Research Article

Relation between serum Endothelin-1 level and Child-Pugh score in patients with liver cirrhosis

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Abstract
Background and aim Liver cirrhosis is a common cause of death in the world. The accurate prognostication of liver cirrhosis is highly important in our practice. The most commonly used method to predict the prognosis of liver cirrhosis is Child-Pugh (CP) score. In this study, we aimed to assess relation between serum endothelin-1 level and Child-Pugh score in liver cirrhosis.
Patients and Methods This is a case-control study that included 60 cirrhotic patients. Patients were subjected to complete medical history & clinical examination & investigations including serum endothelin-1 & pelvi-abdominal ultrasound. Child-Pugh score was calculated for each patient The outcome results obtained for endothelin-1 were used to assess its relation with Child-Pugh score in liver cirrhosis. **Results** There was statistically significant relation between serum endothelin-1 levels and Child-Pugh score (P value < 0.001). Mean value ± standard deviation of serum endothelin-1 level in Child-Pugh classes A, B and C were 2.77 ± 0.22, 3.58 ± 0.32 and 4.44 ± 0.41 pg/ml respectively.
Conclusion Endothelin-1 level has significant relation with Child-Pugh score. Therefore, it has great value in prediction of prognosis of liver cirrhosis.
Keywords Cirrhosis - endothelin-1 – Child-Pugh score.

Abbreviations

ALT	Alanine transaminase	HRP	Horse radish peroxidase
ANOVA	One Way Analysis of Variance	INR	International normalized ratio
AST	Aspartate transaminase	IQR	Inter quartile range
CP	Child-Pugh	IRB	Institutional Review Board
EDTA	Ethylene Diamine Tetra Acetic acid	OD	Optical density
ELISA	Enzyme-linked immunosorbent assay	OLT	Orthotopic liver transplantation
ET	Endothelin	SD	Standard deviation
ETAR	Endothelin A receptor	SPSS	Statistical Package for Social Science
ETBR	Endothelin B receptor	TIPSS	Trans-jugular intra-hepatic porto- systemic shunt
HBV	Hepatitis B virus	TMB	Tetra-methyl-benzidine
HCV	Hepatitis C virus	WBCs	White blood cells

Introduction
Liver cirrhosis has a high morbidity and mortality worldwide. It causes 1.03 million deaths per year in the world[1] and 170,000 deaths per year in Europe[2]. Actual prevalence of liver cirrhosis may be under-estimated, because early phase of liver cirrhosis are often asymptomatic, and most of patients with liver cirrhosis are admitted due to its specific complications. The 1-year mortality of liver cirrhosis varies from 1% to 57% according to occurrence of its complications[3,4]. Liver cell failure affects multiple organ systems and leads to a shortened life expectancy[5]. Liver cirrhosis has different stages that include mild stable compensated cirrhosis, stable cirrhosis with prior decompensation, acutely decompensated cirrhosis and acute-on-chronic liver failure[6]. For a long time, many clinical and biochemical parameters have been suggested to accurately predict the prognosis of cirrhotic patients and correctly assess their short term survival. The number of patients on waiting lists for ortho-topic liver transplantation (OLT) is becoming

higher compared with the number of available donor livers. Correct timing of OLT can reduce the mortality of patients on waiting lists and improve post-transplant survival. Moreover, accurate prognosis of patients with cirrhosis is important so as to determine the plan for their management as well as the choice of major procedures[7,8].

Child-Pugh classification includes total bilirubin, albumin, international normalized ratio (INR) or prothrombin time, hepatic encephalopathy, and ascites[9]. The CP score has been shown to have significant prognostic effect on postoperative complications and mortality rate[10]. This influence was significant by both univariate and multivariate analyses[11]. Child-Pugh class A patients usually show good medium term survival without OLT unless other events occur[12,13] while Child-Pugh class C patients are considered the conventional candidates for the procedure[14]. Child-Pugh class B patients can be considered an intermediate group as they may remain stable for long time or rapidly deteriorate[15].

The endothelins (ETs) are a family of genes consisting of three 21-amino-acid proteins including peptides (ET-1, ET-2 and ET-3) [16]. ETs exert their effects by binding to two G-protein-coupled receptors, ETA receptor (ETAR), and ETB receptor (ETBR), thus triggering the signaling of cells. The effects of endothelins are mediated by many receptors[17]. Earlier studies focused on the effects of ETs on systemic vasoregulation. Recent data suggest that they may regulate blood flow in specific tissues. This is of great importance for the liver, where changes in blood flow may play an important role in many events like portal hypertension, ascites formation and hypoxic damage[18].

Patients & Methods

In this case-control study, 60 patients who attended to Aswan University Hospital in the period from September 2016 to June 2018 were enrolled in the study. The study was performed according to the ethical guidelines of the 1975 Declaration of Helsinki after approval from Institutional Review Board (IRB) for human subject research at Aswan University Hospital (Serial: aswu/84/9/16). A written informed consent was obtained from all enrolled participants before enrolment to the study. Patients with hepatocellular carcinoma or extra-hepatic malignancy were excluded from the study.

All enrolled patients were subjected to complete history taking, through physical examination especially manifestations of cirrhosis and portal hypertension and imaging study in the form of pelvi-abdominal ultrasonography with stress on**;** Size, surface and echopattern of the liver, portal vein diameter and its patency, size of the spleen and detection of porto-systemic collaterals, ascites and detection of its grade[19] by looking for the presence of fluid in five areas of the abdomen namely right upper quadrant (perihepatic and Morrison's pouch), left upper quadrant (perisplenic), right paracolic gutter, left paracolic gutter and pelvis:

- Fluid in 1 location = minimal ascites.
- Fluid in 2 locations = mild ascites.
- Fluid in 3 locations = moderate ascites.
- Fluid in 4 or 5 locations = marked ascites.

Child-Pugh score was calculated for each patient according to the variable parameters[10]:

	Points		
	1	2	3
Bilirubin (mg/dl)	< 2	2-3	> 3
Albumin (g/l)	> 3.5	2.7-3.5	< 2.7
Encephalopathy	None	Minimal	Advanced
Ascites	Absent	Mild	Moderate to marked
Prothrombin time (sec)	< 4	4-6	> 6
Child-Pugh class A :5-6 points. **Child-Pugh class B :7-9 points.** **Child-Pugh class C :10-15 points.**			

Accordingly, patients were categorized into three groups: group I is formed of 12 patients having Child-Pugh class A, group II is formed of 18 patients having Child-Pugh class B and group III is formed of 30 patients having Child-Pugh class C. From every studied person 10 ml of venous blood were drawn, under complete aseptic conditions. Each sample was divided into three portions: - 2 ml of blood into EDTA (Ethylene Diamine Tetra Acetic acid) vacutainer tubes used for complete blood count, done on sysmex XP-300 cell counter - 8 ml in plan tube which were lefted for clotting the centrifuged for serum collection to be used for routine and special parameter estimation. Routine parameter estimation (glucose, renal and liver function tests,…..) all parameters were done on fully automated chemistry analyzer BT-3500 (Italy). The other 2 tubes (2 ml for each) has been freezed at 20℃ for later use for estimation of serum Endothelin-1 (ET-1) level by ELISA Technique. Haemolyzed samples were excluded from the study.

Principle of the ET-1: serum Endothelin-1 (ET-1) by Sandwich ELISA technique; SinoGeneClon Biotech Co., LTD, (limited): Kits adopted on coated microtiter plate with Human ET-1, make solid phase antibody, then add ET-1 to wells (samples, controls and standards). Combine ET-1 antibody with labeled HRP (horse radish peroxidase) to form antibody-antigen-enzyme-antibody complex, after washing completely, add TMB (tetra-methyl-benzidine) substrate solution. TMB substrate becomes blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a stop solution and the color change is measured at a wavelength of 450 nm. The concentration of ET-1 in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve.

Statistical analysis:

The patients were grouped into three groups: group I is formed of 12 patients having Child-Pugh class A, group II is formed of 18 patients having Child-Pugh class B and group III is formed of 30 patients having Child-Pugh class C. Data were collected, coded, revised and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The data were presented as number and percentages for the qualitative data, mean, standard deviations and ranges for the quantitative data with parametric distribution and median with inter quartile range (IQR) for the quantitative data with non-parametric distribution. Chi-square test was used in the comparison between two groups with qualitative data and Fisher exact test was used instead of the Chi-square test when the expected count in any cell found less than 5. The comparison between more than two groups with quantitative data and parametric distribution was done by using One Way Analysis of Variance (ANOVA) test and Kruskall-Wallis test was used in the comparison between more than two groups with quantitative data and non-parametric distribution. Spearman correlation coefficients were used to assess the significant relation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the P-value was considered significant as the following: $P > 0.05$: Non significant, $P < 0.05$: Significant, $P < 0.01$: Highly significant.

The outcome results of endothelin-1 were subjected for statistical analysis in comparison with clinical data & investigations in all patients to assess the relation between serum endothelin-1 level and Child-Pugh class in patients with liver cirrhosis.

Results

This study included 60 patients who attended to Aswan university hospital from September 2016 to June 2018; Mean age was 62.66 ± 8.8 years, 38 patients (63.33%) were men and 18 (30%) were from urban areas. The etiology of liver cirrhosis was either chronic hepatitis B or C virus (**Figure 1**). Based on the Child-Pugh class, the studied patients were categorized into 3 groups; The mean age of patients of group I (Child-Pugh class A) was 67.5 ± 4.9 years; 66.7% of them were male while the mean age of patients of group II (Child-Pugh class B) was 65.6 ± 7.4 years; 61.11% of them were male but as regards to Group III (Child-Pugh class C) their mean age was 61.2 ± 8.6 years and 63.33% of them were male. All patients of groups I, II and III were Hepatitis C virus (HCV) antibody positive. 33.3% of patients of group I were diabetic and 16.7% were hypertensive, however 22.2% of group II and 20% of group III were diabetic and hypertensive. Patients of group I had neither history of ascites

nor lower limb edema, while 66.7% of patients of group II and 60% of patients of group III had history of ascites and lower limb edema with significant difference between the groups (P value = 0.022), on the other hand 50% of group I, 66.7% of group II and 100% of group III complained of bleeding tendency with significant difference between the groups (P value = 0.003). There was statistically significant difference between the study groups as regard size of the spleen with more increase in the spleen size in group III in comparison to groups I & II (P value = 0.009). However, there was no significant difference in the liver size between the study groups (P value = 0.189). Regarding the complete blood count (CBC), the mean ± SD of hemoglobin, White blood cells (WBCs) count and platelets count in patients of group I were 10.85 ± 0.16 g/dl, 12.95 ± 1.59 /mm^3 and 64.00 ± 8.76 /mm^3 respectively, and in patients of group II were 8.73 ± 1.69 g/dl, 4.93 ± 2.23 /mm^3 and 171.00 ± 112.36 /mm^3 respectively, while in patients of group III were 8.9 ± 1.49 g/dl, 4.36 ± 2.76 /mm^3 and 59.20 ± 26.80/mm^3 respectively. There was statistically significant difference between the study groups as regard WBCs & platelets count only (P value = < 0.001). There was statistically significant difference between the study groups as regard serum albumin, bilirubin, prothrombin time and serum creatinine (P value = < 0.001). However, there was no statistically significant difference as regard liver enzymes (ALT & AST). Serum endothelin-1 levels had was statistically significant relation with Child-Pugh class, with mean ± SD in groups I, II and III were 2.77 ± 0.22 pg/ml, 3.58 ± 0.32 pg/ml and 4.44 ± 0.41 pg/ml respectively **(Table 1)**.

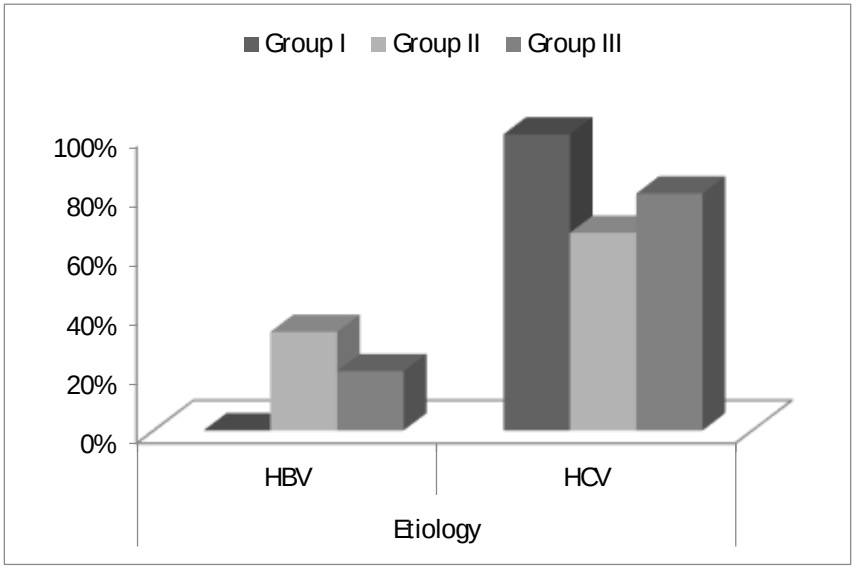

Figure (1): Comparison between the study groups as regard etiology of liver cirrhosis.

Table (1): Relation between serum endothelin-1 level and Child-Pugh class

	Group I		Group II		Group III		one way ANOVA	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**P value**
Endothelin-1 level (pg/ml)	2.77	0.22	3.58	0.32	4.44	0.41	23.153	**< 0.001**
Post hoc test								
	Group I VS Group II		**Group I VS Group III**		**Group II VS Group III**			
Endothelin-1	**0.001**		**0.001**		**0.001**			

Discussion

Cirrhosis is the end-stage of many chronic liver conditions that lead to progressive liver failure and, finally, death[4]. Historically, the severity of cirrhotic liver disease has been calculated using the Child-Pugh (CP) score. The variables used in the calculation of the CP class emerged from clinical experience & follow up. The CP class is highly valuable in determining prognosis in cirrhosis[20].

The endothelins are a group of potent vasoconstrictors[21]. Three endothelin peptides, each consisting of 21 amino acids, have been identified and known as endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). They were included in many normal and pathological processes. Their different effects are dependent on the receptor to which they bind either endothelin-A (ETA) or endothelin-B (ETB)[22,23]. ET-1 is one of the vasoactive substances which is released in response to many stimuli that can affect both the function and structure of the vascular smooth muscle[24]**.** It was observed that ET-1 expression was significantly enhanced in cirrhotic liver tissue in comparison to normal liver tissue. Moreover, activated hepatic stellate cells were shown to be the major sites of ET-1 synthesis. ET-1 has many actions on stellate cells, such as mitogenicity, activation of mitogen activated protein kinase, and a rapid increase in intracellular calcium. All of these effects appear to be mediated by ETA receptors, and studies have shown up-regulation of endothelin receptors in cirrhotic liver[25,26].

The grade of the ET-1 elevation was found to be related to the severity of cirrhosis[27,28] and the presence of ascites[29,30]. A study showed that hepatic ET-1 levels were higher in patients with a Child-Pugh score of 13 or greater than in those with a Child-Pugh score of 12 or less. A statistically significant correlation was found between hepatic ET-1 levels and Child-Pugh score. Therefore, it was suggested that the increase in ET-1 level is, at least in part, related to the severity of cirrhosis[25]**.**

In our study, we found that there was significant relation between ET-1 level and Child class. ET-1 levels in Child-Pugh A, B, C were 2.77 ± 0.22 pg/ml, 3.58 ± 0.32 pg/ml and 4.44 ± 0.41 pg/ml respectively with P value < 0.001. Therefore, it has great value in prediction of prognosis of liver cirrhosis.

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