# DISCUSSION

Cirrhosis is a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules.

The fibrosis causes distortion of the hepatic vasculature and can lead to an increased intrahepatic resistance and portal hypertension. Portal hypertension can lead to oesophageal varices as well as hypo perfusion of the kidneys, water and salt retention and increased cardiac output.[187]

The major clinical consequences of cirrhosis are impaired hepatocyte (liver) function, an increased intra hepatic resistance (portal hypertension) and the development of hepatocellular carcinoma (HCC).

Cirrhosis and its associated vascular distortion are traditionally considered to be irreversible but data suggest that cirrhosis regression or even reversal is possible.[188,189]

In a research done by Stelios et al,[190] This study demonstrates for the first time that human liver cirrhosis induces significant alteration in enterocytes' TJs. These changes might represent an important cellular mechanism for intestinal barrier dysfunction and hyperpermeability in patients with liver cirrhosis.

Campillo etal[191]documented that increased intestinal permeability in patients with liver cirrhosis, particularly in those with septic complications. While a number of investigators have observed normal intestinal permeability in patients with liver cirrhosis.[192,193,194]

By virtue of its anatomical location and its blood supply, the liver is uniquely positioned to confront and interact with those microbes, microbial components and products of microbe-gut interactions that may have traversed the gut barrier and gained access to the blood stream.[195]

In liver disease, an over growth of gram-negatives allied to impaired gut barrier function allows whole organisms, through the process referred to as translocation in which bacterial components arriving in the portal system resulting in a cascade of pro-inflammatory cytokine production leading to liver injury and can progress to fibrosis.[196]

Among the various potential contributions of the microbiota to liver disease, small intestinal bacterial overgrowth (SIBO) has been one of the most extensively studied. Indeed, an altered gut microbiota was first noted by Hoefert in chronic liver disease over80 years ago;[197] since then SIBO has been documented to be common in liver disease, to correlate with its severity and to be linked to minimal and overt encephalopathy and an increased risk for SBP through translocation across the gut wall.[198,199]

Moreover, factors predisposing to SIBO such as altered small bowel motility, increased intestinal permeability, and delayed gut transit have been reported in patients with liver cirrhosis.[193]

Alterations in gastrointestinal motility in cirrhosis have been variably ascribed to the effects of autonomic dysfunction, altered levels of circulating neuropeptides and the effects of inflammatory mediators on gut muscle and nerve.[200,201]

Kirsten et al,[202] found that dysfunction of intestinal epithelial barrier may enhance the risk of translocation of bacteria and bacterial products into the systemic circulation, and thereby contribute to the pathogenesis of chronic liver diseases, cirrhosis and the development of complications.

**Mauro** et al,[203] was found that all patients with cirrhosis and ascites are at risk of developing a bacterial infection of the ascitic fluid, called SBP,[204]even without septic shock, may precipitate circulatory dysfunction with severe liver failure, hepatic encephalopathy and type 1 HRS, causing death of the patient in approximately 20% of cases despite infection resolution with antibiotic treatment.[204]

Rita et al,[205] performed a similar research and found that intestinal permeability evaluation in patients with chronic liver diseases might clarify the significance of intestinal permeability in the pathophysiology of both the progression of liver damage, and the occurrence of complications that accompany liver cirrhosis Alteration in intestinal permeability may be an important factor in the pathogenesis of both the progression of some chronic liver diseases and the onset of some complications in patients with liver cirrhosis.

The gut wall forms a physical/anatomical and immunological barrier. The physical/anatomical barrier of the gut is formed by a monolayer of epithelial cells, originating from multipotent stem cells present in the crypt.

The epithelial cells together with the lamina propria form the mucosa of the intestine.[138,139,144] Tight junctions (TJ) are the major complexes responsible for the adherence of intestinal epithelial cells to each other and are in this context an important part of the intestinal barrier.[144,145]There is also an immunological barrier.

Evaluation of intestinal pathology in patients of all age-groups has been a challenge for clinicians. Numerous patients present with abdominal complaints which are frequently a specific and therefore correspond to pathologies of most intra- and even some extra-abdominal organs.

Laboratory tests and imaging techniques are often helpful in revealing disorders of organs, including the liver, pancreas, heart and kidneys. However, it is currently still difficult to diagnose intestinal pathology in patients presenting with abdominal complaints.[ 207,208]

Ersöz et al,[208]reported that intestinal permeability increased in cirrhotic patients regardless of the grade and etiology of disease.

The current standard technique for assessing intestinal status is endoscopy with bowel biopsy which is often inadequate as it is invasive, sometimes requires sedation, is expensive and only assesses the function of the biopsied fraction Moreover, for patients who are

neutropenic and/or thrombocytopenic, the procedure is physically hazardous and often ethically un acceptable.

Therefore, the aim of this work was to evaluate plasma D-Lactatae, and fatty acid binding protein as non-invasive parameters of gut wall integrity in patients with HCV induced liver cirrhosis.

The study was conducted on one hundred subjects classified into three groups; group I consisted of forty patients with chronic HCV infection and Liver cirrhosis, Group II included forty patients with liver cirrhosis and ascites with spontaneous bacterial peritonitis, group III composed of twenty healthy subjects as controls.

In the present study we observed that (FABP) level was significantly higher in plasma of patients with spontaneous bacterial peritonitis than patients with chronic HCV infection and Liver cirrhosis without peritonitis. Mean Fatty acid binding protein was significantly higher in groups I and II than in group III). In addition, it was found to be significantly higher in group II than in group I .

Plasma/serum, urinary and fecal markers are currently available as useful tools to study the condition of the gut wall in man. Measurement of endogenous cytosolic enterocyte proteins in urine or plasma have been shown to be useful to estimate enterocyte damage.

Fatty Acid Binding Proteins (FABP) comprise a class of low molecular weight (14-15 kDa) cytosolic proteins found in high concentrations in tissues involved in the uptake and consumption of fatty acids.

Intestinal Fatty Acid Binding Protein (I-FABP) is primarily limited to mature enterocytes of the small and large intestine.[149] It circulates in low amounts in the blood stream of healthy individuals. I-FABP is a useful plasma marker for early enterocyte cell death and levels rise rapidly after episodes of acute intestinal ischaemia and inflammation.[149,150]

The level of circulating I-FABP has been reported to correlate with the histological status of the epithelium after intestinal ischaemia-reperfusion in experimental studies.[151,152]

Derikx et al,[209]. performed a research on I-FABP, they found that the plasma level of I-FABP was high after shock ,sepsis and systemic inflammatory response syndrome.

Thuijls et al,[210]evaluated the role of Plasma and especially urinary I-FABP and L-FABP levels and urinary I-BABP levels can improve early diagnosis of intestinal ischemia. Furthermore, plasma I-BABP levels can help in localizing ileal ischemia.

Median plasma concentrations of I-FABP and L-FABP and urinary concentrations of all three markers were significantly higher in patients with proven intestinal ischemia than in patients suspected of intestinal ischemia with other final diagnoses and they found that they can serve as early predictor for treatment success in these patients.

In a research done by Tatsuo et al,[211]a rapid and simple determination method for acute enteritis which can determine acute enteritis in a quantitative and objective manner by determination of intestinal fatty acid-binding protein in the blood performed by an immunochemical method and is preferably an enzyme immunochemical assay, more preferably a sandwich enzyme immunoassay.

Alicja et al,[212] reported that The elevated serum I-FABP concentration in patients with Ulcerative Colitis(UC) may indicate ileitis I-FABP may be a useful marker of the extended inflammatory process.thus supporting the results of the present work.

Breakdown of the mucosal barrier potentially leads to translocation of microbiota or their toxic products. A promising plasma marker, reflecting translocation of bacteria or their products, is D-lactate, which is a metabolic products or component of the commensal bacteria of the gastrointestinal tract. D-lactate is only produced by bacteria as a product of bacterial fermentation.[161]

The present work revealed that Mean Plasma D-lactate was found to be significantly higher in plasma of patients with spontaneous bacterial peritonitis( group II) than in patients with chronic HCV infection and Liver cirrhosis without peritonitis. ( group I), and was found to be significantly higher in group I and II than in group III.

Baseline levels of D-lactate in healthy subjects are very low, but the exact mechanism by which D-lactate enters the bloodstream is unknown.

Increased levels of D-lactate have been correlated with conditions in which the number of bacteria elevates rapidly, including in patients with bacterial overgrowth due to infection, short bowel syndrome and mesenteric ischaemia.[162,163]

Murrayet al,[163]reported that significant elevations in D-lactate levels in patients with mesenteric ischemia compared with controls ($P< 0.00005$), as well as in patients with other forms of abdominal catastrophes ($P < 0.00005$) and with bowel obstruction ($P < 0.0005$). concluding that D-lactate serum levels can aid in diagnosing acute mesenteric ischemia, thus supporting the results of the present work.

However, Han et al,[213] found that a subset of patients with liver cirrhosis can develop elevation of D-lactate in blood, particularly when metabolic acidosis is accompanied.

In the present study, Regarding the relation between plasma D-lactate and FABP level it was found that there is a positive correlation between the two markers in group I and II.

Duzgun et al.[214]showed that there is a positive correlations between serum D-lactate levels in acute appendicitis .and serum D-lactate had the lowest false negative rate among the other parameters. Therefore, they conclude that D-lactate might be a simple and reliable diagnostic marker for appendicitis.

In the present study, the child score was observed to have a statistically significant association with the plasma level of D-lactate and FABP in groups I and II .

In the current study, it was observed that in group I patients there was 45% Child A ,55% Child B while. Among the patients in group II, there was 52.5% Child B and 47.5%were Child C .The plasma level of D-lactate and FABP was significantly higher in Child C patients than in Child B in both groups.

ALSO it was found that Patients with SBP have higher plasma level of D-lactate and FABP than in Patients without SBP which were in agreement with Chang CS et al,[215] who demonstrated that the incidence of bacterial overgrowth of the small intestine was higher in cirrhotic patients with history of SBP than in those without SBP. Small intestine motility dysfunction was more severe in cirrhotic patients with history of SBP.

Impaired motility of the small intestine, causing bacterial overgrowth of the small intestine, may be one of the explanations for recurrent SBP in cirrhotic patients. On the other hand Bac**,** et al,[193] found that small bowel function is maintained to a large extent in patients with advanced liver cirrhosis and portal hypertension .

# SUMMARY

**Liver cirrhosis** is defined histologically as a diffuse process with liver cell necrosis/apoptosis, fibrosis and regenerative nodules. There are several causes of liver cirrhosis, the most common being high alcohol consumption, hepatitis C, hepatitis B, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, and non-alcoholic steatohepatitis.

Cirrhosis results in two major events: hepatocellular failure and portal hypertension.

Important complications of liver cirrhosis include esophageal varices, ascites, hepatic encephalopathy, hepatic failure with jaundice, hepatocellular cancer, and cholangiocarcinoma.

Cirrhosis has also been associated with alterations in the gastrointestinal (GI) tract. A major endoscopic finding is varices most commonly located in the esophagus and/or the fundus of the stomach. Mucosal changes are also frequently encountered upon endoscopic examination of the GI tract in patients with liver cirrhosis.

**Ascites** is the most common major complication of cirrhosis and is an important landmark in the natural history of chronic liver disease. If observed for 10 years, approximately 60% of patients with cirrhosis develop ascites requiring therapy.

**Spontaneous bacterial peritonitis (SBP)** is the most frequent and life-threatening infection in patients with liver cirrhosis.

It is defined by the presence of >250 polymorphonuclear cells (PMN)/$mm^3$ in ascites in the absence of an intra-abdominal source of infection or malignancy.

Bacterial translocation (BT) is the most common cause of SBP. Cirrhosis is associated with structural and functional alterations in the intestinal mucosa that increase permeability to bacteria and bacterial products.

The intestinal tract constitutes a large interface between the outside environment and the human body. This interface has two critical functions: As a filter and as a barrier.

Breakdown of the mucosal barrier potentially leads to translocation of microbiota or their toxic products.

Promising plasma marker reflecting translocation of bacteria or their products, is D-lactate which is a metabolic product or component of the commensal bacteria of the gastrointestinal tract, Baseline levels of D-lactate in healthy subjects are very low.

Increased levels of D-lactate have been correlated with conditions in which the number of bacteria elevates rapidly, including in patients with bacterial overgrowth due to infection, short bowel syndrome, and mesenteric ischaemia.

Measurement of endogenous cytosolic enterocyte proteins in plasma has been shown to be useful to estimate enterocyte damage.

Fatty acid-binding proteins (FABPs) are a class of cytoplasmic proteins that bind long chain fatty acids.

The level of circulating I-FABP has been reported to correlate with the histological status of the epithelium after intestinal ischaemia-reperfusion in experimental studies. Based on this mechanism, many investigators have already reported the relationship between serum I-FABP concentration and small intestinal diseases from early 1990s.

Therefore, the aim of this work was to evaluate plasma D-Lactatae, and fatty acid binding protein as non-invasive parameters of gut wall integrity in patients with HCV induced liver cirrhosis.

This study was conducted on one hundred subjects classified into three groups; group I consisted of forty patients with chronic HCV infection and Liver cirrhosis, Group II included forty patients with liver cirrhosis and ascites with spontaneous bacterial peritonitis, group III composed of twenty healthy subjects as control

**All patients and controls were subjected to the following:**

- Detailed history and thorough clinical examination.
- Laboratory investigations including
  - Routine investigations
    - Complete blood picture.
    - Serum urea and creatinine
    - ESR and CRP
  - Liver function tests and liver enzymes including serum alanine transaminase (ALT), serum aspartate transaminase (AST), serum albumin, serum bilirubin, prothrombin activity alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT).
- HCV Ab by ELISA, HCV RNA by PCR and HBs Ag by ELISA.
- Estimation of plasma D-lactate.
- Estimation of plasma I-FABP.
- Abdominal ultrasonography.

## Statistical analysis of data obtained from the present study revealed the following results:

- As regards haematological findings, Hb level, RBC's count as well as platelets count were lower in groups I and II than in group III.

- As regards liver enzymes, ALT, AST, ALP and GGT were significantly higher in groups I and II than in group III.

- Liver function tests showed significantly lower serum albumin and prothrombin time in groups I and II than in control group and significantly higher serum bilirubin in groups I and II than in the control group.

- Plasma D-lactate level was found to be significantly higher in patients with liver cirrhosis and ascites with spontaneous bacterial peritonitis( SBP) and patients with chronic hepatitis C and liver cirrhosis than in healthy controls, and was found to be significantly higher in patients with SBP than in those with cirrhosis.

- Plasma Fatty acid binding protein level was found to be significantly higher in patients with liver cirrhosis and ascites with spontaneous bacterial peritonitis( SBP) and patients with chronic hepatitis C and liver cirrhosis than in healthy controls and was found to be significantly higher in patients with SBP than in those with cirrhosis .

- There was a significant correlation between plasma D-lactate level and Child Pugh also There was a significant correlation between plasma Fatty acid binding protein level and Child Pugh.

- Plasma Fatty acid binding protein level was also found to correlate positively to plasma D-lactate level.

- The ROC curve for Plasma FABP was significant (p.001) and showed that the cutoff point discriminating control from cases was 2ng/ml, with sensitivity of 92%, specificity of 80%, positive predictive value of 99.1 % and negative predictive value of 0.9% and diagnostic accuracy of 95%.

- The ROC curve for plasma D-lactate was significant (p.001) and showed that at a cut off value of $\geq$ 0.57, diagnostic sensitivity for discriminating control from cases was 0.57 with sensitivity of 96%, specificity of 85%, positive predictive value of 91.8 % and negative predictive value 0f 8.2 and diagnostic accuracy of 95%.

- Thus, at these cut off points, plasma FABP and plasma D-lactate was found to have better diagnostic sensitivity, accuracy and larger AUC (0.89) and (0.96) respectively as regards differentiating healthy controls from cirrhosis cases.