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Serum Endotoxin Level in Cirrhotic Patients with Hepatic Encephalopathy

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Abstract

Background: In cirrhotic liver increased secretion of endotoxin by extrahepatic macrophages may play an important role in the progression of Hepatic encephalopathy (HE). The aim of the present study was to evaluate serum endotoxin in cirrhotic patients with HE and to investigate relation between serum endotoxin level and severity of liver disease and grades of HE. Patient and methods : This study included Four groups: Group I was 25 apparent healthy age and sex matched participants as a control group. Group II was 25 cirrhotic patients without HE. Group III was 25 cirrhotic patients with minimal HE and Group IV was 25 patients with overt HE. Serum endotoxin was measured in the 4 groups. Results: there was a high statistically significant positive correlation between endotoxin level with WBCs, PT, INR, PTT, RBS, ALT, AST, total, direct bilirubin, urea, creatinine, and HE grades, while there was statistically significant negative correlation with Hb, platelets, serum K, Na level. Total proteins, serum albumin, child pugh score and GCS. Endotoxin level can detect 100% of HE patients and excluding 97.3% of cirrhotic patients with no encephalopathy, with 98% accuracy. Conclusion: Serum endotoxins is elevated in patients with HE. Serum endotoxin correlate well with grades of HE.

Keywords: HE, endotoxinaemia, cirrhotic liver

1. Introduction

HE has been considered a continuous spectrum that involves several domains as cognition, emotion, behavior, and biological rhythm [1]. Minimal HE (MHE) represents the mildest form of it in which no clinically overt symptoms of HE is seen, but patients have abnormal neuropsychological and/or neurophysiologic findings indicative of cerebral dysfunction. [2].

The prevalence of this entity varies depending on the Child Pugh score of the population and the diagnostic tests used, but varies from 30% to 84% in various studies. Though MHE has no clinically overt symptoms, it is still clinically relevant because it impairs patients daily functioning and health related quality of life (HRQOL), predicts development of overt HE, and is associated with poor prognosis. Recent observations suggest that inflammatory response may be important in the pathogenesis of HE [3].

In normal circumstances, only small amounts of endotoxin was crossed from the intestinal lumen into the systemic circulation and the absorbed endotoxin was rapidly removed by monocytes, particularly resident Kupffer cells within the liver. However, emerging evidence indicates that chronic, low level elevation of endotoxin levels may play a role in insulin resistant states. Elevated endotoxin levels have been noted as an aggravating factor in alcoholic liver disease, whilst other studies observed that a high-fat meal induces post-prandial low grade endotoxinaemia [5].

The presence and severity of HE was independent of severity of liver disease and ammonia concentration. but were associated with higher levels of markers of inflammation, hyperammonemia, endotoxin, systemic inflammation and central neuroinflammation play synergistic role in pathophysiology of HE [5].

HE is a complex neuropsychiatric syndrome characterized by neuronal inhibition and damage probably by chronic accumulation of endotoxins.

The production of tumor necrosis factor-a is of the earliest events in hepatocyte injury, which trigger cytokine production, damage hepatocytes and Kupffer cells release transforming growth factor b which initiate fibrogenesis as the healing response [6].

Studies have shown that higher plasma levels of endotoxin significantly associated with more blunted motor activities in Sprague-Dawley rats with fulminant hepatic failure induced by intraperitoneal thioacetamide. [7]. There is paucity of data on serum inflammatory markers and serum endotoxin in different grades of HE.

In cirrhotic liver, together with increased secretion of endotoxin by extrahepatic macrophages may play an important role in the progression of hepatic and renal disturbances [8, 9].

The aim of the present study was to evaluate serum endotoxin in cirrhotic patients with HE and to investigate relation between serum endotoxin Level with severity of liver diseases and grades of HE.

2. Patient and Method

A Case control study was conducted on 100 patients in four groups

- **Group I** :apparent healthy age and sex matched participants as a control group
- **Group II** : cirrhotic patients without HE
- **Group III:** cirrhotic patients with minimal HE.
- **Group IV :**.patient with overt HE

2.1. Inclusion criteria

- Both sexes were involved in the study.
- Patients of cirrhosis (age 18–70 years) with HE was included in the study.

- Diagnosis of MHE was based on psychometric HE score (PHES).
- PHES includes digit symbol test, number connection test-A, number connection test-B, serial dotting test, and line drawing test. MHE was diagnosed with the sum of all scores ≤ -4 points.

2.2. Exclusion criteria

The following exclusion criteria were used for all subjects

- Patients with history of gastrointestinal bleeding in the last 6 weeks,
- Active ongoing infection, serum creatinine> 1.5 mg/dL,
- Electrolyte impairment (sodium < 130 meq/L, potassium < 3.5 meq/L or > 5.0 meq/L), use of psychotropic drugs in the last 6 weeks,
- Recent alcohol use (< 6 week),
- Trans jugular intrahepatic Porto systemic shunt or shunt surgery,
- Hepatocellular carcinoma,
- Severe comorbidity such as congestive heart failure, pulmonary disease, neurological and psychiatric problems impairing quality of life, and poor vision precluding neuropsychological assessment was excluded.

Diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic, and liver histologic data whenever available. All patients of cirrhosis was screened for exclusion criteria and the presence of minimal HE (MHE). Child Pugh score was used for assessing the severity of chronic liver diseases. Diagnosis and grading of HE was done according to the West Haven criteria.

Serum electrolytes, renal function tests, blood sugar, viral markers (HBsAg, Anti-HCV antibodies), serum endotoxin ELISA Kit standard curve 0.5EU/L - 200 EU/L and serum endotoxin were measured by ELISA

3. Results

The four groups were matched as regard age and sex, the difference was statistically not significant. While there was a high statistically significant difference among all studied groups regarding weight, HCC, DM and hypertension.

Our result shows a high statistically significant difference among all studied groups regarding laboratory data.

both groups III and IV were close to each other with no significant difference in all laboratory data except regarding pt and serum Na level, and both group I and II were the same with no significant difference regarding WBCs, ptt, serum K and Na level and RBS, while there was a significant difference in between all other groups.

Our result shows a high statistically significant difference among all studied groups regarding liver and kidney function tests.

both groups III and IV were close to each other with no significant difference in liver and kidney function tests except regarding total, direct bilirubin, creatinine and urea, while there was a significant difference in between all other groups.

Our result shows a high statistical significant difference among all studied groups regarding endotoxin and hepatitis C viral Ab.

Our result shows a high statistical significant difference among all studied groups regarding clinical finding, while both group III and IV were close to each other in clinical finding except as regard HE as all patients of group III had minimal encephalopathy versus 0% of group IV (as all patients of group IV suffered of grade I (28%), II (40%), III (20%) and grade IV encephalopathy (12%)).

Our result shows a high statistical significant difference among all studied groups regarding GCS and child pough score.

Regarding child score both groups III and IV were close to each other with no statistical significant difference between them, while regarding GCS both groups I and II were the same with no significant difference, but there was a high statistical significant difference in between all other groups there was a high statistically significant positive correlation between endotoxin level with WBCs, PT, INR, PTT, RBS, ALT, AST, total bilirubin, direct bilirubin, urea, creatinine, INR and HE grades, while there was statistically significant negative correlation with Hb, platelets, serum K, Na level. Total proteins, serum albumin, child pugh score and GCS. endotoxin level can detect 100% of HE patients and excluding 97.3% of cirrhotic patients with no encephalopathy, with 98% accuracy.

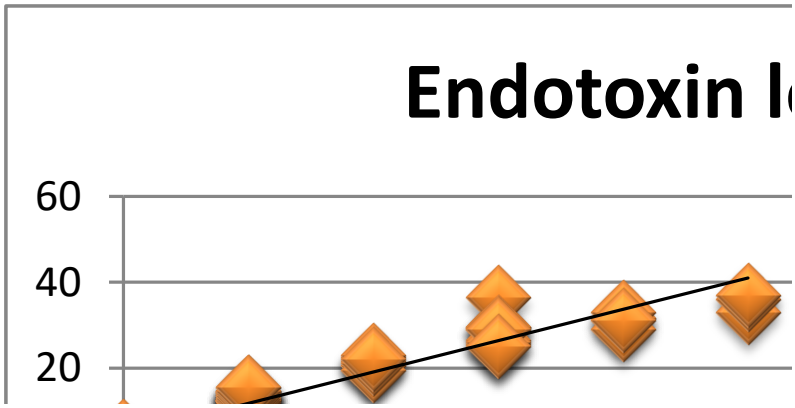

Fig. (1) Positive correlation between grades of HE and endotoxin level.

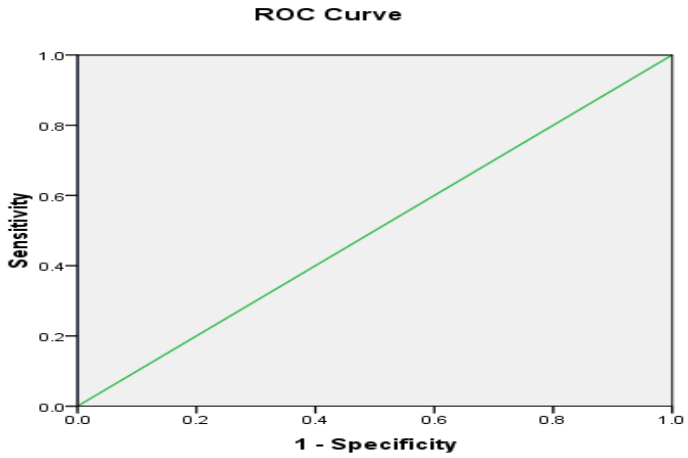

Fig. (2) Receiver operating characteristics (ROC) curve for endotoxin as a predictor of HE.

3. Discussion

In normal conditions, intestinal bacterial antigens, including lipopolysaccharides (LPS) derived from gut flora, are absorbed into the blood and removed by the reticuloendothilial cells of the liver. In liver disease, the pro-inflammatory cytokines are upregulated secondary to liver damage or endotoxemia [10].

Also, **Butterworth,** [11]**, and Wright G, Jalan,** [10] found that endotoxin, hyperammonemia, systemic inflammation and centralcneuroinflammation play synergistic role in pathophysiology of HE.

Coltart et al. [12] found that sterile inflammation by circulating endotoxin from the gut (bacterial translocation) inducing immune dysfunction may have some effect via the release of pro-inflammatory mediators which directly signal to the brain.

Liu et al. [13] have shown that alteration of gut flora by the administration of probiotics and fiber in patients with cirrhosis is associated with improvement in MHE severity, along with a reduction in venous endotoxin and ammonia levels at day 30, in comparison to the control group.

In the present study, etiology of liver cirrhosis was HBV infection ((5.3%) and HCV infection (98.6%).

In disagreement with our study, **Jain et al.** [2] found that the etiology of liver cirrhosis was alcohol (40%), hepatitis B virus infection (25%), hepatitis C virus infection (10%) and cryptogenic cirrhosis (25%). This difference can be explained by different causes of cirrhosis in different populations.

In the present study, there was a high statistically significant positive correlation between endotoxin level with HE grades, total bilirubin, direct bilirubin, urea, creatinine, PT, PTT and INR while there was statistically significant negative correlation with serum albumin, child pough score and GCS.

This came in agreement with **Jain et al.** [2] who found that there is a significant, positive correlation between serum endotoxin with grades of encephalopathy. This suggests that hyperammonemia and inflammation (elevated TNF-alpha, IL-6, IL-18 and serum endotoxin) play a role in the pathogenesis of HE in patients with cirrhosis.

The mechanism of HE remains unclarified, although hyperammonemia and systemic inflammation related to gut dysbiosis have been proposed as contributors.54 Accumulating evidence indicates that systemic inflammation, neuroinflammation and endotoxemia play cardinal roles in the pathogenesis of HE [11]. Endotoxin is

known to increase permeability of the blood-brain barrier and to act on the brain microglia through endothelial cell receptors with the succeeding production of nitric oxide (NO) and prostanoid, which may ultimately enhance the astrocyte swelling in HE [14]

Tarao et al. [15] reported that child pugh score is decreased with increased endotoxin level leading to death occurred within 6 months in 47.8% of the patients with a positive endotoxin test, whereas only 16.7% of those with a negative test died in the same period.

Trebicka et al. [16]**,** found that the severity of cirrhosis correlates with a higher level of serum endotoxin . In an impressive clinical study from Japan in 2005, a retrospective analysis was done on 105 patients with severe alcoholic liver disease. Plasma endotoxin levels increased as the severity increased and decreased as recovery occurred [17].

4. Conclusion

Serum endotoxins is elevated in patients with HE. Serum endotoxin correlate well with grades of HE.

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