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Evaluation of interleukin-6 and ammonia levels in liver cirrhotic patients: a case-control study

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Abstract

Cirrhosis is a progressive chronic liver disease resulting in fibrogenesis. Cirrhosis associated various etiologies can cause increased mortality rate worldwide. The liver cirrhotic patients exhibit systemic inflammation, multiple cytokines are produced and involved in progression of disease. Serum levels of IL-6 and ammonia are associated with complications in patients with liver cirrhosis. To evaluate association between IL-6 and ammonia in prognosis and diagnosis of liver cirrhotic patients. A total of 176 participants were included in this study, out of which 88 were liver cirrhotic patients. Recruited patients were showing various etiological factors. Child-Pugh scoring was used to assess severity of liver cirrhosis. IL-6 and ammonia levels were estimated among all participants. Independent t-tests were used to find the differences between the groups and area under the curve (AUC) were conducted to analyze the data. The mean age of cirrhotic patients was 47.1±6.7 years. The patients were categorized under MELD (29.08±1.599) and Child-Pugh score. As per the Child-Pugh scoring around 38 patients were coming under stage C which is the end stage of liver disease. The liver function specific markers such as AST and ALT showed an AUC of 0.965 and 0.986 respectively. The predictive performance of IL-6 (AUROC, 0.972) and Ammonia (AUROC of 0.980) and significant correlation has been observed during the progression of liver cirrhosis. Study related to combination of markers as non-invasive methods indicate the severity of the disease. IL-6 and ammonia may be considered as diagnostic biomarkers to estimate severity of liver cirrhosis. The potential synergistic interaction between IL-6 and ammonia based standardized testing and interpretation guidelines may enhance improved patient care with cirrhosis and hepatic encephalopathy.

Keywords: Interleukin-6; Ammonia; Child-Pugh score; Hepatic cirrhosis; Hepatic encephalopathy

Full length article **Corresponding Author*, e-mail: *ravikumar.sambandam@avmc.edu.in*

1. Introduction

Cirrhosis is well-defined anatomically as diffuse process with fibrosis and nodule formation, which is the end result of fibrogenesis that occurs due to chronic liver injury. Liver cirrhosis is an 11th cause and leading to increased liver-related mortality worldwide [1]. Approximately 2 million deaths have been reported in the world due to the occurrence of liver diseases. Liver cirrhosis accounts for 1 million deaths per year. The mortality rate of 18.3% in 2 million people suffering from chronic liver disease in India [1-3]. Multiple mechanisms cause injury to the liver further triggering fibrogenesis, necroinflammation, and diffuse regeneration of nodules. The cirrhotic patients exhibit systemic inflammation resulting in elevated production of pro-inflammatory cytokines. The imbalanced immune fibrosis and regeneration of hepatic tissue leading to liver cirrhosis with disrupted liver architecture [4]. Several cytokines have been involved in hepatitis viral infection development that gives rise to chronic liver disease. Chronic liver disease is an advancement in worsening of liver function such as production of clotting factors, detoxification, excretion of bile, and release of other proteins. Alcoholic liver disease, non-alcoholic liver disease (NAFLD), chronic viral hepatitis, genetic causes and autoimmune cases are the most important etiological factors considered in manifestation of liver cirrhosis. Fibrosis is an irreversible process but it could be reversible in the starting phase of development of disease.

The chronic liver injury developing into the fibrotic stage mostly affected by environmental and/or genetic factors [5-6].

Currently liver biopsies, serological investigations and imaging examinations are used to determine clinically examined hepatic lesions. Although most of these serological investigations developed and validated in adult cirrhotic patients, various research groups have been investigated in populations suffering from liver diseases with multiple etiologies. Liver biopsy is the gold standard method to determine the stages of liver fibrosis/cirrhosis [7-8]. However, the risk of complications, increased cost, and invasiveness of this procedure makes it unsuitable for screening and monitoring progression of liver cirrhosis. Therefore, there is an urgent need for non-invasive, accurate, safe and cost-effective marker for staging fibrosis and to detect progression of liver cirrhosis. The liver function tests include alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) considered as traditional biomarkers [9-10]. Aim of the study is to evaluate association between IL-6 and ammonia in prognosis and diagnosis of liver cirrhotic patients. These biochemical findings, the levels of IL-6 in serum as a marker of cause of systemic inflammation in liver cirrhotic patients with higher risk of developing overt hepatic encephalopathy (HE) and leading to increased mortality.

2. Materials and methods

2.1. Study design and patient selection

This was an age and gender matched case-control study. The study was conducted in the department of General Medicine at Aarupadai Veedu Medical College and Hospital, Puducherry from January 2020 to December 2021. The participants with age more than 18 years were enrolled for the study. A total of 88 cirrhotic patients were recruited in the study. The patients who were attending in-patient care of the department of general medicine were enrolled for the study. Patients were recruited based on the inclusion criteria such as clinical symptoms viz. ascites, splenomegaly, encephalopathy, jaundice, and altered biochemical and diagnostic parameters (ALT, AST, IL-6 and ammonia levels). The cases were identified as those who are having one or more than the following evidence suggestive of hepatic cirrhosis with irrespective etiology i.e., lower levels of albumin (<3.5 mg/dl), higher levels of AST and ALT (>55U/L) along with any clinical evidence of hypertension (Abdominal bleeding, Ascites with Spontaneous bacterial peritonitis and hepatic encephalopathy). Patients having HBV, HCV, NAFLD, biliary cirrhosis, kidney related diseases, autoimmune hepatitis and cardiac cirrhosis were recruited. The individuals were showing platelet count of 70x103/µL, measured by a multiplate analyzer also included in the study. the exclusion criteria was strictly followed based on patient's physiological conditions such as, pregnancy, individuals with higher age and complications of diabetes, acute and chronic renal failure, myocardial infarction, cancer, pneumonia and individuals undergoing thrombolytic therapy. Voluntary participants of age and gender matched healthy controls were enrolled for the study to compare with cases. Prior to start the study, approval from the institutional ethical committee (IEC) was obtained. Written informed consent was obtained from all the study participants. All study participants were interviewed for the demographic details such as age, gender, socioeconomic status etc. and were collected by filling up a case record form (proforma).

Laboratory analysis

The study was conducted based on scoring and/or grading helps in assessing the disease severity in liver cirrhotic patients. MELD and child Pugh scoring were applied for all the patients to categorize them based on disease severity. The development of liver cirrhosis associated with etiological conditions such as hepatic encephalopathy, ascites and/or esophageal vertices. 3ml of venous blood was collected from all the study participants in both plane tubes. Collected samples meant for processing primarily samples were centrifuged to 3000 rpm for 10min in 4^0C. Separated serum was used for the assessment of biological parameters. Remaining aliquoted serum samples were stored in -80^0C for future use. Estimation of albumin, total protein, alanine-aminotransferase (ALT), ammonia and aspartate aminotransferase (AST) were done by using an automated biochemical analyzer. The baseline data of characteristics and clinical data was noted for all enrolled patients. Serum levels of IL-6 were estimated by chemiluminescence immunoassay.

Statistical analysis

We entered data electronically by Microsoft excel and data was analyzed by SPSS software (Version 28, IBM corporation, NY, USA). Descriptive data was represented as mean±standard deviation (SD), and continuous variables by student t-test. IL-6 and ammonia were identified and their discriminative ability of cirrhotic patients was analyzed by using area under the curve of receiver operating characteristic (AUROC). Odds ratio and their 95% of confidence intervals were calculated. The p-value ≤0.05 was considered to be statistically significant.

3. Results and Discussions

A total of 176 study participants were recruited which includes 88 cirrhotic patients and 88 healthy controls. The mean age of the study subjects was 47.1±6.7 years in cirrhotic group and similar age matched controls. Out of which 80 (90.9%) males and 8 (9.09%) females both in patients with liver cirrhosis as well as in controls. The medical history of patients with cirrhosis regarding various etiological factors were considered in this study. The biochemical tests of liver enzymes in cirrhotic patients were elevated compared to controls. Albumin levels significantly differ in both the groups. Whereas no difference has been observed in WBC or sodium. The biochemical parameters such as blood urea (54.90±10.573), serum potassium (3.89±0.734), total bilirubin (3.30±3.534), total protein (5.99±0.355), albumin (2.61±0.490), ALP (70.07±40.606), and gamma GT (45.41±34.938) were significantly differ in patients with liver cirrhosis. The oxidative stress parameters included total antioxidant capacity (1.05±0.209), and total oxidant status (14.80±3.288). The total leukocyte and platelet count was 6.51±3.948 and 1.66±0.477 respectively. Detailed

comparison of clinical parameters were displayed in the result part (Table 1). Viral infections such as hepatitis B and hepatitis C are more common in cirrhotic patients. The results indicating that 5 (5.9%) were hepatitis B, 7 (7.9%) were hepatitis B with alcohol, 7 (7.9%) were hepatitis C, and 11 (12.5%) were hepatitis C with alcohol. Among the patients 35 (39.7%) were alcoholic, 14 (15.9%) were non-alcoholic (fig.1). Most of the patients were classified under MELD (29.08±1.599) and Child-Pugh score. The patients showing increased MELD score reflecting the past ascites. The patients suffering from severe ascites and unknown etiology were 49 (55.7%) and 9 (10.2%) in numbers respectively. As per the Child-Pugh scoring described severity of about 38 patients were fit into the stage C which is the end stage of liver disease. The correlation between serum levels of IL-6 and ammonia were significantly vary ($r^2 = 0.012$, $P<0.05$) in patients with hepatic cirrhosis (Fig.2).

ROC analysis was carried out for defining sensitivity and specificity of IL-6, uric acid, ammonia, AST and ALT in cirrhotic patients. The serum IL-6 levels were increasing in cirrhotic patients. Serum IL-6 levels do not significantly vary in patients among the etiological factors. Whereas serum IL-6 levels were significantly increased in cirrhotic patients compared to controls. IL-6 showed an AUC of 0.982 (95% CI: 0.966-0.996). Uric acid showed an AUC of 0.976 (95% CI: 0.959-0.993). Ammonia during the progression of cirrhosis showed an AUC of 0.980 (95% CI: 0.965-0.995). Liver specific markers such as AST and ALT showed an AUC of 0.965 (95% CI: 0.938-0.993) and 0.986 (95% CI: 0.970-1.0) respectively (Table 2 & Fig.3). The odds ratio was calculated to find the association of IL-6 and ammonia as a risk of developing liver cirrhosis 3.8 and 9.9 times ($p<0.001$) respectively which is more likely to be under go end stage of liver disease (Table 3).

The prevalence of cirrhosis ranging from 4.5% to 9.5% based on autopsy reports in multiple population studies worldwide. Cirrhosis is associated with substantial mortality and 10th most leading cause of death among male population. Among the liver cancer patients over 51% were showing mortality worldwide. Hepatic diseases are firmly identified as a priority of public health. The current total of 2 million prevalence of chronic liver disease have been reported in India. Out of 2 million, 18.3% alone contributed to mortality [11-12]. Estimation of cirrhosis varies depending upon the etiological factors identified. Liver diseases including viral hepatitis, alcoholic hepatic disease and nonalcoholic fatty hepatic diseases. In India these mentioned diseases are very common compared to the rest of the world [12-13]. Cirrhosis affects individual lives beyond its medical concerns and it worsens economic status of patients. Therefore it requires attention to vigorously monitor alterations in liver function. Early diagnosis is very important to delay hepatic disease progression and to reduce the frequency of liver cancer [14]. The main risk factors and the major contributors with higher probability to cause liver cirrhosis such as hepatitis B and C, NAFLD and alcoholic hepatic disease. The world is facing the challenges in management of hepatic diseases due to limited resources, healthcare facilities and hepatologists along with higher prevalence of poverty and malnutrition. In India the clinical management of advanced liver disease is more challenging due to confounding factors such as illiteracy, lack of rural healthcare facilities, and reliance in traditional medicine increased prevalence of causal malnutrition [13]. During progression of liver cirrhosis, immune response and other pathogenic factors involved in activation of hepatic stellate cells leading to increased collagen synthesis and reduced degradation of collagen. Moreover liver fibrosis leading to formation of pseudolobules is one of the features of hepatic cirrhosis. Based on etiological characteristics hepatic cirrhosis is categorized into viral, autoimmune, alcoholic, congestive and non-alcoholic liver cirrhosis [15]. In some of the patients, progression of liver cirrhosis cannot be defined by the known etiologies, which is termed as occult hepatic cirrhosis. In developing countries viral hepatitis and alcoholic hepatic diseases are the most serious forms and cause liver cirrhosis [16]. Laboratory examinations may indicate liver panel deterioration (alkaline phosphatase, total bilirubin and serum aminotransferases) and prothrombin time. The biochemical tests for the liver are known as liver function tests. These are the most important clinical tests used to determine occurrence of liver damage and to estimate liver disease progression. Biochemical parameters shown in this study are AST, ALT, uric acid, IL-6, ALP, gamma GT and ammonia were significantly increased compared to controls. These parameters are associated with stages of damage in hepatocytes and epithelial cells of bile ducts further resulting in impaired physiological function of liver and apoptosis.

The invasive liver biopsy is a gold standard method to classify stages of liver fibrosis. Concentration of collagen, glycoproteins, and proteoglycans are significantly increased during hepatic fibrosis. Serum AST, ALT, ALP, IL-6 and uric acid have been used as serological markers in staging liver fibrosis with the help of scoring methods such as MELD and Child-Pugh score [17-18]. In a current study, we analyzed the relationship between general characteristics, with the biochemical examinations of serological markers, and with other various etiological factors. The serological liver function assays were carried out, results were shown that the markers with varying degrees based on different etiologies during liver cirrhosis. Higher transaminase was observed in the patients with viral hepatic liver disease. It may indicate development of hepatic cirrhosis. At present, various non-invasive serological biomarkers are available in assessing liver fibrosis [18]. The AST/platelet ratio index score consists of AST levels and platelet count would be used in evaluating liver fibrosis. The FIB-4 index includes age, AST, ALT and platelet count used to indicate severity of fibrosis and cirrhosis in patients. Assessment of disease severity by using a combination of multiple markers for hepatic diseases should be analyzed to improve the specificity [18-19]. The variation in concentration of albumin was observed, which can cause edema leading to disease severity. On the basis of study results, the clinical factors are considered via medical history and serological investigations of cirrhotic patients. Moreover, the patients with advanced liver disease (NAFLD, past alcohol use and hemochromatosis) showed normal liver panels.

Table 1: Clinical profile of cirrhotic patients

Parameters	Hepatic cirrhosis (n=88)
Age (Years)	47.17±6.716
MELD Score (Points)	29.08±1.599
Blood urea (mg/dL)	54.90±10.573
Serum Potassium (meq/l)	3.89±0.734
Total bilirubin (mg/dL)	3.30±3.534
Total protein (g/dL)	5.99±0.355
Albumin (g/dL)	2.61±0.490
AST (U/L)†	79.83±43.707
ALT (U/L)†	57.48±21.256
ALP (U/L) †	70.07±40.606
GGT (U/L)†	45.41±34.938
Total Antioxidant Capacity (nmol/µL)	1.05±0.209
Total Oxidant Status (µmol H_2O_2 Equiv/L)	14.80±3.288
Total leukocyte count (10^3 / Litre)	6.51±3.948
Platelet count (10^4/Litre)	1.66±0.477
PT (Sec)	20.25±4.562
IL-6 (pg/ml)†	37.69±11.44
Uric acid (mg/dL)†	8.13±1.905
Ammonia (µmol/L)†	51.23±18.282
Child-Pugh Score/Grade	
Grade-A	25 (28.4%)
Grade-B	25 (28.4%)
Grade-C	38 (43.2%)
Ascites	
Mild	22 (25%)
Moderate	17 (19.3%)
Tense	49 (55.7%)
Etiological risk factors	
Alcoholic liver disease	35 (39.8%)
Hepatitis-B	5 (5.7%)
Hepatitis-B & Alcoholism	7 (7.9%)
Hepatitis-C	7 (7.9%)
Hepatitis-C & Alcoholism	11 (12.5%)
Non-alcoholic fatty liver disease	14 (15.9%)
Unknown etiology	9 (10.3%)

Abbreviations: MELD model for end stage of liver disease, AST aspartate aminotransferase, ALT alanine aminotransferase, ALP alkaline phosphate, GGT gamma-glutamyl transferase, PT prothrombin time, IL interleukin. †P≤0.005 Significant.

Table 2: ROC analysis for screening ability of hepatic cirrhosis

Significant parameters	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Cut off
IL-6	0.982 (0.966-0.99)	.966	0.996	13.5
Uric acid	0.976 (0.95-0.99)	.959	.993	4.50
Ammonia	0.980 (0.96-0.99)	.955	.995	13.0
AST	0.965 (0.93-99)	.938	.993	22.5
ALT	0.986 (0.96-0.99)	.970	1.000	28.5

Abbreviations: AUC area under the curve, CI confidence interval, IL interleukin, AST aspartate aminotransferase, ALT alanine aminotransferase

Table 3: Odds ratio for Interlukine-6 and ammonia

Parameters	Subjects with Cirrhosis	Subjects without cirrhosis	Odds ratio	95% Confidence interval		Z Statistic	p -Value
				Lower	Upper		
Interlukine-6	68	20	3.8976	2.0323	7.4746	4.095	$p < 0.0001^{\dagger}$
Normal interlukine-6	41	47					
Hyperammonemia	73	15	9.9011	4.8603	20.1702	6.315	$p < 0.0001^{\dagger}$
Normal Ammonia	29	59					

$^{\dagger}p<0.0001$ Significant

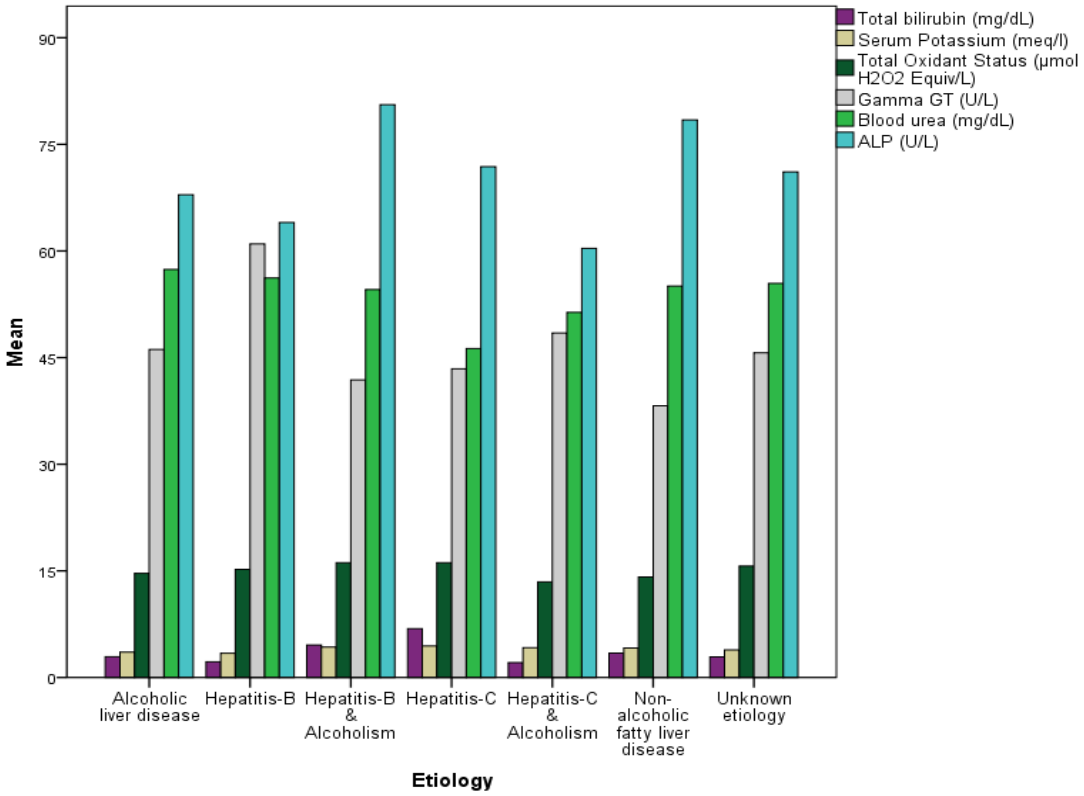

Figure 1: Etiological characteristics of patients with liver cirrhosis based on clinical manifestations

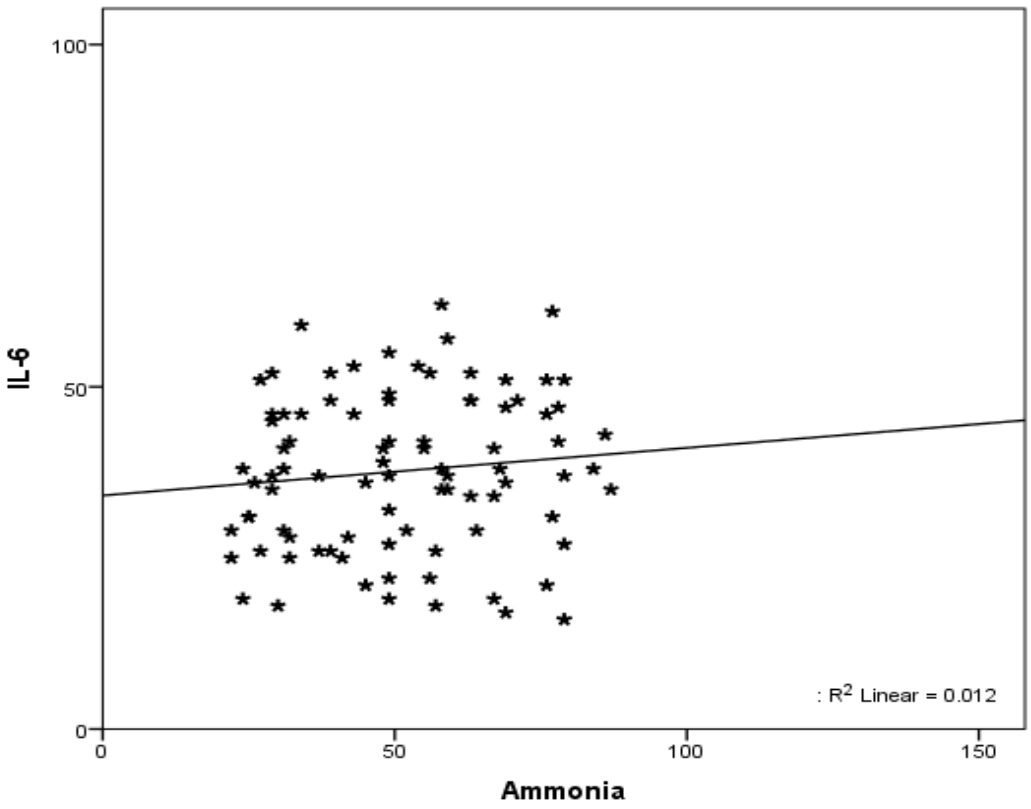

Figure 2: Pearson correlation between interleukin-6 and ammonia in patients with hepatic cirrhosis

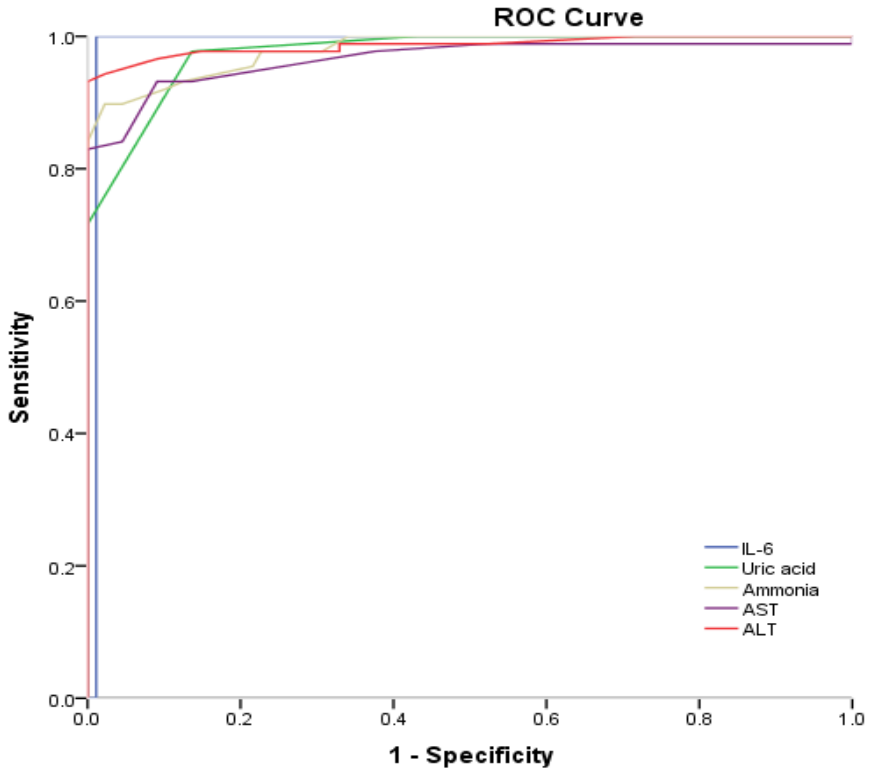

Figure 3: Comparison of area under the curve for interleukin-6 (0.982), uric acid (0.976), ammonia (0.980), and AST (0.965) with ALT (0.986)

Thrombocytopenia is commonly observed in cirrhotic patients. In case of chronic alcohol use related patients showed reduced production of thrombopoietin and higher platelet destruction was observed in patients with hepatitis C virus [20]. The levels of ammonia were widely used and playing an important role in pathogenesis of liver cirrhosis progression to hepatic encephalopathy [21]. The present study indicates that the ammonia levels were correlated with severity of the liver cirrhosis. Alzheimer type II astrocytes appear to be the main targets of ammonia toxicity in the brain. Hyperammonia resulting in cerebral edema/sepsis. There was no doubt that the levels of ammonia were significantly increased in patients with cirrhosis after the pandemic due to increased cytokine storms [22]. Accumulation of ammonia as a toxin in the brain leading to development of HE. HE is a common neurologic impairment in cirrhotic patients [23]. The criteria of west haven grading is subjected to assess cognitive status of cirrhotic patients. If signs of cirrhosis complications such as hepatic encephalopathy are able to be identified by the primary care workers, it reduces delays in patient care [24]. IL-6 is a pleitropic cytokine produced during inflammatory response. The elevated levels of IL-6 was observed in patients with cirrhosis. IL-6 levels with Child-Pugh scoring were associated with severity of liver cirrhosis. A study reported that IL-6, an inflammatory cytokine was associated with Child-Pugh score. The elevated levels of IL-6 followed by regulation of STAT3 cascades promotes activation of hepatic stellate cells (HSCs). Further develops oxidative stress leading to tissue damage to disease progression. Association of levels of inflammatory cytokines with other etiologies of cirrhosis examined [25]. Prystupa et al. study also found that the levels of IL-6 are correlated with severity of liver cirrhosis along with Child-Pugh scoring [26]. The hepatitis infections along with inflammation cause increased risk of developing cirrhosis. Multiple studies reported that the proportionate levels of IL-6 with viral hepatic infections [27]. In the present study we found that the association of serum IL-6 levels with cirrhotic patients may prone to develop HE. The study reported that the levels of both IL-6 and IL-10 were prominent in the acute phase of infection [25-28]. Our study also reported the higher association of IL-6 and development of liver cirrhosis. The elevated levels of IL-6 was observed in cirrhotic patients may further developing into HE. The patients with HE are showing infections, GI bleed and alkalosis, which may further worsen the disease. The pathophysiology of development of HE is poorly understood. The hyperammonia is negatively affected by systemic inflammation. IL-6 is elevated in multiple pathological conditions. Whereas IL-6 and ammonia levels exerts an effect on the central nervous system (CNS) in HE. IL-6 interrupts blood-brain barrier via increasing CNS-derived endothelial cell permeability further leading to higher ammonia influx into the CNS [29]. Study intended to estimate IL-6 and ammonia levels in cirrhotic patients significant similarly study reported that IL-6 and hyperammonia levels were contributing to hepatic encephalopathy. Due to reduced clearance of ammonia encourages IL-6 to penetrate through the blood brain barrier further inducing edema leading to development of hepatic encephalopathy. Furthermore ROC suggested that IL-6 and ammonia may be good indicators for progression of hepatic cirrhosis. The present study has some potential limitations. First, data was collected from tertiary care centers from inpatients. Recruited patients belonged to advanced liver cirrhosis conditions. Outpatients with compensated cirrhosis likely to be excluded from the study may lead to bias regarding severity in liver cirrhotic patients. Second, the present study discussed the pathogenesity of IL-6 along with previously reported studies. But other pro-inflammatory mediators such as IL-1β and α-interferon play a major role in pathogenesis in progression of liver cirrhosis to hepatic encephalopathy. Third, with a smaller sample size, all cirrhotic patients were enrolled in a single tertiary care center. In future, evaluation of IL-6 and ammonia levels required larger samples from various health care centers.

4. Conclusions

For many years the cirrhotic patients showed asymptomatic due to delayed diagnosis. Our findings suggested elevated levels of liver enzymes such as ALT, AST, ALP, GGT, inflammatory cytokine like IL-6 and ammonia in patients with cirrhosis compared to healthy controls. Resulting in indication of progression of liver cirrhosis to development of hepatic encephalopathy. IL-6 and ammonia may be considered as a diagnostic biomarker to estimate severity of liver cirrhosis. However this study was a tertiary care center study and the clinical findings need to be evaluated in larger scale studies for further validation. Studies related to combination of markers as non-invasive methods indicate the severity of the disease.

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Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

The study was approved by the human ethical committee at the Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), and all procedures were following the declaration of Helsinki.

References

[1] P. Ginès, A. Krag, J.G. Abraldes, E. Solà, N. Fabrellas, P.S. Kamath. (2021). Liver cirrhosis. Lancet. 398(10308): 1359-1376.

[2] D. Mishra, K.R. Dash, C. Khatua, S. Panigrahi, P.K. Parida, S.K. Behera, R.K. Barik, S. Pradhan, S.K. Sahu, B. Thakur, S.P. Singh. (2020). A Study on the Temporal Trends in the Etiology of Cirrhosis of Liver in Coastal Eastern Odisha. Euroasian journal of hepato-gastroenterology. 10(1): 1-6.

[3] D. Mondal, K. Das, A. Chowdhury. (2022). Epidemiology of Liver Diseases in India. Clinical liver disease. 19(3): 114-117.

[4] D. Dhar, J. Baglieri, T. Kisseleva, D.A. Brenner. (2020). Mechanisms of liver fibrosis and its role in liver cancer. Experimental biology and medicine (Maywood, N.J.). 245(2): 96-108.

[5] D. Schuppan, N.H. Afdhal. (2008). Liver cirrhosis. Lancet. 371(9615): 838-851.

[6] V. Arroyo, R. Moreau, P.S. Kamath, R. Jalan, P. Ginès, F. Nevens, J. Fernández, U. To, G. García-Tsao, B. Schnabl. (2016). Acute-on-chronic liver failure in cirrhosis. Nature Reviews Disease Primers. 2: 16041.

[7] P. Thampanitchawong, T. Piratvisuth. (1999). Liver biopsy:complications and risk factors. World Journal of Gastroenterology. 5(4): 301-304.

[8] N.H. Afdhal, D. Nunes. (2004). Evaluation of liver fibrosis: a concise review. The American Journal of Gastroenterology. 99(6): 1160-74.

[9] G. Gheorghe, S. Bungău, G. Ceobanu, M. Ilie, N. Bacalbașa, O.G. Bratu, C.M. Vesa, M.A. Găman, C.C. Diaconu. (2021). The non-invasive assessment of hepatic fibrosis. Journal of the Formosan Medical Association. 120(2): 794-803.

[10] K. Patel. (2010). Noninvasive tools to assess liver disease. Current Opinion in Gastroenterology. 26(3): 227-33.

[11] D. Mondal, K. Das, A. Chowdhury. (2022). Epidemiology of Liver Diseases in India. Clinical liver disease. 19(3): 114-117.

[12] P.S. Mukherjee, S. Vishnubhatla, D.N. Amarapurkar, K. Das, A. Sood, Y.K. Chawla, C.E. Eapen, P. Boddu, V. Thomas, S. Varshney, D.S. Hidangmayum, P. Bhaumik, B. Thakur, S.K. Acharya, A. Chowdhury. (2017). Etiology and mode of presentation of chronic liver diseases in India: A multi centric study. PLoS One. 12(10): e0187033.

[13] P.J. Thuluvath, A. Saraya, M. Rela. (2021). An Introduction to Liver Disease in India. Clinical liver disease. 18(3): 105-107.

[14] S.K. Asrani, H. Devarbhavi, J. Eaton, P.S. Kamath. (2019). Burden of liver diseases in the world. Journal of hepatology. 70(1): 151-171.

[15] A.J. Sanyal, M.L. Van Natta, J. Clark, B.A. Neuschwander-Tetri, A. Diehl, S. Dasarathy, R. Loomba, N. Chalasani, K. Kowdley, B. Hameed, L.A. Wilson, K.P. Yates, P. Belt, M. Lazo, D.E. Kleiner, C. Behling, J. Tonascia. (2021). Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease. The New England journal of medicine. 385(17): 1559-1569.

[16] J. Wiegand, T. Berg. (2013). The etiology, diagnosis and prevention of liver cirrhosis: part 1 of a series on liver cirrhosis. Deutsches Arzteblatt international. 110(6): 85-91.

[17] R. Bataller, D.A. Brenner. (2005). Liver fibrosis. The Journal of clinical investigation. 115(2): 209-218.

[18] K.S. Nallagangula, S.K. Nagaraj, L. Venkataswamy, M. Chandrappa. (2018). Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression. Future science OA. 4(1): Fso250.

[19] Q.M. Anstee, L. Castera, R. Loomba. (2022). Impact of non-invasive biomarkers on hepatology practice: Past, present and future. Journal of hepatology. 76(6): 1362-1378.

[20] C. Torruellas, S.W. French, V. Medici. (2014). Diagnosis of alcoholic liver disease. World Journal of Gastroenterology. 20(33): 11684-11699.

[21] C. Hu, K. Huang, L. Zhao, F. Zhang, Z. Wu, L. Li. (2020). Serum ammonia is a strong prognostic factor for patients with acute-on-chronic liver failure. Scientific Reports. 10(1): 16970.

[22] D. Ragab, H. Salah Eldin, M. Taeimah, R. Khattab, R. Salem. (2020). The COVID-19 Cytokine Storm; What We Know So Far. Frontiers in Immunology. 11.

[23] S. Chiriac, C. Stanciu, C. Cojocariu, A.M. Singeap, C. Sfarti, T. Cuciureanu, I. Girleanu, R.A. Igna, A. Trifan. (2021). Role of ammonia in predicting the outcome of patients with acute-on-chronic liver failure. World journal of clinical cases. 9(3): 552-564.

[24] N. Verma, R.K. Dhiman, A. Choudhury, S. Taneja, A. Duseja, V. Singh, M. Al Mahtab, H. Devarbhavi, A. Shukla, Q. Ning, S.S. Hamid, A.S. Butt, W. Jafri, S.S. Tan, J. Hu, D. Zhongping, S. Treeprasertsuk, G.H. Lee, H. Ghazinyan, L.A. Lesmana, A. Sood, V. Midha, O. Goyal, D.J. Kim, C.E. Eapen, A. Goel, H. Tao, X. Shaojie, N. Yuemin, A.K. Dokmeci, M. Sahu, A. Singh, A. Arora, A. Kumar, R. Kumar, V.G.M. Prasad, A. Shresta, J. Sollano, D.A. Payawal, S. Shah, P.N. Rao, A. Kulkarni, G.K. Lau, S.K. Sarin. (2021). Dynamic assessments of hepatic encephalopathy and ammonia levels predict mortality in acute-on-chronic liver failure. Hepatology International. 15(4): 970-982.

[25] I. Rey, R. Effendi-Ys. (2021). Association Between Serum IL-6, IL-10, IL-12, and IL-23 Levels and Severity of Liver Cirrhosis. Medical archives (Sarajevo, Bosnia and Herzegovina). 75(3): 199-203.

[26] A. Prystupa, P. Kiciński, J. Sak, A. Boguszewska-Czubara, A. Toruń-Jurkowska, W. Załuska. (2015). Proinflammatory Cytokines (IL-1α, IL-6) and Hepatocyte Growth Factor in Patients with Alcoholic Liver Cirrhosis. Gastroenterology research and practice. 2015: 532615-532615.

[27] T. Lan, L. Chang, L. Wu, Y.-F. Yuan. (2015). IL-6 Plays a Crucial Role in HBV Infection. Journal of clinical and translational hepatology. 3(4): 271-276.

[28] C. Labenz, G. Toenges, Y. Huber, M. Nagel, J.U. Marquardt, J.M. Schattenberg, P.R. Galle, J. Labenz, M.A. Wörns. (2019). Raised serum Interleukin-6 identifies patients with liver cirrhosis at high risk for overt hepatic encephalopathy. Alimentary pharmacology & therapeutics. 50(10): 1112-1119.

[29] D.R. Aldridge, E.J. Tranah, D.L. Shawcross. (2015). Pathogenesis of hepatic encephalopathy: role of ammonia and systemic inflammation. Journal of clinical and experimental hepatology. 5(Suppl 1): S7-S20.