findings and median ARFI shear wave velocity scores of the liver and the spleen were entered into a coded – numbered proforma (Appendix 1). Informed consent was obtained from the patient / patient`s relative prior to ultrasound in accordance with the ethical guidelines of Helsinki declaration and approved by the Institutional review board of the hospital. The consent form and the patient's information sheet are attached in Appendix 2.

VARIABLES:

The various variables studied were patient's age, gender, BMI, clinical presentation, haemoglobin, platelet count, serum bilirubin, serum albumin, AST, ALT, PT with INR, CHILD PUGH score. On imaging, the B- mode ultrasound parameters studied were liver size, liver echotexture, liver surface, liver edge, periportal echogenicity, portal vein size, size of spleen, spleen area (product of maximum spleen length and spleen width at the hilum), presence of collaterals and ascites. ARFI elastography parameters measured were liver stiffness (LS), spleen stiffness (SS) and ratio of spleen stiffness and liver stiffness (LS/ SS).

ULTRASOUND SCANNER:

Acoustic radiation force impulse (ARFI) elastography is a radiation force based imaging method combined with conventional B mode ultrasound (Acuson S 2000, SEIMENS Medical Solutions). The 'Virtual Touch Tissue Quantification' (VTQ) provided by SEIMENS quantifies the tissue stiffness by shear wave velocity of acoustic radiation force impulse displacement within the human tissues. It is represented as m/sec and is proportional to the square root of the tissue elasticity.

To obtain a baseline signal, an initial ultrasonic pulse at diagnostic intensity level is transmitted by the transducer. Subsequently, a short duration (0.3 s) high intensity acoustic 'pushing pulse' is applied by the transducer, followed by a series of diagnostic intensity pulses. The diagnostic pulses are used to track the displacement of the tissues caused by the pushing pulse. The response of the tissues to the radiation force is assessed by conventional B-mode imaging pulses, and are represented shear wave velocity measurements as m/ sec.

PROTOCOL FOR LIVER AND SPLEEN STIFFNESS MEASURMNENT BY ARFI ELASTOGRAPHY:

ARFI elastography Virtual Touch Tissue Quantification imaging is performed with curved array 4 MHz, B-mode ultrasound transducer. Conventional ultrasound was performed in all patients.

For liver stiffness (LS) assessment the patient lies in the dorsal decubitus position with the right arm maximally abducted. The right lobe of the liver is assessed through the intercostal approach. The patient was asked to hold the breath for 1-2 seconds while measurements were taken. The Region of interest (ROI) cursor was placed 2-3 cm below the liver capsule not exceeding a depth of 6 cm from the liver capsule. The gall bladder, visible vessels and the biliary radicals were avoided within the ROI cursor. Total of 10 measurements were taken from segments 5 and 8 of the liver. The median shear wave velocity measurement was taken for analysis. The interquartile range between the measurements for accurate assessment was taken to be less than 25%. (13).

For splenic stiffness assessment (SS), the patient lies in the right lateral decubitus position with left arm in maximum abduction. The ROI was placed 2-3 cm below the splenic capsule and 10 measurements were taken from the mid and the lower pole of the spleen. The median splenic shear wave velocity measurement was taken for analysis.

The ratio of splenic stiffness (SS) to liver stiffness (LS) ratio was taken. The best parameter to predict the stage of liver fibrosis, to differentiate NCPF from other causes of cirrhosis and best parameter to predict clinically significant portal hypertension using ARFI elastography was studied.

ARFI ELASTOGRAPHY SHEAR WAVE VELOCITY (SWV) CUT-OFF VALUES:

LIVER FIBROSIS STAGE	**ARFI SWV LIVER STIFFNESS (LS) CUT OFF VALUES :**
Normal (F0)	1. 2 cm / sec
Mild fibrosis (F1)	1.21-1.34 cm / sec
Significant fibrosis (F2- F3)	1.35- 1.86 cm / sec
Cirrhosis	> 1.87 cm / sec

Table1: ARFI elastography liver stiffness (LS) velocity ranges corresponding to grades of liver fibrosis (18).

NCPF	**ARFI SWV CUT-OFF VALUES**
Liver stiffness (LS)	<1.65 cm / sec
Spleen stiffness (SS)	>2.69 cm /sec
SS/ LS	>1.53

Table2: ARFI elastography cut off values of LS, SS and SS/LS to indentify NCPF from other causes of cirrhosis (25).

SPLEEN	**ARFI SWV SPLEEN STIFFNESS (SS) CUT- OFF VALUES :**
Normal	<2.0 cm / sec
Cirrhosis	>2.51 cm /sec
Presence of esophageal varices on endoscopy	>3.16 cm / sec
Significant esophageal varices on endoscopy	>3.3 cm / sec

Table 3: ARFI elastography spleen stiffness (SS) velocity ranges (31).

ARFI ELASTOGRPAHY OF LIVER

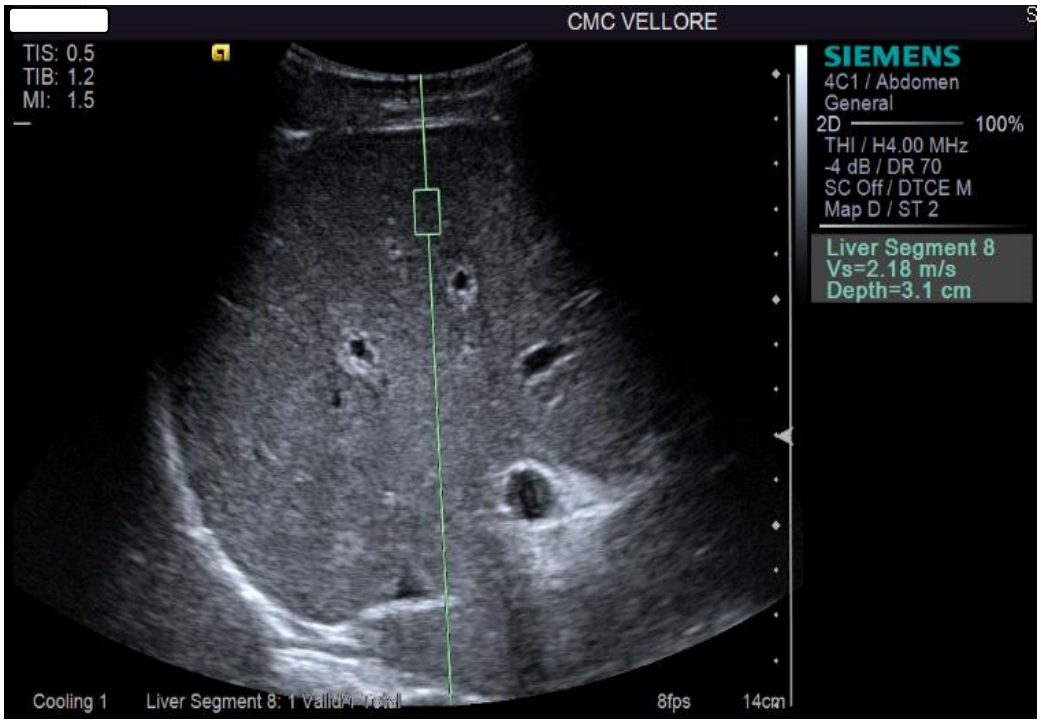

Figure1: ARFI elastography of the liver showing the ROI placement for liver stiffness assessment (LS).

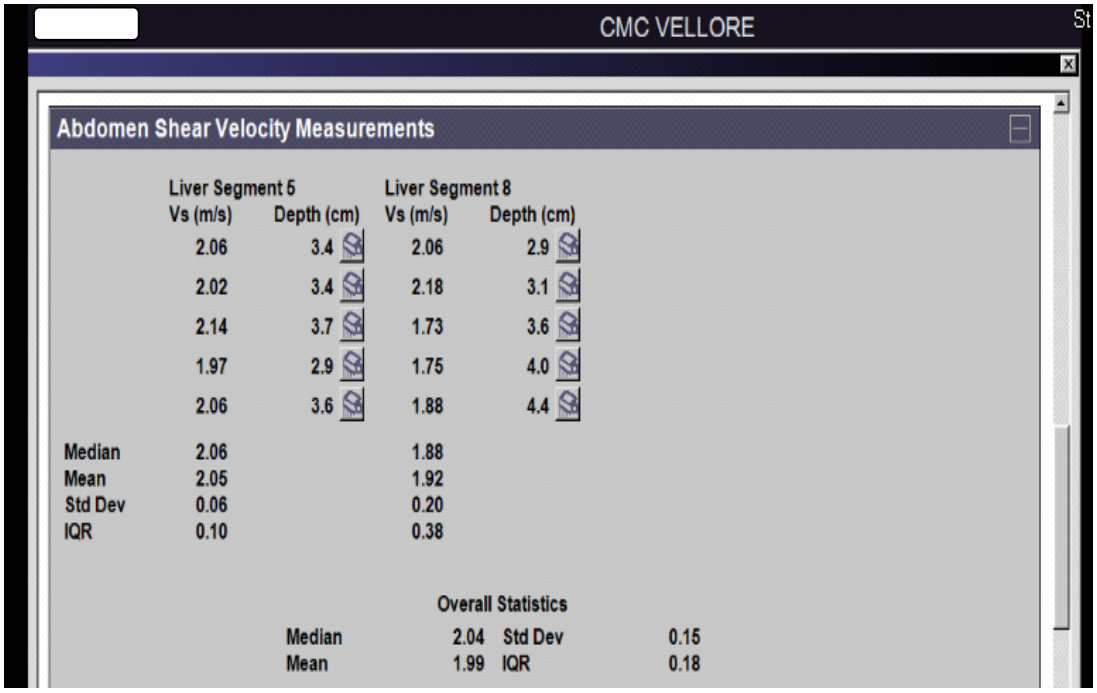

Figure 2: Sample report of the liver stiffness (LS) measurements taken by ARFI elastography.

ARFI ELASTOGRAPHY OF THE SPLEEN

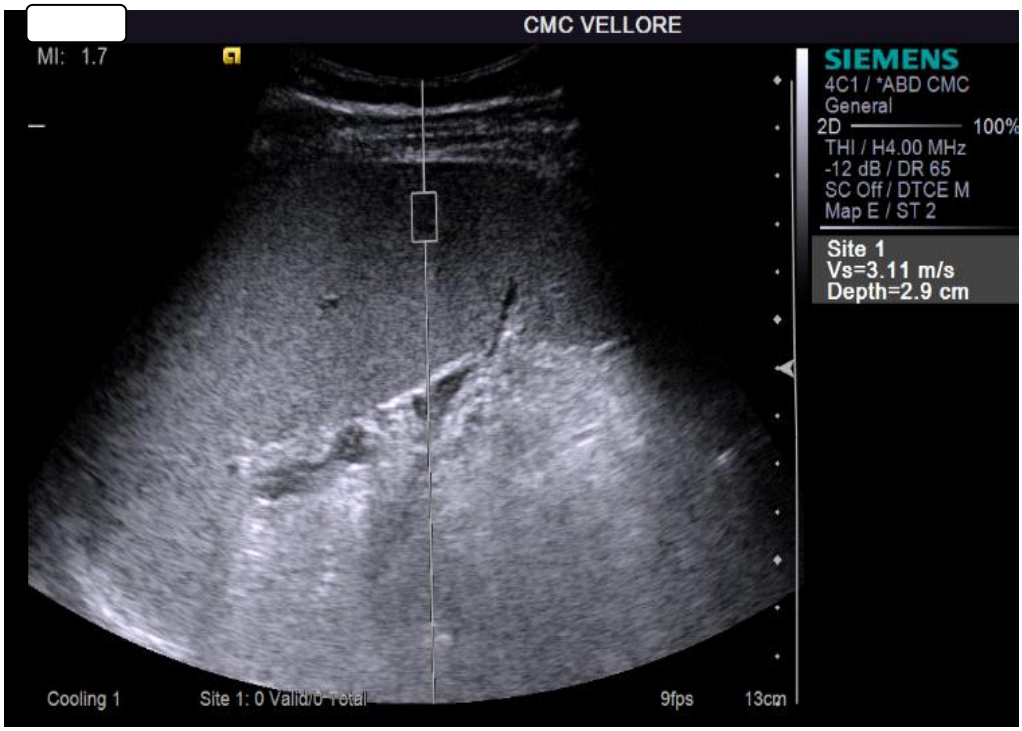

Figure3: ARFI elastography of the spleen showing the ROI placement for spleen stiffness assessment (SS).

REFERENCE STANDARDS:

The reference standard for staging liver fibrosis was the liver biopsy. For assessing clinically significant portal hypertension, the reference standards were HVPG for patients who underwent TJLB and by the presence of high risk esophageal varices on endoscopy.

LIVER HISTOPATHOLOGY:

Liver biopsy was taken as the gold standard to grade the liver fibrosis. The patients underwent liver biopsy either by percutaneous ultrasound guided liver biopsy, trans-jugular liver biopsy or percutaneous blind liver biopsy. The fibrosis on liver biopsy for graded as nil, mild, moderate, severe and cirrhosis corresponding to METAVIR staging of F0-F4. > Significant liver fibrosis was taken as >F2 stage on histopathology (18).

HEPATIC VENOUS PRESSURE GRADIENT (HVPG):

HVPG was taken as the gold standard for measuring portal pressures. Measurements were taken using catheter wedge method for the subgroup of patients who underwent Transjugular liver biopsy (TJLB). Clinically significant portal hypertension (CSPH) was taken as HVPG > 10 mm of Hg (44, 45).

ENDOSCOPY:

Clinically significant portal hypertension (CSPH) on endoscopy was represented by the presence of high risk esophageal varices or severe portal hypertensive gastropathy on endoscopy. Liver stiffness cut off of > 1.67 cm/ sec and spleen stiffness cut-off of > 3.3 cm/ sec was used (31).

STATISICAL ANALYSIS:

Statistical analysis was performed with SPSS version 18.0 software; $\alpha = .05$ was considered the significance level. Quantitative liver stiffness variables were expressed as mean SD. The agreement between the grades of liver fibrosis on biopsy and liver stiffness (LS), spleen stiffness (SS) and ratio of spleen stiffness to liver stiffness (SS/LS) was assessed using kappa statistics. Spearman correlation was done to assess the liver stiffness velocity scores with grades of liver fibrosis on biopsy. Mann-Whitney u test is performed to see whether the stiffness values changes between significant and non-significant liver fibrosis. Chi-square test/ fisher's exact test was used to analyze the association between stiffness grades and ultrasound parameters.

RESULTS:

STUDY POPULATION:

48 cases with preliminary diagnosis of cryptogenic cirrhosis were studied. 39 cases had liver biopsy. 11 cases (28%) were proven to be NCPF on biopsy. The rest of the 28 cases were autoimmune chronic liver disease, cryptogenic cirrhosis, Wilson's disease, drug induced hepatitis, Non alcoholic fatty liver disease, Thalassemia and HBV related chronic liver disease. 9 cases were clinically suspected to have NCPF who did not undergo liver biopsy were also studied.

58 cases of portal hypertension of any etiology were studied to assess the correlation of ARFI elastography of the liver and the spleen with the presence of high risk esophageal varices on endoscopy. This group includes 39 liver biopsy cases, 9 suspected NCPF cases without liver biopsy and 10 cases of portal hypertension due to other known etiologies

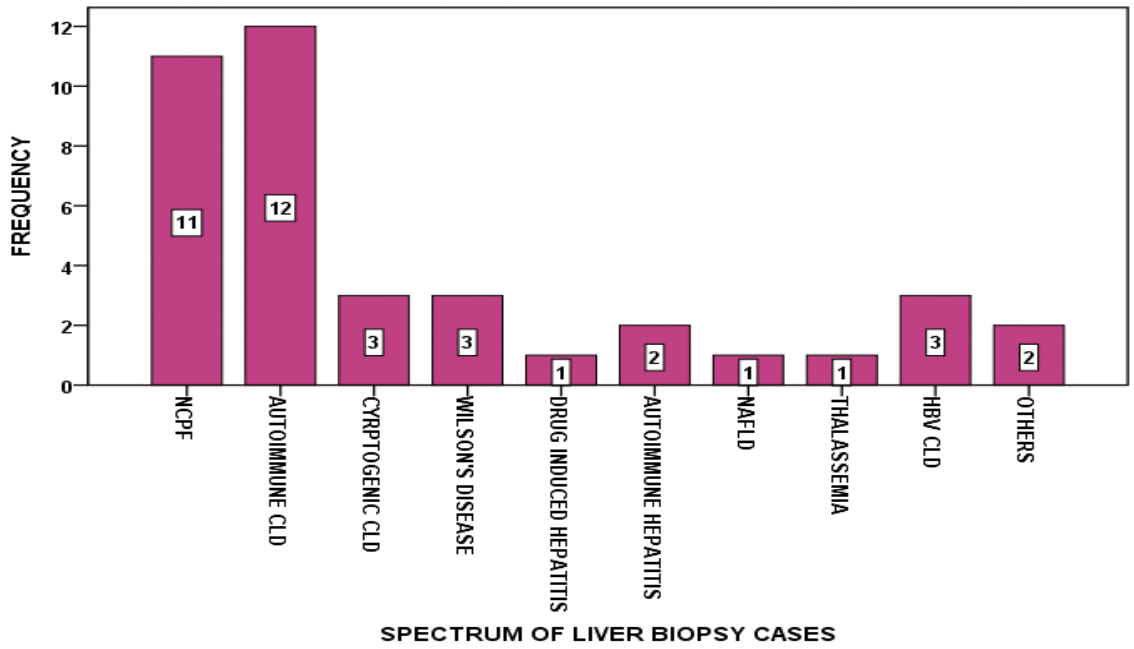

Figure 4: Graph showing the frequency and the spectrum of the final diagnosis on liver biopsy.

AGE DISTRIBUTION: Of the 39 cases of liver biopsy, the median age was 35 years, and minimum age of 6 and maximum age was 65 years.

SEX DISTRIBUTION:

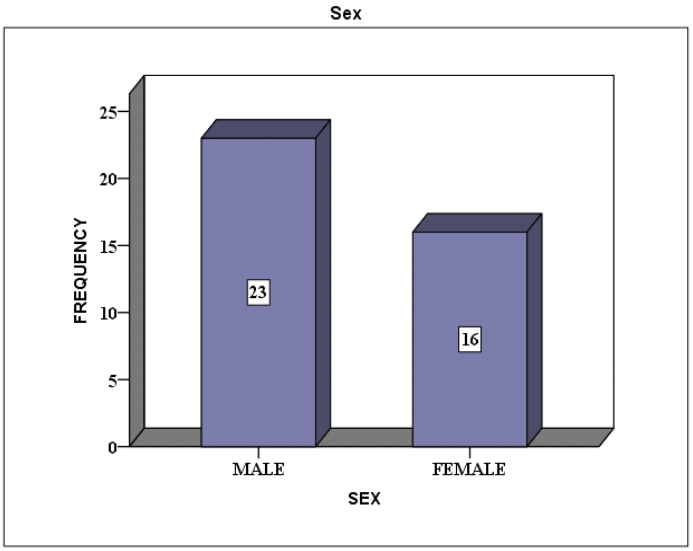

Figure 5: Graph showing the sex distribution between the study populations.

Of the 39 patients 23 were male and 16 were female.

GEOGRAPHIC DISTRIBUTION:

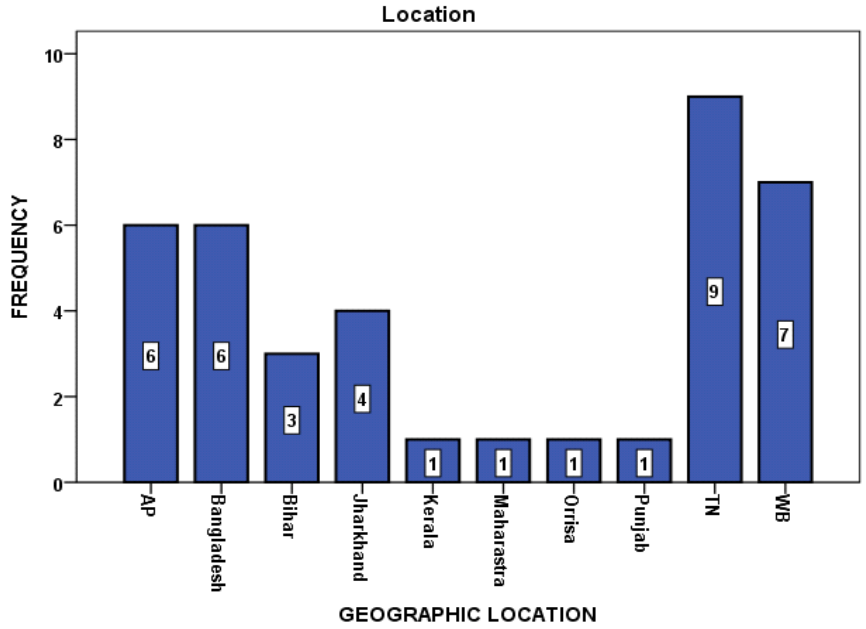

Figure 6: Graph showing the spectrum of geographic distribution of the liver biopsy patients.

Of the 39 patients, 9 were from Tamil Nadu, 7 from West Bengal, 6 from Bangladesh, 6 from AP,4 from Jharkhand, 3 from Bihar, and one each from Kerala, Maharashtra, Orissa and Punjab.

CLINICAL FEATURES:

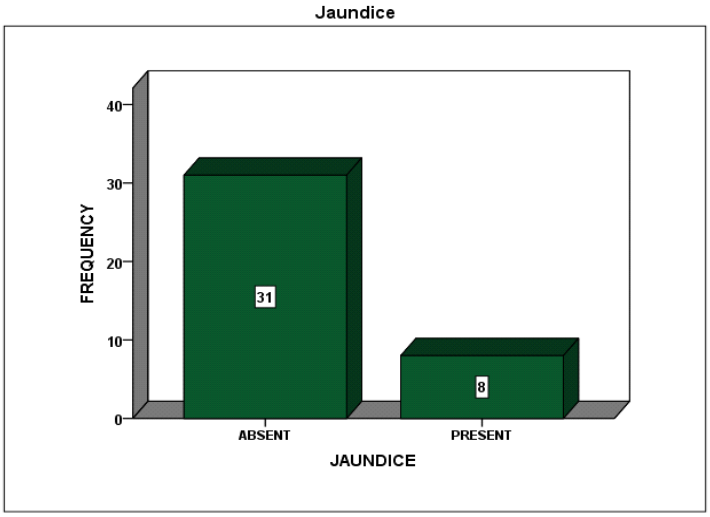

Figure 7: Graph showing the frequency of liver biopsy patients with jaundice at the time of the study.

Only 8 of the 39 patients had jaundice at the time of the study.

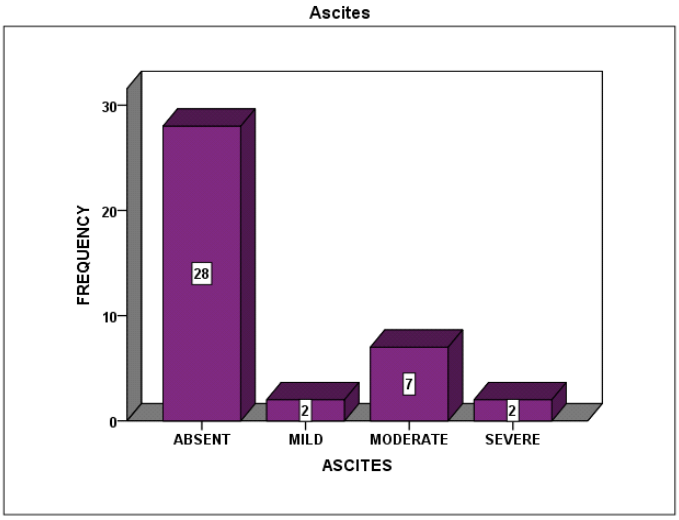

Figure 8: Graph showing the frequency of liver biopsy patients with ascites at the time of the study.

11 patients had ascites at the time of the study.

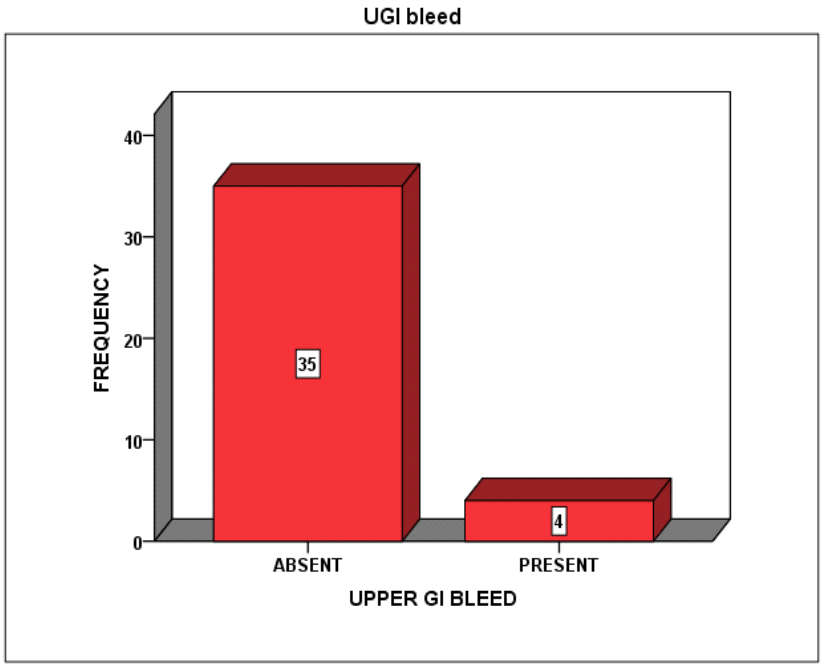

Figure 9: Graph showing the frequency of liver biopsy patients with recent upper gastrointestinal (GI) bleed at the time of the study.

4 patients had recent upper GI bleed before the study.

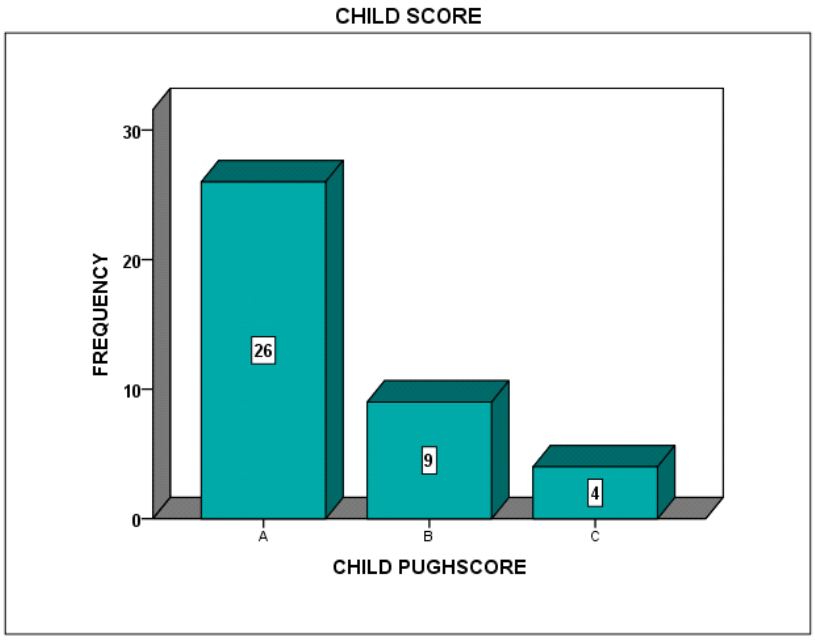

Figure 10: Graph showing the frequency of liver biopsy patients with the grades of CHILD PUGH score at the time of the study.

26 patients (66.7%) were CHILD A, 9 patients were CHILD B and 4 patients (10.3%) were CHILD C.

GRAY SCALE ULTRASOUND EVALUATION OF LIVER AT THE TIME OF ARFI ELASTOGRAPHY:

The gray scale parameters analysed were in the liver were size, echotexture, surface, edge, volume redistribution and the presence of periportal echogenicity.

Of the 39 patients, 24 had shrunken liver, 32 had coarse echotexture, 29 had surface irregularity, 20 had blunted liver edge, 16 had volume redistribution and 4 had periportal echogenicity.

INDICATORS OF PORTAL HYPERTENSION ON GRAY SCALE ULTRASOUND AT THE TIME OF ARFI ELASTOGRAPHY:

The ultrasound parameters evaluated were the presence of portal vein dilatation (>13 mm), splenomegaly (>13 cm) and the presence of porto-systemic collaterals**.** 14 patients had dilated portal vein, 28 had splenomegaly and 7 had porto-systemic collaterals.

LIVER STIFFNESS (LS) SHEAR WAVE VELOCITY MEASUREMENTS BY ARFI ELASTOGRAPHY:

Grades of liver fibrosis	**NIL (F0)**	**MILD (F1)**	**MODERATE (F2-3)**	**CIRRHOSIS (F4)**
Number of cases	3	19	3	14
Median LS velocity	1.18	1.87	2.09	2.61
Mean LS velocity	1.23	1.96	2.4	2.66
Standard deviation	0.10	0.75	1.00	0.59
Minimum velocity	1.17	1.0	1.59	1.54
Maximum velocity	1.36	3.5	3.52	3.73

Table 4: The liver stiffness (LS) median, mean velocities, standard deviation, and the velocity ranges as compared to the grades of fibrosis on biopsy. Shear wave velocities in cm/ sec.

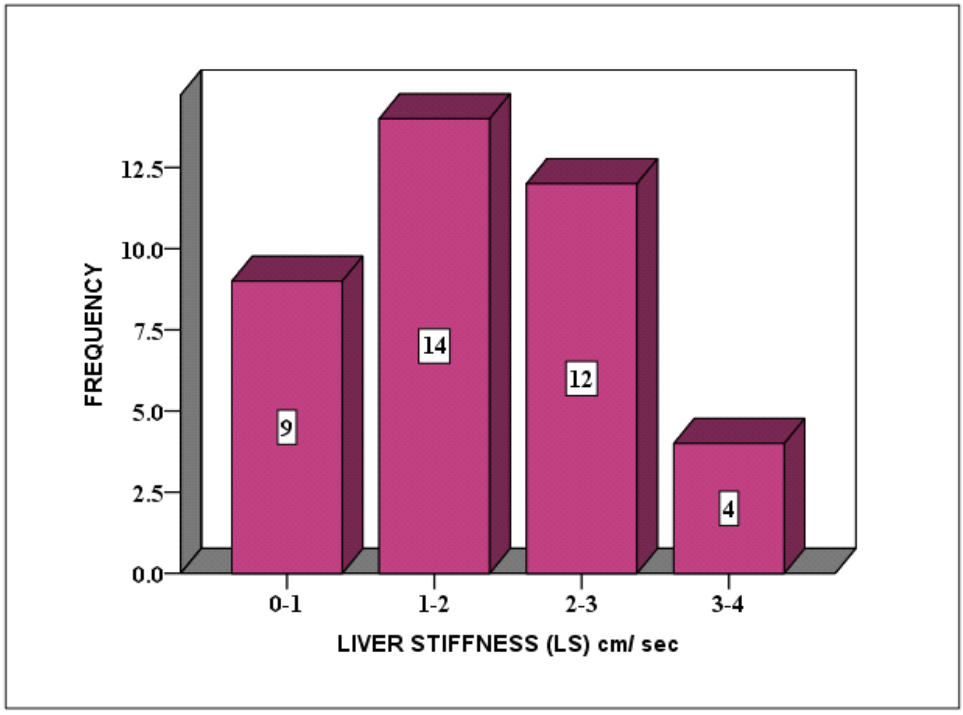

Figure 11: Graph showing the frequency of liver stiffness (LS) velocity ranges among the liver biopsy patients.

Of the 39 patients, the liver stiffness (LS) velocity ranges were 3 normal velocities, 19 had mild fibrosis range, 3 had moderate fibrosis range and 14 had cirrhotic range.

SPLEEN STIFFNESS (SS) SHEAR WAVE VELOCITY MEASUREMENTS BY ARFI ELASTOGRAPHY:

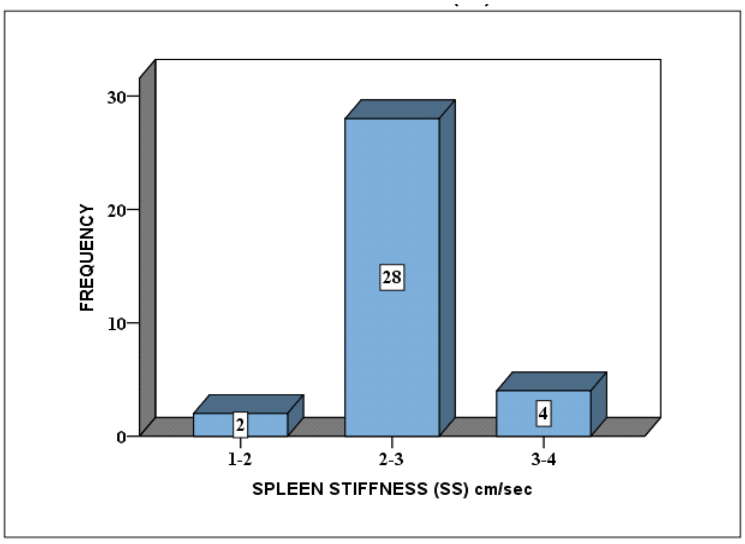

Figure 12: Graph showing the frequency of spleen stiffness (SS) velocity ranges among liver biopsy patients.

Grades of liver fibrosis	NIL (F0)	MILD (F1)	MODERATE (F2-3)	CIRRHOSIS (F4)
Number of cases	3	17	3	14
Median LS velocity	NA	2.92	3.01	3.05
Standard deviation	NA	0.41	0.57	0.31
Minimum velocity	NA	2.47	2.35	2.59
Maximum velocity	NA	3.82	3.43	3.72

NA- Spleen stiffness was not assessed.

Table 5: Median velocities, standard deviation, and the velocity ranges of spleen stiffness (SS) as compared to the grades of liver fibrosis on biopsy.

Of the 39 patients, the spleen stiffness (SS) velocity ranges were not assessed out of which 1 had splenectomy and 2 patients could not co-operate for the study, 17 had mild fibrosis range, 3 had moderate fibrosis range and 14 had cirrhotic range.

ROUTES OF LIVER BIOPSY:

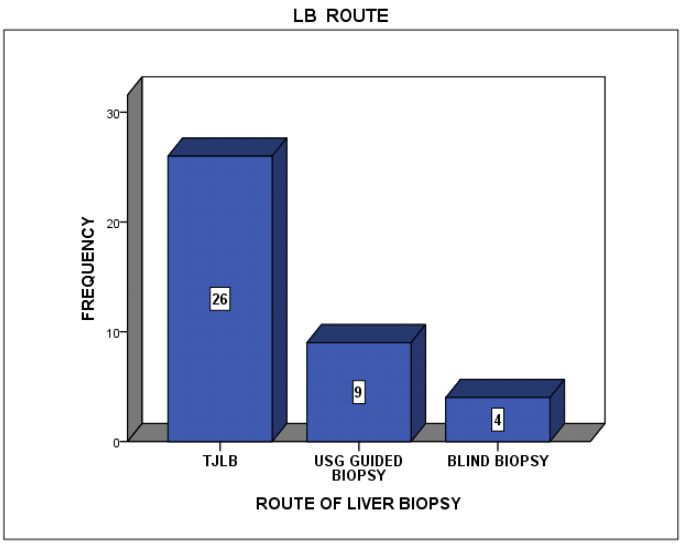

Figure 13: Graph showing the frequency of the various routes of liver biopsy.

Majority of the patient's i.e 26 had Transjugular liver biopsy, 9 had ultrasound guided and 4 had percutaneous blind liver biopsy.

TIME INTERVAL BETWEEEN ARFI AND LIVER BIOPSY:

Of the 39 patients, 32 (82%) had recent biopsy (less than 1 week from the ARFI elastography study) and 7 patients (18%) had liver biopsy in the past ranging from 1 to13 years. Among the NCPF group, 5 patients had recent biopsy and 6 patients had biopsy in the past, between 4 to 13 years.

GRADES OF LIVER FIBROSIS ON HISTOPATHOLOGY:

Of the 39 patients who underwent liver biopsy, 3 patients (7.7%) had no evidence of fibrosis (F0), 19 patients (48.7%) had mild fibrosis (F1), 3 patients (7.7%) had moderate fibrosis (F2-3) and 14 patients (35.9%) had cirrhosis (F4) on histopathology. In this study, 17 patients (43.6%) had significant fibrosis i.e greater than or equal to F2.

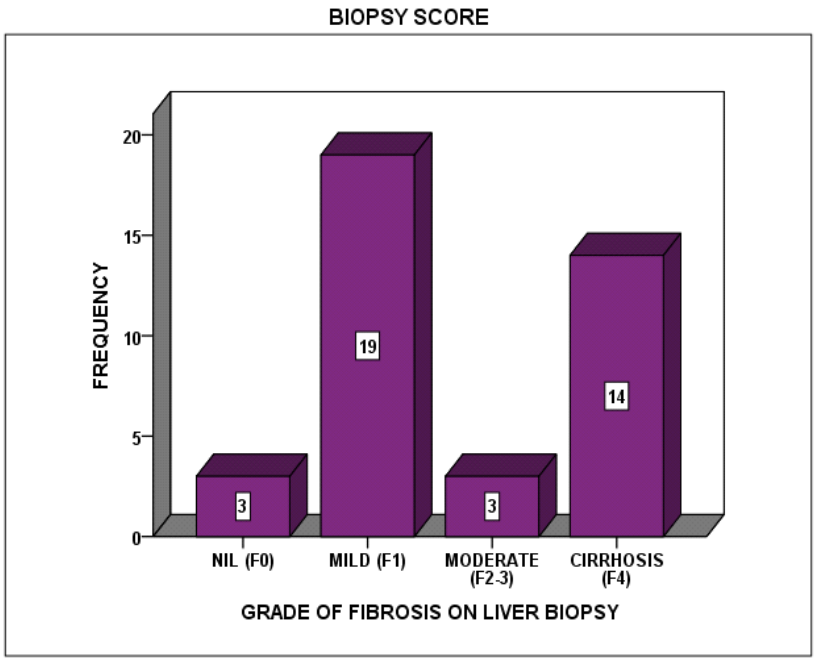

Figure 14: Graph showing the frequency various grade of liver fibrosis on histopathology.

HEPATIC VENOUS PRESSURE GRADIENT (HVPG) MEASUREMENTS:

18 patients had HVPG measurements taken during the liver biopsy. Out of the 18 patients, 11 patients had clinically significant portal hypertension (HVPG greater than or equal to 10 mm of Hg). The mean HVPG value was 10.33 mm of Hg with a range from 1-23 mm of Hg. Majority of the patients had catheter wedge method of HVPG measurement.

GRADING OF ESOPHAGEAL VARICES ON ENDOSCOPY:

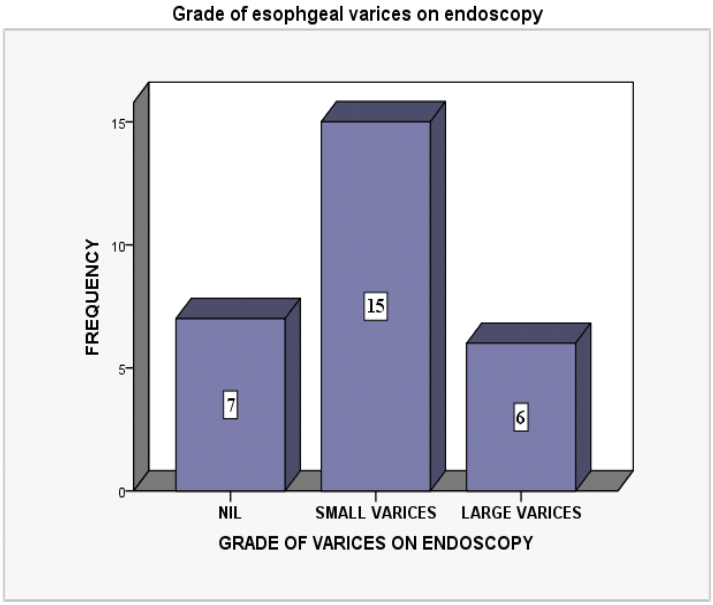

Figure 16: Graph showing the frequency grades of esophageal varices on endoscopy among liver biopsy patients.

Of the 39 patients, 28 patients underwent endoscopy for evaluation of esophageal varices. 6 (15.4%) patients had large esophageal varices representing clinically significant portal hypertension. Of the 39 patients, 12 (30.8%) had prior endoscopic variceal ligation (EVL). Among the 6 patients with large esophageal varices, 2 had prior EVL.

1. DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO IDENTIFY NONCIRRHOTIC PORTAL FIBROSIS (NCPF) FROM CIRRHOSIS WITH PORTAL HYPERTENSION:

Among the 39 cases with a preliminary diagnosis of cryptogenic cirrhosis that underwent liver biopsy, 11 were biopsy proven cases of NCPF and 28 causes of liver cirrhosis due to other causes with portal hypertension.

The mean age of the NCPF group was 34 years with male: female sex ratio of 7:4. The average platelet count was 69,000. Most NCPF patients had normal liver functions.

	NCPF GROUP	**LIVER CIRRHOSIS GROUP**
Number of cases	11	28
Gender (M:F)	7:4	15:13
Age * yrs	34	36
Platelet count *	69,000	90,000
AST*	38	51
ALT	23.5	36
Total bilirubin*	1.4	2.1
Albumin*	4.1	3.6
PT-INR*	1.1	1.18
Esophageal varices	8	13
EVL	3	7
Ascites Absence	10	17

Table 6: Comparison of the demographic, biochemical and clinical parameters of NCPF and liver cirrhosis group.*Median value, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, PT- INR- Prothrombin time with international normalized ratio, EVL- endoscopic variceal ligation in the past.

There is only one published study in literature recently from Japan which has studied the use of ARFI elastography of the liver and spleen to identify NCPF (25). No Indian studies have been published. The cut-off values suggested by the Japanese study were liver stiffness (LS) < 1.65 cm/sec, spleen stiffness (SS) > 2.69 cm / sec and ratio of SS and LS (SS/LS) > 1.53 to identify NCPF from other causes of liver cirrhosis (25).

In the present study, NCPF group 45.5% (n=5) had LS < 1.65 cm/ sec and 54.5 %(n=6) had LS > 1.65 cm/ sec, 70% (n=7) had SS > 2.72 cm / sec and 63% (n=7) had SS/LS > 1.53 cm/sec.

In the liver cirrhosis group, 21.4% (n=6) had LS< 1.65 cm/ sec and 71.4% (n=20) had LS > 1.87 cm / sec i.e in the cirrhotic velocity range. 79 % (n=19) had SS >2.72 cm/sec and 25% (n=5) had SS/LS > 1.53 cm/ sec.

ARFI ELASTOGRAPHY CUT-OFF	**NCPF GROUP (n=11)**	**LIVER CIRRHOSIS GROUP (n=28)**
LS < 1.65 cm/ sec	45.5% (n=5)	21.4% (n=6)
SS > 2.69 cm / sec	70% (n=7)	79 % (n=19)
SS/LS > 1.53 cm/ sec	63% (n=7)	25% (n=5)
EITHER LS or SS or SS/LS	90% (n=10)	78% (n=22)

Table 7: Table showing the percentages and the frequency of cases among NCPF and liver cirrhosis group according to the cut-off described in the Japanese study.

In the present study, the median LS, SS and SS/ LS velocities in NCPF group were 1.75 cm/ sec, 3.11 cm/sec and 1.81 cm/ sec respectively. The median LS, SS, SS/ LS in the cirrhosis group were 2.5 cm/ sec, 2.53 cm/ sec and 1.22 cm/ sec respectively.

	NCPF	LIVER CIRRHOSIS
Median LS	1.75	2.5
Median SS	3.11	2.95
Median SS/ LS	1.81	1.23

Table 8: Median ARFI elastography velocities of liver stiffness (LS), spleen stiffness (SS) and the ratio of spleen stiffness to liver stiffness (SS/LS) in NCPF and liver cirrhosis group. Median velocities in cm/sec.

Each liver biopsy patient was coded as test positive by ARFI elastography for LS, SS, and SS/LS according to the cut off values which are mentioned in the literature. The sensitivity, specificity, PPV, NPV and likelihood ratios were calculated.

COMBINATION OF EITHER LS*, SS* AND SS/LS*	NCPF	LIVER CIRRHOSIS
TEST POSITIVE BY ARFI	10	22
TEST NEGATIVE BY ARFI	1	6
TOTAL CASES	11	28

Table 9: Two by two table showing the number of biopsy proven NCPF and liver cirrhosis that were positive for LS, SS, and SS/LS cut off values by ARFI elastography. * Liver stiffness (LS), spleen stiffness (SS) and the ratio of spleen stiffness to liver stiffness (SS/LS).

ARFI elastography of the liver (LS) had 36 % sensitivity, 93% specificity, PPV of 90%, NPV of 78.8 % and kappa of 0.28 and p value of 0.04 to identify NCPF from other liver cirrhosis which was statistically significant.

Spleen stiffness (SS) had 70% sensitivity, 20% specificity, PPV of 26.9% and NPV of 62.5%, kappa of -0.63 and p value of 0.5 which was not statistically significant. The SS/ LS ratio had 70% sensitivity, 79.2% specificity, PPV of 58.3%, NPV of 86.4% with kappa of 0.46 and p value of 0.06 which was statistically not significant. The kappa agreement with the liver biopsy for LS, SS, and SS/LS was low.

The combination of either LS, SS or SS/ LS had sensitivity of 82%, specificity of 21.4%, PPV of 29%, NPV of 75% and kappa of 0.02 (p=0.8).

ARFI VARIABLES	**SENSITIVITY**	**SPECIFICITY**	**PPV**	**NPV**
LS*	36%	93%	90%	44.8 %
SS*	70%	20.8%	26.9%	62.5%
SS/LS*	70%	79.2%	58.3%	86.4%
COMBINATION OF LS, SS AND SS/LS	82 %	21.4%	29%	75%

Table 10: Comparison of LS, SS and SS/LS and the combination of either of these parameters to identify NCPF from other causes of cirrhosis with portal hypertension.*LS- Liver stiffness, SS- spleen stiffness, SS/LS ratio- Spleen stiffness / Liver stiffness.

Among the NCPF group, Spearman correlation (rho) for LS with liver biopsy was 0.3 (p=0.31) and for SS with liver biopsy was -0.28 with p=0.5 which were statistically not significant.

Among the liver cirrhosis group, Spearman correlation (rho) for LS with liver biopsy among was 0.57 (p=0.01) which was statistically significant. Spearman correlation (rho) of SS with liver biopsy was -0.15 (p=0.4) which was statistically not significant.

Among the 9 patients who were clinically suspected to have NCPF but did not undergo liver biopsy, 6 (66.67%) had LS < 1.65 cm/ sec, 6 (66.67%) had SS > 2.69 cm/ sec and 6 (66.67%) had SS/LS >1.53 cm/ sec which are suggestive of NCPF on ARFI elastography.

The best individual ARFI elastography parameter to identify NCPF from other causes of cirrhosis was found to be the ratio of spleen stiffness to liver stiffness (SS/LS) with good sensitivity, specificity, negative predictive value and good kappa correlation. The combination of LS, SS or SS/LS had the maximum sensitivity but had low specificity.

2. DIAGNOSTIC ACCURACY ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO DIFFRENTIATE SIGNIFICANT LIVER FIBROSIS FROM NON-SIGNIFICANT LIVER FIBROSIS:

LIVER BIOPSY		LS	SS	LS x SS	SS/ LS
NOT SIGNIFICANT	N	22	17	17	17
	Mean	1.8614	3.0206	6.1359	1.70276
	Median	1.7250	2.9200	5.2900	1.52000
	Std. Deviation	.74484	.41814	2.57121	.689253
	Minimum	1.00	2.47	3.08	.860
	Maximum	3.50	3.82	11.41	3.080
SIGNIFICANT	N	17	17	17	17
	Mean	2.6212	3.0482	7.9841	1.23571
	Median	2.5900	3.1200	7.9800	1.21000
	Std. Deviation	.65018	.34877	2.28189	.370352
	Minimum	1.54	2.35	4.31	.660
	Maximum	3.73	3.72	13.35	2.040

Table 11: Mean and Median velocities of liver stiffness (LS), spleen stiffness (SS), LS x SS and SS/LS measurements with standard deviation and shear wave velocity ranges to differentiate significant liver fibrosis from non-significant liver fibrosis.

Based on the prior published literature the liver stiffness (LS) velocity cut-off value of 1.35 cm/ sec, was taken to differentiate significant fibrosis from non-significant fibrosis (18).

LS was found to have 100 % sensitive to identify significant liver fibrosis (>F2), but has a low specificity of 30.4%. The positive predictive value (PPV) was 45.2 % and negative predictive value was 100% with kappa of 0.33, (p=0.05) and Likelihood ratio of 1.57.

The spleen stiffness cut-off value of 2.51 cm/ sec was taken to differentiate cirrhosis from lesser grades of liver fibrosis (30). ARFI elastography of spleen was found to have 100% sensitivity, 10% specificity, PPV of 43.7%, NPV of 100% and kappa of 0.08 to identify liver cirrhosis.

The ratio of spleen stiffness (SS) to liver stiffness (LS) cut off value of 1.53 cm/ sec was taken to differentiate significant fibrosis from non significant fibrosis. ARFI elastography measurements of SS/ LS were found to have 76.5% sensitivity, 47% specificity, PPV of 59%, NPV of 66.7% and kappa of 0.23 (P= 0.1) The best parameter to identify significant fibrosis was the combination of both liver and spleen elastography i.e SS/ LS ratio.

Both the liver stiffness and spleen stiffness measured by ARFI elastography have 100 % sensitivity to identify significant fibrosis but have low specificity. They have 100 % NPV and low PPV. The combination of SS/ LS was found to have higher specificity of 47% and 76.5% specificity. The p value of LS was 0.002, SS was 0.68, LS x SS was 0.034 and SS/ LS was 0.049. The liver stiffness (LS), product of LS and SS and ratio of SS/ LS were found to statistically significant differentiate between significant from non-significant liver fibrosis. The spleen stiffness was not found to be useful to identify significant liver fibrosis.

ARFI	ARFI	SENSITIVITY	SPECIFICITY	PPV	NPV	Kappa	P value
LS*	<1.35	100 %	30.4%	45.2%	100%	0.3	0.002
SS*	>2.51	100%	10%	43.7%	100%	0.08	0.68
SS/LS*	>1.53	76.5%	47%	59%	66.7%	0.23	0.049

Table 12: Comparison of LS, SS and SS/LS to differentiate significant liver fibrosis from non-significant liver fibrosis.*LS- Liver stiffness, SS- spleen stiffness, SS/LS ratio- Spleen stiffness / Liver stiffness. Column 2 indicates the ARFI cut off values in cm/ sec.

	LS	SS	LS x SS	SS/ LS
Mann-Whitney U	82.500	132.000	83.000	87.500
Wilcoxon W	335.500	285.000	236.000	240.500
Z	-2.960	-.431	-2.118	-1.964
Asymp. Sig. (2-tailed)	.003	.667	.034	.050
P value	.002	.683	.034	.049

Table 13: Mann- Whitney U test for liver stiffness (LS), spleen stiffness (SS), product of LS and SS and SS/ LS to differentiate between significant liver fibrosis from non-significant liver fibrosis.

DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF THE LIVER TO IDENTIFY GRADES OF LIVER FIBROSIS ON BIOPSY:

The liver stiffness (LS) velocity measurements were correlated with the grades of liver fibrosis on biopsy. The ARFI liver stiffness velocity cut-off for mild fibrosis (F0-F1) was taken as < 1.34 cm/ sec, moderate fibrosis (F2-3) as 1.35-1.86 cm/ sec and greater than 1.87 cm/ sec for cirrhosis (F4) (18).

There were 19 patients who had mild fibrosis (48.7%), 3 with moderate fibrosis (7.7%) and 14 with cirrhosis (35.9%). Of the 19 patients with mild fibrosis, only 6 patients (31.6%) were accurately identified as mid fibrosis by ARFI elastography and 13 patients (69.3%) had velocities greater than 1.35 cm/ sec. Of the 3 patients with moderate fibrosis on biopsy, 1 (33.3%) patient had LS velocity in the moderate fibrosis range and 2 (66.7%) had velocities in cirrhotic range. Of the 39 patients with cirrhosis on biopsy, 25 (64.1%) had LS velocities in the cirrhotic range, 6 (15.4%) in the moderate fibrosis range and 8 (20.5%) had velocities in the mild fibrosis range.

The sensitivity of ARFI elastography to identify no fibrosis was 66.7%, 31.6% for mild fibrosis, 33.3% for moderate fibrosis and 64.1% for cirrhosis.

Spearman correlation (rho) of the liver stiffness (LS) with the fibrosis grades on liver biopsy was 0.54 with p value of < 0.001 which was statistically significant. This states that as the liver fibrosis increases the liver stiffness velocity also increases.

The liver stiffness measurement by ARFI elastography has higher sensitivity to identify moderate fibrosis and cirrhosis and low sensitivity to identify mild fibrosis.

ARFI LIVER STIFFNESS (LS) VELOCITY (cm/ sec)	LIVER BIOPSY GRADE			
	NIL (F0)	MILD (F1)	MODERATE (F2-3)	CIRRHOSIS (F4)
<1.35	2	6	0	8
	66.7%	31.6%	.0%	20.5%
1.35-1.86	1	3	1	6
	33.3%	15.8%	33.3%	15.4%
>1.87	0	10	2	25
	.0%	52.6%	66.7%	64.1%
TOTAL	3	19	3	39
	100.0%	100.0%	100.0%	100%

Table 14: Comparison of liver stiffness (LS) velocities by ARFI elastography with grades of fibrosis on liver biopsy.

3A. DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO IDENTIFY CLINICALLY SIGNIFICANT PORTAL HYPERTENSION AS COMPARED TO HEPATIC VENOUS PRESSURE GRADIENT (HVPG):

Clinically significant portal hypertension is defined as HVPG > 10 mm of Hg. ARFI elastography liver stiffness cut off of > 1.67 cm / sec and spleen stiffness cut off value of >3.3 cm / sec were taken to identify clinically significant portal hypertension (31).

Total 18 patients who underwent liver biopsy had HVPG measurements. Among the NCPF group, 8 patients had HVPG measurements, 37.5% (n=3) had HVPG > 10 mm Hg. With LS, one case (33%) among the 3 was identified to have HVPG > 10 mm of Hg and with SS 2 (66.7%) out of 3 cases had CSPH. In the liver cirrhosis group, 10 patients had HVPG measurements, 40% (n=4) had HVPG > 10 mm of Hg. Using LS, 2 out of 4 cases were identified and using SS none were identified.

VARIABLE	**ARFI cm/ sec**	**SENSITIVITY**	**SPECIFICITY**	**PPV**	**NPV**	**KAPPA**
LS*	>1.67	57%	27%	33%	50%	-0.01
SS*	>3.3	14.3%	90%	50%	60%	0.04

Table 15: Comparison of LS and SS velocities to identify clinically significant portal hypertension as compared to HVPG. *LS- Liver stiffness; SS- Spleen stiffness. Column 2 indicates the ARFI cut off values in cm/sec.

Liver stiffness (LS) was found to have 57% sensitivity, 27% specificity, PPV of 33%, NPV of 50% and kappa of -0.13 (p=0.49) and Spleen stiffness(SS) was found to have 14.3% sensitivity, 90% specificity, PPV of 50%, NPV of 60% and kappa of 0.04, (p-0.78). Both LS

and SS were found to have low sensitivity with low kappa agreement and were statistically not significant to identify clinically significant portal hypertension as compared to HVPG.

3B. DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO IDENTIFY CLINICALLY SIGNIFICANT PORTAL HYPERTENSION AS COMPARED TO HIGH RISK ESOPHAGEAL VARICES ON ENDOSCOPY:

58 cases of portal hypertension of any etiology were studied to assess the correlation of ARFI elastography of the liver and the spleen with the presence of high risk esophageal varices on endoscopy. 51 patients had recent endoscopy out of which 13 (25.4%) had high risk esophageal varices. This group includes 39 liver biopsy cases, 19 causes of portal hypertension without liver biopsy, among which 9 were suspected NCPF cases and 10 cases had known etiologies for portal hypertension.

ARFI elastography liver stiffness cut off of > 1.67 cm / sec and spleen stiffness cut off value of > 3.3 cm / sec was taken which was found to represents high risk esophageal varices on endoscopy to identify clinically significant portal hypertension (31).

In the NCPF group, 8 of the 11 patients had esophageal varices on endoscopy, 2 (25%) had high risk esophageal varices. Both the patients (100 %) with high risk esophageal varices were identified with LS and with SS one (50%) among the 2 cases was identified. 3 patients were treated with prior endoscopic variceal ligation.

In the liver cirrhosis group with liver biopsy, 4 (14%) out of the 28 patients had high risk esophageal varices. 2 (50%) were identified using LS and with SS none were identified.

In the group of third group of patients with portal hypertension, 7 (36%) out of 19 had high risk esophageal varices. Only 2(10%) were identified using LS and 3 (42.8%) were identified with SS.

Overall, Liver stiffness (LS) was found to have 46.5% sensitivity, 26.4% specificity, PPV of 10%, NPV of 56% and (p= 0.07) which was statistically not significant. Spleen stiffness was found to have 31% sensitivity, 81.25% specificity, PPV of 40%, NPV of 74.3% and (p=0.38) which was statistically not significant. Both LS and SS were found to have low sensitivity with low kappa agreement and statistically not significant to identify large esophageal varices and clinically significant portal hypertension.

VARIABLE	**ARFI**	**SENSITIVITY**	**SPECIFICITY**	**PPV**	**NPV**	**P value**
LS*	>1.67	46.5%	26.4%	10 %	56%	0.07
SS*	>3.3	31%	81.25%	40%	74.3%	0.38

Table 16: Comparison of LS and SS velocities to identify clinically significant portal hypertension as compared to endoscopy. *LS- Liver stiffness; SS- spleen stiffness. Column 2 indicates the ARFI cut off values in cm/sec.

DISCUSSION:

Non cirrhotic portal fibrosis (NCPF)/ Non cirrhotic intrahepatic portal hypertension (NCIPH) or idiopathic portal hypertension (IPH) is a rare disease of unknown etiology characterized by portal hypertension and splenomegaly. They can present with esophageal varices, portal hypertensive gastropathy, liver function disorders, anaemia, ascites and encephalopathy but does not lead to cirrhosis or hepatocellular carcinoma. On routine imaging, NCPF can mimic cryptogenic cirrhosis and hence can be misdiagnosed. Liver biopsy is essential for the diagnosis. The importance of making accurate diagnosis of NCPF is that these patients have excellent prognosis when treated adequately for portal hypertension.

ARFI elastography is a novel ultrasound based technology which is combined with B-mode ultrasound. It quantifies the stiffness of the tissues by using acoustic push pulses and measures the shear wave velocities in the tissues which are proportional to the degree of stiffness of the target organ. ARFI is superior to transient elastography which is based on A-mode imaging and requires higher skill for the examiners.

NCPF is termed as “obliterative portal sclerosis” as there is portal sclerosis in and thin fibrous septa in the absence of cirrhosis on histopathology. The occlusion of peripheral portal veins in the liver causes increase in blood flow to the spleen, causing pulp hyperplasia, congestion from increased blood flow and fibrosis within the spleen resulting in splenomegaly and portal hypertension.

There is only one study published in literature from Japan by Furuichi et al. (25) who studied the use of ARFI elastography of liver and spleen in NCPF and as compared to liver biopsy. The study included 17 NCPF patients, 25 liver cirrhosis, 20 chronic hepatitis and 20 normal controls. In the present study, 39 cases underwent liver biopsy with a preliminary diagnosis

of cryptogenic cirrhosis, out of which 11 had NCPF and 28 had cirrhosis of other causes after liver biopsy that were the controls.

	NCPF/ IPH **JAPANESE STUDY**	**NCPF** **PRESENT STUDY**	**LIVER CIRRHOSIS** **JAPANESE STUDY**	**LIVER CIRRHOSIS** **PRESENT STUDY**
Number of cases	17	11	25	28
Gender (M:F)	11:6	7:4	18:7	15:13
Age * yrs	40	34	71	36
Platelet count *	86,000	69,000	98,000	90,000
AST*	27.5	38	42	51
ALT	17.5	23.5	29	36
Total bilirubin*	1.15	1.4	1.02	2.1
Albumin*	4.1	4.1	3.6	3.6
PT-INR*	1.06	1.1	1.1	1.18
Esophageal varices	12	8	18	13
EVL	6	3	7	7
Ascites Absence	17	10	25	17

Table 17: Summary of the demographic, clinical and biochemical parameters of the present study as compared to the Japanese study. *Median value.

The mean age in the present study among NCPF group was 34 years as compared to the 40 years in the Japanese study. The liver function tests were almost similar, except the AST levels in the present study were slightly higher, median value being 38 IU/L. 8 of the 11 patients had esophageal varices on endoscopy and 3 had been treated with endoscopic variceal ligation. The patients demographic, clinical and biochemical parameters have been compared with the Japanese study summarized in table 16.

In NCPF, the fibrogenesis enlargement of the spleen causes passive congestion which is probably the cause of increase in spleen stiffness. The increase in liver stiffness in NCPF is due to the small amount of peripheral periportal fibrosis. Seijo et al. studied the use of transient elastography in IPH and compared to hepatic venous pressure gradient (HVPG) (27). They reported that the liver stiffness in IPH is lower than the liver cirrhosis. Thus, NCPF/ IPH is a disease with soft liver and hard spleen and hence the spleen/ liver stiffness ratio is increased (25).

In the present study, the liver stiffness (LS), spleen stiffness (SS) and SS/ LS ratio cut-offs were taken from the Japanese study (28). The median liver stiffness (LS) was 1.75 cm/ sec which was higher than the Japanese study. The increase in the liver stiffness is probably due to the fact that some NCPF cases were diagnosed few years earlier and had increase in peripheral periportal liver fibrosis as compared to the newly diagnosed cases. Among the liver cirrhosis group, the median liver stiffness was comparable with the Japanese study.

	NCPF/ IPH **JAPANESE STUDY**	**NCPF** **PRESENT STUDY**	**LIVER CIRRHOSIS** **JAPANESE STUDY**	**LIVER CIRRHOSIS** **PRESENT STUDY**
Median LS	1.56	1.75	2.44	2.5
Median SS	3.88	3.11	3.18	2.95
Median SS/ LS	2.47	1.81	1.3	1.23

Table 18: Comparison between the liver stiffness (LS), spleen stiffness (SS) and SS/LS ratio among NCPF and cirrhosis group in the present study and the Japanese study. Median velocities in cm/sec.

The sensitivity of liver stiffness in the present study to identify NCPF was 36% as compared to 65% in the Japanese study. However the specificity of 93%and the positive predictive value (PPV) of 90 % are comparable with the prior study. This indicates that assessment of liver by itself in NCPF is not adequate to identify NCPF from cirrhosis. However as the specificity is 93%, if the liver stiffness velocity is elevated i.e greater than 1.65 cm/ sec, the probability of the patient having NCPF is very low.

LIVER STIFFNESS (SS)	**NCPF/ IPH** **JAPANESE STUDY**	**NCPF** **PRESENT STUDY**
Sensitivity	65 %	36 %
Specificity	92%	93%
PPV	85%	90%
NPV	79 %	48%

Table 19: Comparison of the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of liver stiffness (LS) between the Japanese study and the present study.

The spleen area (product of the maximum length into shortest diameter) of the present study and the Japanese study are similar in the NPCF group. The median spleen stiffness (SS) velocity in the present study was 3.11 cm/ sec which was lower than the Japanese study of 3.88 cm/ sec. The correlation of the spleen area with the spleen stiffness in this study was not found to be statically significant which was also reported by the Japanese study. This suggests that the spleen size may not reflect the extent of spleen fibrosis and degree of portal hypertension. The sensitivity and specificity of spleen stiffness to identify NCPF in this study were found to 70 % sensitivity and 21 % specificity as compared with 100 % and 95% in the Japanese study. This may due to the lower sample size in this study and also due to the lack

of any Indian studies for comparison. More studies are required to further study the cause for lower splenic stiffness in Indian population.

	NCPF/ IPH **JAPANESE STUDY**	**NCPF** **PRESENT STUDY**	**LIVER CIRRHOSIS JAPANESE STUDY**	**LIVER CIRRHOSIS PRESENT STUDY**
Spleen area cm 2	102.5	101.6	44	102.5
Spleen stiffness cm/ sec	3.88	3.11	3.18	2.95

Table 20: Comparison between the spleen area and splenic stiffness in the present study and the Japanese study.

SPLEEN STIFFNESS (SS)	**NCPF/ IPH** **JAPANESE STUDY**	**NCPF** **PRESENT STUDY**
Sensitivity	100 %	70%
Specificity	95%	21%
PPV	94 %	27%
NPV	100 %	62.5%

Table 21: Comparison of the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of spleen stiffness (SS) between the Japanese study and the present study.

Among the NCPF group, Spearman correlation (rho) for LS with liver biopsy was 0.3 and p=0.31 which was statistically not significant. Spearman correlation of SS with liver biopsy was -0.28 with p=0.5 which was statistically not significant. This may be due to the low sample size.

The spleen to liver stiffness ratio (SS/LS) had 70%, sensitivity, 79% specificity, PPV of 58.3% and NPV of 86.4%. The sensitivity in this study was lower than the Japanese study, however the specificity was higher. The combination of either LS, SS or SS/ LS had sensitivity of 82%, specificity of 21.4 %, PPV of 29%, NPV of 75%, kappa of 0.02 (p=0.8) to identify NCPF from other liver cirrhosis. The best individual parameter of ARFI elastography to identify NCPF from other causes of cirrhosis was found to be the ratio of spleen stiffness to liver stiffness (SS/LS) with good sensitivity, specificity, negative predictive value and good kappa correlation which was also found to be the best parameter in the Japanese study.

SS/ LS	**NCPF/ IPH** **JAPANESE STUDY**	**NCPF** **PRESENT STUDY**
Sensitivity	100 %	70%
Specificity	72%	79.2%
PPV	71%	58.3%
NPV	100 %	86.4 %

Table 22: Comparison of the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of spleen stiffness to liver stiffness ratio (SS/ LS) between the Japanese study and the present study.

Hence ARFI elastography of the liver and the spleen in combination have good sensitivity and can be used as screening tool for non invasive detection on NCPF patients from other causes of liver cirrhosis. Currently liver biopsy is required for accurate diagnosis. Larger multicentric studies among Indian population are required as none are available at present. In future ARFI elastography of the liver and spleen can be used as of the diagnostic criterion for non- invasively diagnosis of NCPF patients.

DIAGNOSTIC ACCURACY ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO DIFFRENTIATE SIGNIFICANT LIVER FIBROSIS FROM NON SIGNIFICANT LIVER FIBROSIS:

According to a large meta-analysis by Nierhoff et al, the ARFI shear wave velocity cut-off values of liver are 1.35 m/s for the diagnosis of significant fibrosis, 1.61 m/s for the diagnosis of severe fibrosis and 1.87 m/s for the diagnosis of liver cirrhosis. The mean diagnostic accuracy of ARFI of the liver expressed as the AUROC was 0.84 (DOR, 11.54) for the diagnosis of significant fibrosis ($F \geq 2$), 0.89 (DOR, 33.54) for the diagnosis of severe fibrosis ($F \geq 3$) and 0.91 (DOR, 45.35) for the diagnosis of liver cirrhosis ($F = 4$). The meta-analysis revealed good diagnostic accuracy of the ARFI elastography of the liver for the staging of $F \geq 2$ and $F \geq 3$, and excellent diagnostic accuracy for $F = 4$ (21).

In the present study, LS was found to have 100 % sensitive to identify significant liver fibrosis (>F2), but has a low specificity of 30.4%. The positive predictive value (PPV) was 45.2 % and negative predictive value was 100% with kappa of 0.33, (p=0.05) and Likelihood ratio of 1.57 which was statistically significant. Thus the use of ARFI in identifying significant liver fibrosis in this study is comparable to the already published studies.

ARFI elastography of spleen was found to have 100% sensitivity, 10% specificity, PPV of 43.7%, NPV of 100% and kappa of 0.08 to identify liver cirrhosis. The spleen stiffness was not found to statistically significant to identify significant liver fibrosis.

The ratio of spleen stiffness (SS) to liver stiffness (LS) cut off value of 1.53 cm/ sec was taken to differentiate significant fibrosis from non significant fibrosis. ARFI elastography

measurements of SS/ LS was found to have 76.5% sensitivity, 47% specificity, PPV of 59%, NPV of 66.7% and kappa of 0.23 (P= 0.1) The best parameter to identify significant fibrosis was the combination of both liver and spleen elastography i.e SS/ LS ratio. The liver stiffness (LS), product of LS and SS and ratio of SS/ LS were found to statistically significant differentiate between significant from non significant liver fibrosis.

The sensitivity of ARFI elastography to identify no fibrosis was 66.7%, 31.6% for mild fibrosis, 33.3% for moderate fibrosis and 64.1% for cirrhosis. The liver stiffness measurement by ARFI elastography had higher sensitivity to identify moderate fibrosis and cirrhosis, and low sensitivity to identify mild fibrosis. The sensitivity and specificity in this study may be low due to the smaller sample size.

Ye et al. found significant linear correlation (Spearman $\rho = 0.87$; $P < .001$) between liver stiffness and the fibrosis stage (29). In the present study Spearman correlation of the liver stiffness (LS) with the grades of fibrosis on liver biopsy was $\rho = 0.54$ with p value of < 0.001 which was statistically significant. This states that as the liver fibrosis increases the liver stiffness velocity also increases which is similar to the studies published in literature.

DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY OF LIVER AND SPLEEN TO IDENTIFY CLINICALLY SIGNIFICANT PORTAL HYPERTENSION AS COMPARED TO HEPATIC VENOUS PRESSURE GRADIENT (HVPG) AND HIGH RISK VARICES ON ENDOSCOPY:

Rifai et al. studied 125 patients and found that splenic stiffness is better than liver stiffness to predict significant portal hypertension (AUORC 0.90 vs 0.68). The cut-off used was > 1.67 cm/ sec for LS (26). Rifai et al. also found that spleen stiffness is not useful to predict portal hypertension. The SS cut-off value for predicting portal hypertension was 3.29 m/s, with 47% sensitivity and 73% specificity (63).

In the present study, liver stiffness (LS) was found to have 57% sensitivity, 27% specificity, PPV of 33%, NPV of 50% and kappa of -0.13 (p=0.49) and Spleen stiffness(SS) was found to have 14.3% sensitivity, 90% specificity, PPV of 50%, NPV of 60% and kappa of 0.04, (p-0.78) . Both LS and SS were found to have low sensitivity with low kappa agreement and were statistically not significant to identify clinically significant portal hypertension as compared to HVPG. This may be due to the fact that the number of patients who underwent HVPG measurements was small.

There are very few studies which analysed the use of liver and spleen stiffness to identify significant esophageal varices. Vermehren et al. found that ARFI of spleen is better than ARFI of the liver for esophageal varices (63). Study done by Ye et al. showed that liver stiffness did not correlate with the grade of esophageal varices (29). Takuma et al, found that SS had the greatest diagnostic accuracy for the identification of patients with high-risk esophageal varices, as compared to liver stiffness and other non-invasive parameters and is independent of the etiology of cirrhosis. SS cut-off value of 3.30 m/s had 99.4% negative

predictive value, 98.9% sensitivity, 72.1% accuracy, and 0.018 negative likelihood ratio. SS values less than 3.3 m/s ruled out the presence of high-risk varices in patients with compensated or decompensated cirrhosis (31). Singh et al. a systematic review and meta-analysis of 9 studies, SS detected the presence of clinically significant esophageal varices with 81% sensitivity, 66% specificity, a positive LR of 2.5, a negative LR of 0.2, and a diagnostic odds ratio of 12.6. Due to the different elastography techniques and study locations there was significant heterogeneity among the studies. They proposed that the current techniques of measuring splenic stiffness are limited to detect esophageal varices (32).

In this study, liver stiffness (LS) was found to have 46.5% sensitivity, 26.4% specificity, PPV of 10%, NPV of 56% and ($p= 0.07$) which was statistically not significant. Spleen stiffness was found to have 31% sensitivity, 81.25% specificity, PPV of 40%, NPV of 74.3% and ($p=0.38$) which was statistically not significant. Both LS and SS were found to have low sensitivity with low kappa agreement and statistically not significant to identify large esophageal varices and clinically significant portal hypertension. Further Indian studies with larger sample size are needed to for assessing ARFI elastography of the liver and spleen to diagnose esophageal varices.

CONCLUSIONS:

1. ARFI elastography of the liver and the spleen in combination have good sensitivity to identify Noncirrhotic portal fibrosis (NCPF) from cirrhosis with portal hypertension as compared to liver biopsy. The best elastography parameter was the spleen stiffness to liver stiffness ratio. Liver and spleen stiffness independently are not adequate. ARFI elastography can be used a screening tool to non-invasively diagnose and monitor patients with NCPF, however lacks good specificity.

2. ARFI elastography of the liver and the spleen stiffness to liver stiffness ratio have good sensitivity to identify significant liver fibrosis as compared to liver biopsy. There is good correlation with liver stiffness velocities and grades of liver fibrosis. ARFI elastography can be used as good screening test to diagnose and monitor disease progression in patients with chronic liver disease.

3. ARFI elastography of the liver and spleen have low sensitivity and low specificity to identify clinically significant portal hypertension as compared to hepatic venous pressure gradient (HVPG) measurements and the presence of high risk esophageal varices on endoscopy.

LIMITATIONS:

- NCPF is an uncommon disease. The number of biopsy proven cases of NCPF recruited in this study is small (n=11). Further case recruitment is on-going.
- Only one published study in literature from Japan, no Indian data for comparison.
- ROC curves could not be drawn as the areas under the ROC curve was low due to low sample size. Hence cut-off velocities were taken from the already published studies.

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APPENDIX 1: CLINICAL RESEARCH FROM.

Study number:

Name:
Age:
Gender:
Hospital number:
Address:
Contact number:
Height:
Weight:
BMI:

Clinical Parameters:
Asymptomatic:
Jaundice:
Ascites:
Past history of upper GI bleed:
Others:
CHILD PUGH'S score:
Any other co-morbid illness:

Blood Parameters:
Haemoglobin:
WBC count:
Platelet count:
Prothrombin time:
Serum total bilirubin:
Serum direct bilirubin:
Serum total protein:
Serum albumin:
AST:
ALT:
Alkaline phosphatase:
PT-INR:

Imaging findings:
USG:
Liver size:
Liver echotexture:
Volume redistribution:
Surface irregularity:
Periportal echogenicity:
Hepatic veins:
Portal vein size:
Portal vein thrombosis:
Splenic vein size:
Spleen size:
Spleen area:

Collaterals:

ARFI Elastography:
Liver stiffness median velocity (LS) in m/sec:
Spleen stiffness median velocity (SS) in m/sec:
SS/LS

Method of liver biopsy:
Percutaneous blind biopsy:
USG guided liver biopsy:
Transjugular liver biopsy (TJLB):
With TJLB was HVPG done:

HVPG value:
Method of HVPG measurement:

Gastroscopy report:
Esophageal varices
Grade of esophageal varices:
Any prior procedure:
Other findings:

Liver histopathology report:

APPENDIX2: INFORMED CONSENT

Department of Radio diagnosis, Christian Medical College, Vellore

Information sheet

You are being requested to participate in a study to see if new ultrasound technique called acoustic radiation forced impulse (ARFI) elastography ultrasound, which can help in non-invasively assess the presence of liver fibrosis i.e liver stiffness and also to stage the liver disease. Presently the presence and stage of liver fibrosis is best assessed by a liver biopsy. By this study, we may be able to assess the liver fibrosis early and would help your treating doctor to give appropriate therapy.

How does ARFI help in assessing of liver and spleen in NCPF?

We have observed that there is difficulty in identifying the exact cause of your liver problem with the routine blood tests. Using ARFI, we study the stiffness of the liver and spleen which can tell us the probable cause and the degree of liver involvement. However, we have only used this for a few people and we need to use it on more people to be sure that it really helps.

Does ARFI have any side effects?

There are no known side-effects. This additional ultrasound scan will take 10 minutes.

If you take part what will you have to do?

If you agree to participate in this study, there will be no change in the other treatments and investigations that you will be having. During the scan, you will be asked to hold your breath for few times. You will be expected to come for follow up with your doctor after the liver biopsy. No additional blood tests will be done as a part of this study.

Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

What will happen if you develop any study related injury?

This scan does not involve injections or radiation and it is completely non invasive. So, we do not expect any procedure related injury. However you can immediately report to us.

Will you have to pay for the ARFI?

You will **not** be charged for this additional scan. All other investigations, as requested by your doctor will continue in the usual manner. How much you pay for these investigations will not change and this has nothing to do with your participation in this study.

What happens after the study is over?

You may or may not benefit from this study. Once the study is over, if we come to a conclusion that the investigation is beneficial in assessing the presence and stage of liver fibrosis, we will be able to use this technique in assessing and prognosticating patients in future. Your doctor may also use this on you again on follow up to assess disease progression if required.

Will your personal details be kept confidential?

The results of this study will be published in a medical journal but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission.

CONSENT TO TAKE PART IN THIS STUDY

Study Title:

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____, son /daughter of _____

Declare that I have read/been read to the information sheet provided to me regarding this study and have clarified any doubts that I had. [ ]

(Please tick boxes)

I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

I understand that my identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree to take part in this study [ ]

Name:

Signature/thumb impression

Date:

Name of witness:

Relation to participant:

Date: