

# A study of serological markers and lipid profile in non-alcoholic liver cirrhosis patients

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**Abstract:** Cirrhosis is the end result of chronic liver damage, associated with altered serum biomarkers and lipid profile. However, only few studies regarding serum biomarkers and plasma lipid profile in non-alcoholic liver cirrhosis subjects have been undertaken in Pakistan. This study aimed to evaluate the degree of alterations of tumor markers and lipid profile in liver cirrhosis patients and in normal healthy individuals. Levels of serological markers and plasma lipid pattern was measured in liver cirrhosis patients and in sex and age matched normal healthy individuals (n=46). Tumor marker alpha-fetoprotein (AFP) was measured by ELISA, whereas plasma lipid profile and biomarker alanine aminotransferase (ALT) was determined by colorimetric assays. In patients with cirrhosis significant increase was observed in serum AFP and ALT levels when compared with healthy individuals (p<0.05). The triglyceride levels were found to be significantly higher, while LDL-C was significantly lower (p<0.05) in liver cirrhosis patients. There was no significant difference in the plasma concentration of cholesterol, total lipids and HDL-C in liver cirrhosis patients as compared with normal individuals. AFP and ALT are useful markers in the diagnosis of cirrhosis and can be used for the detection of hepatocellular carcinoma (HCC) in cirrhosis patients. The assessment of plasma lipids and lipoprotein pattern is also important for prognostic evaluation of patients with cirrhosis.

**Keywords:** Cirrhosis, lipids, AFP, lipoproteins, serum biomarkers, ALT.

## INTRODUCTION

Cirrhosis is an advanced stage of liver fibrosis, usually occurred in response to chronic liver injury and is accompanied by distortion of the hepatic architecture. In Cirrhosis formation of scar tissues, impairs liver's ability to function optimally and to replace its damaged hepatocytes (Schuppan and Afdhal, 2008). Cirrhosis if untreated can lead to portal hypertension, ascites, GIT bleeding, hepatic encephalopathy and development of hepatocellular carcinoma (HCC) (Suk and Kim, 2015). Multiple etiological factors can contribute to the development of cirrhosis. The causative factors includes excessive alcohol consumption (alcoholic liver disease), chronic viral hepatitis (such as HBV, HCV and HDV), metabolic genetic disorders (such as hereditary hemochromatosis, Wilson's disease or glycogen storage diseases), drug induced injury, bile duct disorders and nonalcoholic steatohepatitis (NASH) (Wiegand and Berg, 2013; Tsochatzis *et al.*, 2014).

In developing countries like Pakistan liver cirrhosis is highly prevalent and is considered as one of the leading cause of hepatic mortality & morbidity amongst Pakistani population (Memon and Zaki, 2013; Ali *et al.*, 2009). In Pakistan, unlike developed countries incidence of non-alcoholic cirrhosis is more prevalent and viral hepatitis has been identified as the most common cause of cirrhosis (Almani *et al.*, 2008; Ullah *et al.*, 2012).

Clinicians considered liver biopsy as a gold standard for the accurate diagnosis and evaluation of cirrhosis. However, non-invasive serological markers are important for an early diagnosis of cirrhosis, as well as monitoring of diseases progression and treatment responsiveness (Schuppan and Afdhal, 2008).

Alpha-feto protein (AFP) is an onco-developmental serum glycoprotein, which is produced in the visceral endoderm of the yolk sac cells and liver of the fetus during pregnancy (Gillespie and Uversky, 2000; Mizejewski, 2001). Serum AFP concentration decreases gradually after birth up to the levels that are usually undetectable. However, in adult life elevated AFP levels can be seen in certain malignant conditions (Gillespie and Uversky, 2000). AFP has been considered as the ideal serological biomarker for detecting hepatocellular carcinoma (HCC) and elevated levels of AFP was also found in cirrhosis and other chronic liver diseases (Lai *et al.*, 2012). Furthermore, persistent increased AFP levels were also detected in other non-hepatic tumors including pancreatic carcinoma, gastric carcinoma, and germ cell tumors (Gillespie and Uversky, 2000).

Hepatic enzyme alanine aminotransferase (ALT) is involved in protein metabolism and catalyzes the transamination reaction (Kim *et al.*, 2008). ALT has been used as a marker of hepatic health (Giannini *et al.*, 2005) and several studies have reported the significance of ALT activity as an indicator of liver function. These population

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based studies showed a significant association between ALT levels and resultant mortality from liver disease (Kim *et al.*, 2008). To our knowledge, only few studies were carried out to evaluate the liver cirrhosis by analyzing serum AFP and ALT levels and contribution from Pakistan is scarce.

Liver being the metabolic hub of the body, have a critical role in the synthesis, transport, and storage of lipids and lipoproteins. Therefore, abnormalities in serum lipids and lipoproteins concentrations have been commonly observed in liver diseases (Luo *et al.*, 2010, Mehboob *et al.*, 2010). There are discrepancies in the result of the lipid profiling studies of liver cirrhosis patients. Some studies observed a decreased trend in lipid profile with the severity of the liver disease (Ghadir *et al.*, 2010; Subhan *et al.*, 2012), while some have reported an opposite or no change in the pattern of liver cirrhosis patients (Sen *et al.*, 2013). This discrepancy in the result of these studies could be due to differences in the etiology of hepatocellular injury.

We aimed to analyze the subclinical significance of AFP and ALT in patients of non- alcoholic liver cirrhosis, and also to evaluate the influence of cirrhosis on plasma lipids and lipoproteins profile.

## MATERIALS AND METHODS

### Sample collection

Blood samples under standardized fasting condition were collected after taking consent from 46 nonalcoholic liver cirrhosis patients and sex and age matched normal healthy individuals. Aliquots of serum and plasma samples were made for different analytical parameters and stored at -20°C until analyzed.

### Estimation of AFP and ALT

AFP was analyzed by automated ELISA technique on Cobas Core (Roche-Diagnostics, Basil). Determination of serum ALT was carried out with IFCC recommended method on chemistry analyzer Hitachi 912 (Roche-Diagnostics).

### Analysis of lipid profile

Plasma lipid profile (Total lipids, triglyceride and cholesterol) was analyzed using the Randox kits (Randox Laboratories Ltd., UK). Low density lipoprotein cholesterol (LDL-C) was determined by colorimetric method, while high density lipoprotein cholesterol (HDL-C) was estimated by precipitation method using commercial Randox kits (Randox Laboratories Ltd., UK). Plasma Lipoproteins were separated by agarose gel electrophoresis and the relative mobility ratios of LDL & HDL cholesterol was determined by densitometer.

## STATISTICAL ANALYSIS

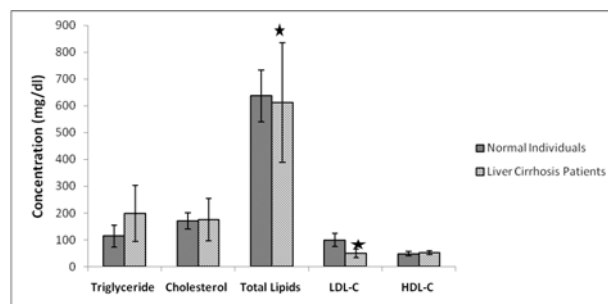
The data was analyzed using the Statistical Package for Social Sciences (SPSS) software. Student's t-test was used to compare the subjects and control and a p value <0.05 is considered statistically significant. All results were presented as Mean  $\pm$  SD unless otherwise mentioned.

## RESULTS

### Tumor marker AFP and enzyme ALT

In liver cirrhosis patients, serum concentration of AFP was significantly higher ( $p < 0.05$ ), i.e.,  $279.4 \pm 13.1$  IU/ml (Mean  $\pm$  SEM) as compared to controls, i.e.  $2.5 \pm 0.2$  IU/ml (Mean  $\pm$  SEM) (table 1). In 11 patients (25%), serum AFP levels were found to be greater than 200 IU/ml.

Similarly when compared with the control individuals, serum concentration of ALT in cirrhotic patients were significantly higher ( $p < 0.05$ ) (table 1), Mean ALT levels in patients and controls were  $1226.3 \pm 27.1$  IU/L (Mean  $\pm$  SEM) and  $20.6 \pm 1.6$  IU/L (Mean  $\pm$  SEM), respectively as shown in fig. 1.



**Fig. 1:** Comparison of lipid profile of liver cirrhosis patients with normal individuals. Values are Mean  $\pm$  SD. Normal individuals were compared with liver cirrhosis patients by independent t-test; \* indicate the significant difference between the two groups ( $p < 0.05$ ).

### Plasma lipid profile

Results are presented in table 2 and fig. 1. The triglyceride levels in liver cirrhosis patients were found to be significantly higher ( $p < 0.05$ ) i.e.  $199.3 \pm 223.8$  mg/dl than those in healthy controls, i.e.  $113.9 \pm 41.6$  mg/dl. There was no significant difference in the plasma levels of cholesterol and total lipids in liver cirrhosis patients and controls. Plasma LDL-C levels in liver cirrhosis patients was significantly lower ( $p < 0.05$ ) i.e.  $49.6 \pm 16.1$  mg/dl as compared to the controls i.e.  $99.3 \pm 24.9$  mg/dl. In contrast, when compared with controls, there was no significant difference observed in the plasma HDL-C levels of liver cirrhosis patients.

Agarose gel electrophoresis of plasma lipoproteins showed two bands corresponding to LDL and HDL in

both controls and liver cirrhosis subjects (fig. 2). Relative LDL concentration was lowered in the observed band of liver cirrhosis patients than those of the healthy subjects ( $p < 0.05$ ), i.e.  $58.0 \pm 6.31\%$  and  $62.90 \pm 10.8\%$  respectively. Relative HDL concentration is non-significantly higher in liver cirrhosis patients when compared with controls, i.e.  $42.0 \pm 6.3\%$  and  $39.10 \pm 6.6\%$  respectively.



**Fig. 2:** Agarose gel electrophoresis of plasma lipoproteins of both normal healthy individual (N) and liver cirrhosis subjects (Patients 1 to 9).

## DISCUSSION

In Pakistan end-stage liver disease burden, morbidity and mortality due to chronic liver diseases, cirrhosis, hepatic malignancies & liver failure is very high (Ali *et al.*, 2009). Liver cirrhosis occurred as a consequence of chronic hepatic injury and is characterized by altered liver architecture and replacement of hepatocytes by scar tissue. As a result normal functioning of liver is impaired, which altered the lipid profile and some serum biomarker levels in cirrhotic subjects (Schuppan and Afdhal, 2008).

The current study attempted to describe the abnormality of serum biomarkers (AFP and ALT) and lipid levels among the patients of non-alcoholic liver cirrhosis. A total of 46 non-alcoholic subjects of both sexes participated in the present study.

In our study elevated AFP and ALT levels were noted in patients with liver cirrhosis as compare to controls. Similar results were reported by other studies where they have found an elevated serum AFP and ALT values in cirrhosis patients (Goldstein *et al.*, 1999; Arrieta *et al.*, 2007). A significant positive correlation has been shown between AFP and ALT values (Goldstein *et al.*, 1999). In cirrhosis subjects, increased AFP levels may indicate the loss of normal hepatic architecture and altered interaction

between hepatocytes (Arrieta *et al.*, 2007; Goldstein *et al.*, 1999).

AFP has been widely used as a tumor marker for hepatic carcinoma. In adults, elevated AFP levels up to pathological range have been found to be correlated with HCC (Lai *et al.*, 2012). AFP levels can also be elevated in benign liver diseases including chronic hepatitis and cirrhosis (caused by different etiologies). Another clinical utility of AFP is its utilization for surveillance of HCC in patients with chronic liver diseases (Gillespie and Uversky, 2000; Lai *et al.*, 2012).

In present study, some of the patients had highly elevated AFP levels (greater than 200 IU/ml) this may be due to the development of HCC in these patients. As many studies have found that AFP levels were higher in liver cirrhosis patients that developed HCC as compare to liver cirrhosis patients without HCC. Many investigators have shown that the presence of elevated AFP levels in liver cirrhosis patients increases the risk of the development of HCC in them (Arrieta *et al.*, 2007, Abbasi *et al.*, 2012, Lok *et al.*, 2009). It has been shown that an elevated AFP level has high diagnostic significance for HCC and the risk of presence of HCC is greater than 90% in case of AFP levels of  $>200\text{ng/ml}$  (Di Carlo *et al.*, 2012). Liver cirrhosis patients with highly elevated AFP levels require regular screening with ultrasonography for the detection of HCC in these patients (Lopez, 2005).

Hepatic disorders lead to major quantitative and qualitative modifications in lipid homeostasis (Luo *et al.*, 2010). In our study, triglycerides levels were significantly increased in cirrhosis patients compared to healthy individuals. Several studies reported a change in lipid profile among liver cirrhosis patients of different etiologies. Cross sectional studies carried out in USA and Brazil has found an increased concentration of triglycerides in patients of non-alcoholic fatty liver disease (NAFLD) (Clark, 2006; Leite *et al.*, 2009). Studies from South Asian region particularly from India, had reported that the subjects with non-alcoholic cirrhosis had significantly higher values of total cholesterol and triglycerides than controls (Sen *et al.*, 2013). However, some other studies reported a decreased in serum triglyceride levels in cirrhosis (Mehboob *et al.*, 2010).

In present study, plasma LDL-C levels were significantly decreased whereas no significant change in total lipids, cholesterol, and HDL-C were seen in cirrhosis patients. It has been reported that there is an inverse relation between the serum LDL-C and severity of liver damage and therefore low LDL-C is expected in cirrhotic patients (Phukan *et al.*, 2013). Few studies have shown a decrease in levels of HDL-C, LDL-C and total cholesterol in case of liver cirrhosis (Phukan *et al.*, 2013). Similar results were observed in a Nigerian study, where LDL-C levels were higher in controls compared with cirrhotic patients (Okeke *et al.*, 2010).

**Table 1:** Comparison of serological markers in normal individuals and liver cirrhosis patients

	Parameters	Normal Individuals(n)	Liver Cirrhosis Patients(n)
1	Alpha-fetoprotein (IU/ml)	2.5±0.2(20)	279.4±13.1(46)*
2	Alanine aminotransferase (IU/L)	20.6±1.6 (20)	1226.3±27.1 (46)*

**Table 2:** Comparison of lipid profile in normal individuals and liver cirrhosis patients

	Parameters	Normal Individuals(n)	Liver Cirrhosis Patients(n)
1	Triglyceride (mg/dl)	113.9±41.6 (46)	199.3±104.7 (44)*
2	Cholesterol (mg/dl)	170.7±30.5 (46)	176.0±78.6 (44)
3	Total Lipids (mg/dl)	636.4±97.4 (46)	612.1±223.8 (43)
4	LDL-C (mg/dl)	99.3±24.9 (46)	49.6±16.1 (13)*
5	HDL-C (mg/dl)	48.2±8.2 (46)	52.2±7.1 (13)
6	LDL-C (%)	62.9±10.8 (20)	58.0±6.3 (20)
7	HDL-C (%)	39.1±6.6 (20)	42.0±6.3 (20)

Data are presented as Mean ±SD.\* indicate the significant difference between the two groups (p<0.05)

Small sample size and no histological diagnosis of cirrhosis were some limitations of the study. Despite the limitations, the study provides a baseline for future studies on lipid abnormalities and serum biomarkers in non-alcoholic cirrhotic patients of different etiologies.

## CONCLUSION

This study suggests that AFP and ALT are useful markers in the diagnosis of cirrhosis and can be used for the detection of HCC in cirrhosis patients. The assessment of plasma lipids and lipoprotein pattern is also important for prognostic evaluation of patients with cirrhosis.

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