Assessment of CD163 as a Predictor of Esophageal Varices in Patients with Liver Cirrhosis

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Abstract: Introduction and study aim: Portal hypertension is one of the most important complications of liver cirrhosis. Endoscopic screening of all patients with liver cirrhosis would result in a large number of unnecessary additional burdens to endoscopic units. Activation of Kupffer cells is involved in the pathogenesis of portal hypertension. Soluble (s) CD163 is a macrophage scavenger receptor and a specific marker for macrophage activation in portal hypertension. This study was designed to assess soluble plasma (s) CD163 as noninvasive parameter for detection of esophageal varices in Child A compensated cirrhotic hepatitis C patients. **Patients and Methods**: This study included 86 subjects among of them 70 (Child A) post hepatitis C compensated cirrhotic patients in whom fibroscan were above 13 Kps (F4) and 16 healthy individuals as a control group who were enrolled in Hepatology Department and outpatient clinic at kafr Elsheikh liver research center at the period from June 2014 to June 2015. Upper gastrointestinal endoscopy was done to detect esophageal varices (EVs) and simultaneously serum soluble (s) CD163 measurement by ELISA was assessed in all individuals. **Results**: There was increase in soluble (s) CD163 in cirrhotic patients with and without esophageal varices, fairly three times more than control groups (10.46±1.73and 5.47±0.72 Vs 2.85±0.33 mg/L) (P value=0.001). In addition, (s) CD163 is nearly doubled in patients with esophageal varices (mean=10.46±1.73mg/L) than patients without varices (mean=5.47±0.72mg/L) (p value=0.001). By multivariate analysis of all studied parameters in cirrhotic patients, presence of EVs was associated with a low platelet count (p=0.02), high body mass index (p=0.029), low hemoglobin (p =0.001), low albumin (p=0.001), increased PV diameter (p =0.002), increased spleen size (p=0.001), high Child A score (in A6 than A5) (p =0.001), high FIB4 score (p =0.003), high Fibroscan results in (KPs) (p=0.001), high serum (S) CD163 level (p =0.001). **Conclusion:** (s) CD163 as serum marker of portal hypertension could potentially predict the presence of esophageal varices in Child A compensated cirrhotic hepatitis C patients.
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Key words: compensated liver cirrhosis, esophageal varices, serum soluble (s) CD 163.

1. Introduction

Complications of liver cirrhosis, is associated with development of a hyperdynamic circulation and complications such as Portal hypertension, which is considered as one of the most important ascites, hepatic encephalopathy and esophageal varices **[1].** Estimated prevalence of esophageal varices is approximately 50% of liver cirrhosis. The risk of bleeding from varices is 25%-35% with majority of the initial bleeding occurring within 1 year from variceal detection **[2].** The prevalence of esophageal varices among cirrhotic patients is variable, ranging from 24% to 80% **[3].** Esophagogastroduodenoscopy (EGD) remains the gold standard for diagnosis and grading of EV and for the evaluation of the risk of bleeding but it has a series of disadvantages that makes long-term surveillance problematic: it is unpleasant for the patient and requires both complex logistics and qualified medical staff. Therefore, there is a strong need for another, less invasive set of investigations that have the ability to select patients with a higher risk of bleeding who will benefit from a therapeutic EGD, from those with low risk, who will not benefit at all **[4].** Several studies have evaluated the noninvasive markers of esophageal varices in patients with cirrhosis, such as the platelet count, FibroTest, spleen size, portal vein diameter, transient elastography of the liver, and more recently, transient elastography of the spleen **[5].** Circulating soluble CD163, originating from activated Kupffer cells is increased in cirrhosis with increasing hepatic venous pressure gradient (HVPG), and it is an independent

predictor for HVPG. These findings support a primary role of macrophage activation in portal hypertension, and may indicate a target for biological intervention **[6].** Also, Kupffer cells were activated in patients with liver cirrhosis in parallel with their portal hypertension and suggest that Kupffer cell activation is a constitutive event that may play a pathogenic role for portal hypertension**[7].** Yang and his colleagues identify a high Serum CD163 level as a new independent predictor of the presence of esophageal varices **[8],** and another study demonstrated that sCD163 is an independent predictor of variceal bleed and death in cirrhotic patients **[9].**

2. Patients and Methods:

After an Informed written consent and approval of Benha faculty of medicine ethical committee of research, 70 compensated cirrhotic Child A post hepatitis C patients and 16 age and sex-matched unrelated healthy subjects as normal control group were randomly selected from Hepatology Department and outpatient clinic at kafrElsheikh liver research center at the period from June 2014 to June 2015. Cirrhosis based on Transient elastography (Fibroscan) above 13 kpa (F4) ± liver biopsy when available.

Inclusion criteria:

Patients older than 18 years of age chronically infected with HCV [HCV-RNA detectable by polymerase chain reaction (PCR) in blood ≥6 months] and cirrhosis (liver stiffness measurement ≥13KPa) that enrolled in the National Committee for Control of Viral Hepatitis (NCCVH) for preparation of antiviral therapy. Only compensated cirrhotic Child –Pugh A patients were included.

Exclusion criteria:

Patients with advanced cirrhosis (Child-Pugh classes B and C), other causes of liver disease, hepatocellular carcinoma, portal vein thrombosis, previous or current treatment beta-blockers, diuretics, or other vasoactive drugs, parenteral drug addiction or alcohol abuse in the last year. Because of soluble CD163 is increased with activation of macrophages, which is a characteristic of tissues responding to inflammation and infectious diseases **[10].** All patients with current infectious and/or chronic diseases (renal, respiratory, rheumatic diseases) were also excluded. All cases were assessed by history taking, through clinical examination and laboratory investigations. After an overnight fast all patients underwent an ultrasound examination with single viewer operator in supine position using device Toshiba, Aplio with convex probe 3,5 MHz to detect the presence of liver cirrhosis (irregular surface, coarse texture, attenuated hepatic veins, relative enlargement of caudate lobe ***[11],*** signs of portal hypertension (presence of abdominal collaterals or splenomegaly) and portal vein diameter.

Upper gastrointestinal (GIT) endoscopy was done by the same endoscopist using Olympus GIF Q - 180 gastroscopy after fasting for at least 6 hours in left lateral position with sedation by midazolum 5mg ampoule with examining esophagus for varices occurrence, size and risk signs of bleeding (red wale sign & cherry red spots) and duodenum till second part and stomach for portal hypertensive gastropathy and fundal varices. Esophageal varices were graded according to their size classification and according to Italian grading of esophageal varices as follows: grade1: Small, less than one third of the radius of the esophagus, grade 2: medium, one third to two thirds of the radius of the esophagus and grade 3: large, greater than two thirds of the radius of the esophagus **[12].** N.B. Control group had performed upper gastrointestinal endoscopy for diagnostic purposes (e.g. epigastric pain).

A single operator examination procedure using FibroScan® (FS) (Echosens, Paris, France)with a medium probe and software version 1.30 for liver stiffness (LS) measurements are performed on the right lobe of the liver in intercostal position which prevents direct compression of the liver that would eventually affect LS values. The standard examination was done using the M probe while the XL probe was used in patients with technical difficulties (e.g. obese patients)**[13].**

Measurement of liver stiffness (LS) by transient elastography (TE) has moderate accuracy in diagnosis and staging of fibrosis. The following table shows the relation between Fibroscan reading in K Pascal and the stage of fibrosis.

Table (1): Relation between Fibroscan reading in K Pascal and the stage of fibrosis showing cutoff levels for HCV patients[**14]**

Fibrosis stage (Metavir)	Fibroscan score (kpa)
F0	0 till 5.4
F0-F1	5.5 till 5.9
F1	6 till 6.9
F1-F2	7 till 8.7
F2	8.8 till 9.4
F3	9.5 till 12.4
F3-F4	12.5 till 14.4
F4	≥14.5

FIB4 calculated for all cases as follows = [age (years) x AST (IU/L)]/ platelet count (10^9/L) x ALT (IU/L)1/2**/[15]**

Blood samples were drawn from patients on the same day upper GIT screening for varices was carried out. Blood from one 9 ml EDTA-coated tube was

separated by centrifugation and plasma was stored at −80 °C. Serum sCD163 was analyzed in duplicate samples of frozen serum by the use of in-house sandwich enzyme-linked immunosorbent assays using a STAT-FAX 2100 ELISA-analyzer (Gama trade, USA). Control samples and serum standards with concentrations that ranged from trace amounts to purified CD163 were included in each run. The limit of detection (lowest calibrator) was 2.5mg/L. Soluble CD163 is resistant to freezing at -80 C for 16 months**[8].**

Statistical analysis:

Statistical presentation and analysis of the present study was conducted by SPSS V.16 Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation or median, range and IQR. Chi square test (X2), Fisher's exact test, "Z" test, Spearman's correlation coefficient (rho), student "t" test, Man Whitney U test, ROC curve, ANOVA and Krauskal Wallis test were used as tests of significance.

3. Results

Study was conducted on 86 cases among of them 70 compensated cirrhotic patients (Child A) post-hepatitis C and 16 healthy subjects as control group.

Table (2): Basic characteristics of the studied groups.

Variable			No (N=86)	%
The studied groups	Group (A)	Cirrhotic without esophagealvarices(EVs)	31	36.0
	Group (B)	Cirrhotic with esophagealvarices(EVs)	39	45.3
	Group (C)	Controls	16	18.6
Gender		Male	52	60.5
		Female	34	39.5
		Mean ±SD	**Minimum**	**Maximum**
Age (years)		52.1±8.75	29	65
BMI (kg/m^2)		26.8±2.1	22	34

Table (3): Comparing the studied groups regarding gender

Sex		Group			Total	
		Cirrhotic without OVS	Cirrhotic with OVS	Controls		
Male	Count	18	24	10	52	**X^2=0.12 & P=0.94 (NS)**
	% within group	58.1%	61.5%	62.5%	60.5%	
Female	Count	13	15	6	34	
	% within group	41.9%	38.5%	37.5%	39.5%	
Total	Count	31	39	16	86	
	% within group	100.0%	100.0%	100.0%	100.0%	

This table shows that there was no statistically significant relation between sex and esophageal varices presence.

Table (4): Comparison between the studied groups with and without varices and control group regarding their Age and body mass index.

Variable	Cirrhotic without EVs (N=31)		Cirrhotic with EVs (N=39)		Control group (N=16)		ANOVA	p
	Mean	± SD	Mean	± SD	Mean	± SD		
Age (ys)	54.9	6.64	50.7	7.28	50.2	13.6	2.53	0.086 (NS)
BMI	26.1	2.21	27.5‡	2.19	26.5	1.26	3.71	0.029 (S)

This table shows that there was NO statistically significant relation between Age and esophageal varices presence. This table shows that there was statistically significant relation between increased BMI and esophageal varices presence.

Table (5): Comparison between patients with and without varices regarding to fasting blood glucose, CBC and serum creatinine.

Variable	Cirrhotic without EVs (N=31)		Cirrhotic with EVs (N=39)		St.'t"	p
	Mean	± SD	Mean			
FBS (mg/dl)	102.1	36.3	98.3	7.9	0.63	0.53 (NS)
Hb% (gm/dl)	13.3	1.82	11.4	0.56	6.56	<0.001 (HS)
WBCs (/mm^3)	5.3	2.47	4.95	1.47	0.74	0.46 (NS)
PLTs (/mm^3)	106.7	34.4	90.02	24.48	2.37	0.02 (S)
Creatinine (mg/dl)	0.83	0.13	0.84	0.14	0.32	0.75 (NS)

This table shows that there was high statistically significant relation between Hb % and esophageal varices presence. There was inverse relation between platelets count and presence of esophageal varices, also there was statistically significant relation between low platelets count and esophageal varices presence.

Table (6): Comparison between patients with and without varices regarding their liver function tests.

Variable	Cirrhotic without EVs (N=31)		Cirrhotic with EVs (N=39)		St."t"	p
	Mean	± SD	Mean	± SD		
T. bilirubin (mg/dl)	0.90	0.38	1.21	1.49	1.11	0.27 (NS)
AST(IU/L)	72.1	27.55	71.8	26.64	0.05	0.95 (NS)
ALT(IU/L)	57.1	27.20	54.2	25.13	0.46	0.64 (NS)
Albumin (gm/dl)	3.83	0.48	3.24	0.18	6.67	< 0.001 (HS)
ALP(mg/dl)	126.2	37.1	135.5	28.83	1.18	0.74 (NS)
PC%	81.0	11.38	80.9	6.95	0.049	0.96 (NS)
AFP	3.96	1.95	4.05	3.51	1.23*	0.22 (NS)

This table shows that there was no statistically significant relation between liver enzymes, PC %, ALP, AFP, T. bilirubin and esophageal varices presence, while shows high statistically significant relation between decreased ALBUMIN level and presence of esophageal varices.

Table (7): Comparison between patients with and without varices regarding their HCV RNA level.

Variable	Cirrhotic without EVs (N=31)		Cirrhotic with EVs (N=39)		Z of Mann Whitney test	p
	Mean	± SD	Mean	± SD		
HCV RNA (x 1000)	441.2	771.34	458.5	1253.79	1.78	0.075 (NS)

This table shows that there was NO statistically significant relation between HCV RNA and esophageal varices presence.

Table (8): Comparison between patients with and without varices regarding their schistosoma abs.

			group		Total	Z Test & P
			Cirrhotic without EVs	Cirrhotic with EVs		
Schisto. titre	**-Ve**	**No.**	16	31	47	2.47 &
		%	51.6%	79.5%	65.7%	0.04 (NS)
	+Ve	**No.**	15	8	23	2.07 &
		%	48.4%	20.5%	34.3%	0.085(NS)
Total		**No.**	31	39	70	
		%	100.0%	100.0%	100.0%	

This table shows that there was NO statistically significant relation between Schistosoma Abs level and esophageal varices presence.

Table (9): Comparison between cirrhotic with out and cirrhotic with EVs regarding US findings.

Variable		Cirrhotic without EVs (N=31)		Cirrhotic with EVs (N=39)		Fisher's test/ St. "t"	P
		No.	%	No.	%		
Liver size	Normal	31	100	39	100	-----	------
	Enlarged	0	0	0	0		
Echogenecity	Normal	0	0	0	0	-----	------
	Cirrhotic	31	100	39	100		
Spleen	Normal	7	22.6	1	2.6	7.0	0.022 (S)
	Enlarged	22	71.0	36	92.3		
	splenectomy	2	6.5	2	5.1		
PV diameter (mm)	Mean	12.1		13.0		3.19	0.002 (S)
	± SD	0.84		1.34			
Splenic size(cm)	Mean	14.9		16.9		4.28	<0.001 (HS)
	± SD	2.04		1.67			

There was statistically significant relation between spleen size, PV diameter and esophageal varices presence

ROC curve for sensitivity and specificity of splenic size for prediction of esophageal varices.

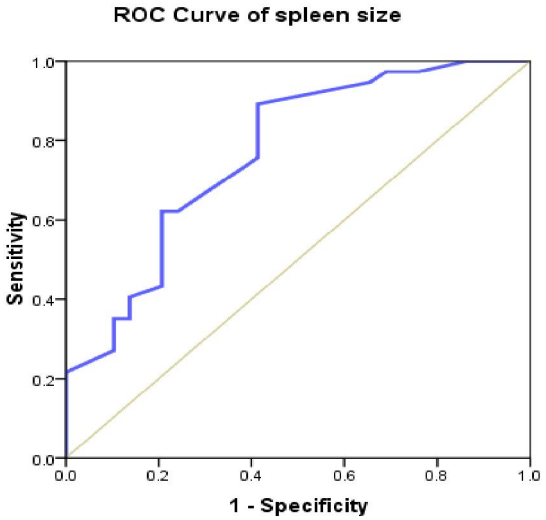


Figure (8): The best cutoff value of splenic size in prediction of esophageal varices was ≥15.9 with AUC (Area under the curve) 0.77, sensitivity 75.7%, specificity 58.6%, positive predictive value 70 %, negative predictive value 65.4%.

ROC curve for sensitivity and specificity of portal vein diameter for prediction of esophageal varices.

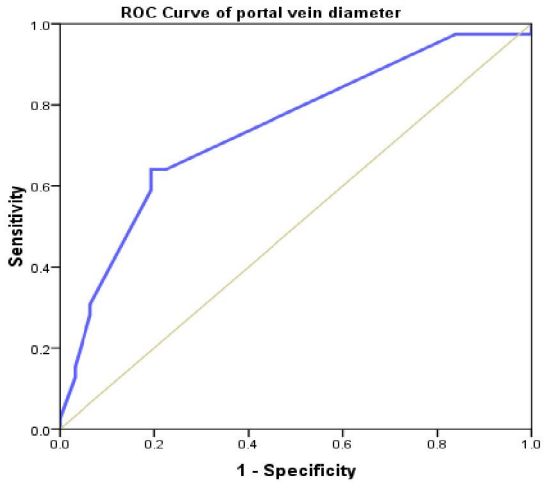


Figure (9): The best cutoff value of portal vein diameter in prediction of esophageal varices was ≥13.5 with AUROC (Area under the curve) 0.74, sensitivity 64.1%, specificity 80.6%, positive predictive value 80.6%, negative predictive value 64.1%.

Table (11): Comparison between patients with and without varices regarding their FIB 4

Group	No.	FIB 4		MWU test	P
		Mean	± SD		
Cirrhotic without EVs	31	4.64	1.54	2.95	0.003 (S)
Cirrhotic with EVs	39	6.67	3.47		

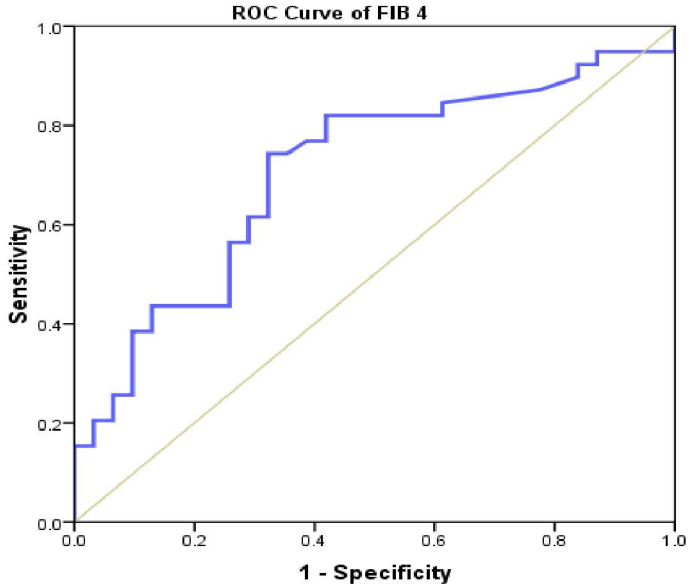
This table shows that there was statistically significant relation between FIB4and esophageal varices presence.

Table (10): Comparison between patients with and without varices regarding their Child-Pugh classification.

	Total	group			
		Cirrhotic with OVS	Cirrhotic without OVS		
X^2= 20.4 & P<0.001 (HS)	40	13	27	Count	A5
	57.1%	33.3%	87.1%	% within group	CHILD Score
	30	26	4	Count	A6
	42.9%	66.7%	12.9%	% within group	
	70	39	31	Count	Total
	100.0%	100.0%	100.0%	% within group	

This table shows that there was high statistically significant relation between Child-Pugh A classification and esophageal varices presence.

ROC curve for sensitivity and specificity of FIB4 for prediction of esophageal varices.



76.9%, specificity 61.3%, positive predictive value 71.4%, negative predictive value 67.9%.

Figure (12): The best cutoff value of FIB4 in prediction of esophageal varices was ≥4.68 with AUROC (Area under the curve)0.71, sensitivity

Table (12): Comparison between patients with and without varices regarding their FibroScan.

Variable	Cirrhotic without EVs (N=33)		Cirrhotic with EVs (N=34)		St. 't'	P
	Mean	± SD	Mean	± SD		
Fibroscan(Kps)	18.1	1.9	25.9	2.94	12.7	<0.001 (HS)

This table shows that there was high statistically significant relation between Fibroscan and esophageal varices presence. ROC Curve for Sensitivity and Specificity of Fibroscan for prediction of esophageal varices.

Table (13): Comparison between patients with and without varices regarding their CD 163 levels

Variable	Cirrhotic without EVs (N=31)		Cirrhotic with EVs (N=39)		Controls (n=16)		KWT	P
	Mean	± SD	Mean	± SD	Mean	± SD		
CD- 163 (mg/l)	5.47	0.72	10.46	1.73	2.85	0.33	72.6	<0.001 (HS)

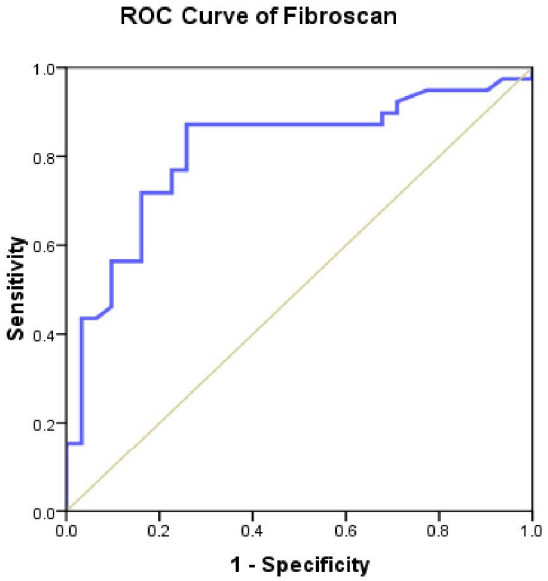


Figure (14): The best cutoff value of Fibroscan in prediction of esophageal varices was ≥20.75with AUROC (Area under the curve) 0.81, sensitivity 87.2%, specificity 74.2%, positive predictive value 80.9%, negative predictive value 82.1%

This table shows that there was High statistically significant relation between CD-163 level and esophageal varices presence.

ROC Curve of CD-163 for early diagnosis (prediction) of esophageal varices**.**

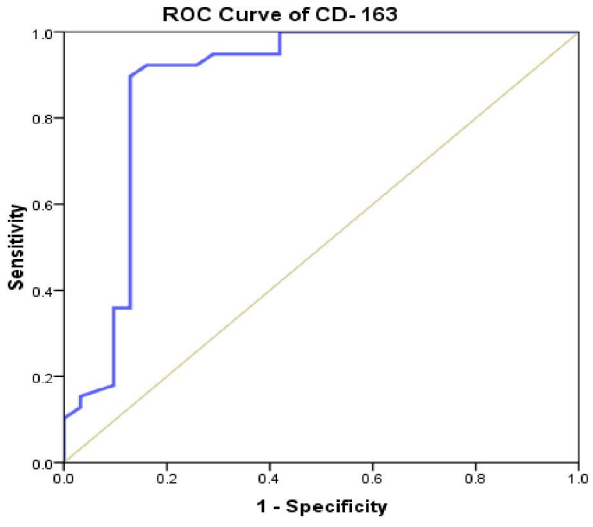


Figure (16): The best cutoff value of serum (CD163) in prediction of esophageal varices was ≥ 7.5 with AUROC (Area under the curve) 0.88, sensitivity 92.3%, specificity 83.9%, positive predictive value 87.8%, negative predictive value 89.6%.

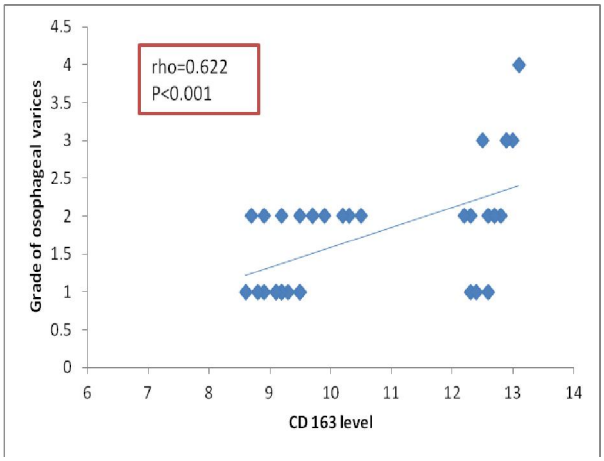


Figure (17): Comparison between CD-163 levels and grading of esophageal varices in group (B) cirrhotic with EVs.

This figure shows that serum sCD163 level is increased with increasing grades of esophageal varices in group (B).

Table (14) shows that high body mass index, low hemoglobin level, low albumin level, high PV diameter, high Spleen size, low PLT count and high child score high FIB4, high Fibroscan results in(KPs), High serum(CD163)level are significant predictors of esophageal varices in studied cases by multivariate analysis.

4. Discussion

Chronic liver diseases and cirrhosis are now being recognized as an important cause of morbidity and mortality world-wide. Established cirrhosis has a 10-year mortality of 34-66% [**16**]. Portal hypertension is one of the main consequences of cirrhosis. It can result in severe complications, including bleeding of esophagogastric varices [**12**]. Esophageal varices (EV) are present in 40% of patients with compensated liver cirrhosis and in 60% of those with decompensated disease, having a constantly progressive evolution; once discovered they need to be under constant surveillance **[17**]. The annual rate of incidence of new varices is 7-8%, with a similar rate of transition from small to large EV. The major risk that threatens the prognosis of a patient with EV is massive upper digestive bleeding, knowing that the first bleeding episode is associated with a 40% mortality rate **[18].** Performing an esophagogastroduodenoscopy (EGD) still remains the best way to diagnose and evaluate esophageal and gastric varices and the risk of variceal bleeding [**19].** EGD is however expensive for the health system and unpleasant for the patient, especially so when it has to be repeated frequently, within the framework of a screening program. Therefore, non-invasive methods are required to

diagnose presence and grading of esophageal varices in patients with hepatic cirrhosis and in this respect, we have evaluated the role of serum soluble (s) CD163 in diagnosis of esophageal varices in Child A cirrhotic chronic hepatitis C patients. Serum (s) CD163 is a macrophage lineage-specific hemoglobin haptoglobin scavenger receptor and a specific marker for macrophage activation **[20, 21]** Serum (s) CD163 is shed into the circulation in a soluble form (sCD163) after Toll-like receptor activation by a similar mechanism as TNF-α **[22]**. Serum concentrations of soluble CD163 are accordingly elevated during conditions of macrophage activation and proliferation *[23,24]*. Circulating CD163 originating from activated Kupffer cells is increased in cirrhosis with increasing hepatic venous pressure gradient (HVPG), and it is an independent predictor for HVPG. These findings support a primary role of macrophage activation in portal hypertension, and may indicate a target for biomarker for diagnosis of esophageal varices***[8].***

Table (14): Multivariate logestic regression analysis for factors associated with diagnosis of esophageal varices.

Variable	Odds Ratio	95% CI	P
BMI (≥ 27)	1.31	0.74-7.8	0.22
Hb% (≤ 12)(gm/dl)	4.9	2.6-9.1	0.002 (HS)
PLT (≤ 100.000)(mm^3)	3.8	2.01-10.6	0.003 (S)
AST (≥ 60)(IU/L)	4.1	0.8-10.3	0.091 (NS)
ALT (≥ 50)(IU/L)	2.7	0.6-11.2	0.11 (NS)
Albumin (≤ 3.75)(gm/dl)	6.2	2.0-10.5	<0.001 (HS)
ALP (≥ 130)(mg/dl)	2.1	0.89-13.7	0.19 (NS)
PC% (≤ 75%)	2.3	1.9-15.6	0.17 (NS)
CD-163 (≥ 7.5)(mg/l)	9.1	4.8-17.3	<0.001 (HS)
Fibroscan (≥ 20.8)(KPs)	8.8	5.0-13.6	<0.001 (HS)
HCV ab (+VE)	2.09	0.92-6.4	0.19 (NS)
Schisto ab +ve	3.3	1.09-9.9	0.007 (NS)
PV diameter(≥13.5) (mm)	6.9	3.7-14.6	<0.003 (S)
Splenic size (≥ 15.9) (cm)	4.9	2.8-17.5	<0.001(HS)
Child score A6	2.8	1.05-9.1	<0.001(HS)
FIB 4 (≥ 4.68)	3.0	1.5-13.1	0.027 (S)

This study was conducted on 86 cases among them 70 cirrhotic patients (Child A) post hepatitis C and 16 cases as normal control group who attended the Hepatology department and outpatient clinic at KafrELsheikh liver research center. In this study we aimed to identify sCD163, as an easy biochemical marker, for predicting the presence of EVs in compensated cirrhotic Child A post hepatitis C patients.

In the present study, although only Child A compensated cirrhotic patients were included, there was statistically significantly higher relation between increased child pugh class and presence of varices as shown in table(10) and this is in agreement with ***Madhotra et al., 2002*[24]** who found a significant relation between the presence of varices and increased Child score. Thus, the more advanced the liver disease (according to Child classification), the more likely the presence of varices. Child-Pugh score is a well-validated classification for the degree of hepatic dysfunction in patients with cirrhosis. In this study, it was statistically significant lower serum albumin in patients with esophageal varices than patients without varices (p value = 0.001). As regard serum albumin is considered the most important circulating protein and reflects the synthetic function of the liver ***(25).*** Hypoalbuminemia was one of the factors that were associated with the presence of large esophageal varices *as reported by* ***Chang, et al., 2007 and Kazemi et al., 2006*** *who* reported that serum albumin was lower in patients with esophageal varices than patients without varices and there was statistically significant difference**[26&27].**

The current study showed that platelet count was significantly lower in patients with esophageal varices (mean=89.500) than those without esophageal varices (mean=110.300) with (P = 0.005). That was stated **by*Agha et al., (2009)*** who found that platelet count was statistically significantly lower in patients with esophageal varices compared to patients without varices.[28] Our results were also in agreement with the results r**eported by*Kazemi et al., 2006 and Esmat et al., 2012 [27&29]***. This may also attributed to thrombopoietin which is a cytokine synthesized within the liver that promotes thrombopoiesis and decreased level of this cytokine has been demonstrated in patients with chronic liver disease and cirrhosis ***[30].*** In addition, splenic sequestration accounts for most of

the thrombocytopenia and up to 90% of the platelet may be sequestrated in the spleen. Platelet sequestration in the spleen is associated with the immune destruction of platelets mediated by platelet bound immunoglobulin G (lgG). So, platelet count correlates with spleen size and it is the main factor in thrombocytopenia in portal hypertension ***[31].***

Measurement of splenic size by ultrasonography is considered a non-invasive predictive indicator of the development of esophageal varices in liver cirrhosis **[29].** Doppler ultrasonography (US) imaging provides a real-time, inexpensive, and repeatable examination of the portal system and allows estimation of both arterial and venous flow. It is considered the first-line imaging technique in patients with cirrhosis. Portal vein diameter, portal blood velocity and congestion index, spleen size, flow pattern in the hepatic veins, and the presence of abdominal portosystemic collaterals are all US parameters previously thought to have with prognostic significance but all with poor sensitivity and specificity **[32]**One large study proposed prothrombin activity of less than 70%, portal vein diameter greater than 13 mm, and platelet count < 100×10^9 as noninvasive predictive tools to discriminate cirrhotic patients with and without oesophageal varices (OVs)**[17]**. As regard spleen size, we found that it was statistically significant in patients with esophageal varices (mean =16.9) than those without esophageal varices (mean =14.9) and (p=0.001). These result agreed with the results of Kazemi *et al., 2006* who found significant difference between patients without esophageal varices in comparison to patients with esophageal varices as regarg spleen size [27]. Our results showed statistically significant difference as regard portal vein diameter between patients with esophageal varices (mean=13) than those without oesophagealvarices(mean=12.1) and (p value=0.002), These results agreed with the study of *Schepis etal.*(2001)**(17).**

Measurement of *liver stiffness* using fibroscan is a very attractive non-invasive parameter for assessment of fibrosis in chronic liver diseases, being reproducible, operator-independent and well correlated with the degree of fibrosis **[33**]. It has already been demonstrated that in patients with histopathologically confirmed cirrhosis, the grade of EV is directly correlated with the degree of liver fibrosis, an increase of the latter being translated in a rise in the size of the former **[27].** With these perquisites in mind, we have tried to evaluate if there is a cut-off value for liver fibrosis which can be used to predict presence or evolution of EV **[6].** In this study liver stiffness measurement was significantly higher in patients with esophageal varices than those with no varices. For a cutoff value (20.75 KPa), sensitivity was 87.2%, specificity was 74.2%, PPV was 80.9%, NPV was 82.1% and accuracy was 58.2%. *Castera et al., (2009)* found that when using cutoff value (21.5 kPa) to predict esophageal varices, the sensitivity was 76%, specificity 78%, positive predictive value(PPV) 68% negative predictive value(NPV) 84%; while on using a cutoff value (30.5 kPa) to predict large EV, the sensitivity was 77%, specificity 85%, PPV 56% and NPV 94%.**[34].** *Stefanescu et al., (2011)* analyzed results and noticed that when using cutoff value (19 kPa), it was possible to predict esophageal varices with sensitivity 84%, specificity 32.3%, PPV 72.4% and NPV 48.9%. When using cutoff value (38 kPa), it was possible to predict LEV (≥ grade 2) with an acceptable sensitivity and specificity (76% and 80% respectively), but the positive predictive value did not exceed 54**%[35].**

Fib-4was confirmed as a good noninvasive marker of liver fibrosis for chronic hepatitis C, with performances similar to the Fibro Test. Fib-4 was also tried for the prediction of esophageal varices in patients with liver cirrhosis.***[35&36].*** The results of the current study showed that for diagnosis of esophageal varices, the Fib 4 score showed statistically significant value in prediction of varices (p value=0.003(and at cutoff value ≥4.68 the sensitivity was 76.9%, specificity was 61.3%, PPV was 71.4%, NPV was 67.9% and accuracy 67%. *Sebastiani et al., (2010)*used FIB-4 for diagnosis of esophageal varices and found that the sensitivity was 70 %, specificity was 59%, PPV was 70%, NPV was 57% and accuracy was 66% and the best cutoff value for Fib-4 was (≥3.5). While, for prediction of large EV they found that the sensitivity was 62 %, specificity was 60%, PPV was 29%, NPV was 85% and accuracy was 61% and the best cutoff value for Fib-4 was ≥4.3.***[36].*** *Stefanescu et al., (2011)* used Fib-4for the diagnosis of presence of esophageal varices and found that at a cutoff value (≥3.98), the sensitivity was 66.2%, specificity 54%, PPV 75.4% and NPV 43%. While, for the diagnosis of large esophageal varices, the cutoff value was (≥6.75), the sensitivity was 45.5%, specificity was 77.3%, PPV was 45.6% and NPV was 77.3**%.[35].**

Soluble CD163 is a specific marker of activated macrophages, another potential biomarker for PH in cirrhosis. The activation of Kupffer cells may be involved in PH by the release of vasoconstrictor substances. Recently, **Gronbaek et al**., **2012**, have shown that sCD163 plasma concentration in cirrhosis is almost three times higher than in controls, and sCD163 was an independent predictor of the hepatic venous pressure gradient.[**6**]. Yang et al. found that serum sCD163 level was elevated in patients with cirrhosis complicated by esophageal varices, and this marker could potentially be used to predict the presence of EVs in clinical practice. Similar to

previous reports, There was increase in soluble (s) CD163 in cirrhotic patients with and without esophageal varices, fairly three times more than control groups (10.46±1.73and 5.47±0.72 Vs 2.85±0.33 mg/L) (P value=0.001). In addition, (s) CD163 is nearly doubled in patients with esophageal varices (mean=10.46±1.73mg/L) than patients without varices (mean=5.47±0.72mg/L) (p value=0.001). in our study. Moller et al.,2007&Holland et al.,2011 excitingly, found good abilities of serum sCD163 level to distinguish cirrhotic patients with EVs from those without EVs, with good sensitivities and specificities by ROS analysis**.[37-38].**

On performing multivariate logistic regression analysis, it was found that high body mass index, low hemoglobin level, low albumin level, low PLT count, high PV diameter, high Spleen size, high child score, high FIB4, high Fibroscan results in(KPs), High serum (CD163) level are significant predictors of esophageal varices as shown in table(4).

The correlation study by univariate and multivariate analysis revealed that serum sCD163 level is a candidate biomarker for prediction of EVs of cirrhosis in our clinical practice. In conclusion, serum (s) CD163 in patients with hepatitis C virus cirrhosis (Child A) significantly increased with the presence of esophageal varices. It could be used as non-invasive parameter for predicting esophageal varices, but not sufficient to be good negative test or surrogate marker alone, still endoscopy is the gold standard for diagnosis of varices.

The study has limitations, the analysis was carried out in a small number of patients, and it will be interesting to determine whether this association holds true also in larger groups of patients with HCV cirrhosis and in patients with liver disease of other origins. Lack of data on other variables, such as direct measurement of portal hypertension by HVPG, also could affect the interpretation of our findings. Finally, we cannot exclude the possibility that hidden abuse of alcohol may be responsible for the presence of EV in a few subjects.

References

1. Garcia-Tsao G, Sanyal AJ, Grace ND, Carey W, and the Pratice Guidelines Committee of the American Association for the Study of Liver Diseases, the Practice Parameters Committee of the American College of Gastroenterology. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. HEPATOLOGY 2007;46:922-938.
2. Jensen DM. Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. Gastroenterology 2002;122:1620–30.
3. de Franchis R. Portal Hypertension. Proceedings of the IVth Baveno International Consensus Workshop on Methodology of Diagnosis and Treatment in Portal Hypertension. Blackwell Science, Oxford, UK 2005; 154-158.
4. Binţinţan, A., Chira, I., R., Vasile Virgil Binţinţan, V. V., Nagy, G. Z., et al (2015): Value of hepatic elastography and Doppler indexes for predictions of esophageal varices in liver cirrhosis. *Med Ultrason, Vol. 17, no. 1, 5.*
5. Colecchia A, Montrone L, Scaioli E *et al*(2012): Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 143: 646–54.
6. *Gronbaek, H.; Sandahl, T. D.; Mortensen, C.; Vilstrup, H.; Moller, H. J. and Moller, S. (2012):* Soluble CD163, a marker of Kupffercell activation, is related to portal hypertension in patients with liver cirrhosis. Aliment. Pharmacol. Ther; 36:173-180.
7. Holland-Fischer P, Gronaek H, Sandahl TD, Moestrup SK, Riggio O, Ridola L, AagaardNK, Moller HJ, Vilstrup H (2011) Kupffer cells are activated in cirrhotic portal hypertension and not normalised by TIPS. Gut 60:1389–1393.
8. *Yang, Y. Y.; Hou, M. C.; Lin, M. W.; Chen, P. H.; Liao W. C.; et al (2013):* Combined platelet count with sCD163 and genetic variants optimizes esophageal varices prediction in cirrhotic patients. Journal of Gastroenterology and Hepatology; 28:112-121.
9. Waidmann O, Brunner F, Herrmann E, Zeuzem S, Piiper A, Kronenberger B (2013) Macrophage activation is a prognostic parameterforvariceal bleeding and overall survival in patients with liver cirrhosis. J Hepatol 58:956–961.
10. Etzerodt, A. and Moestrup, S. K.,(2013) CD163 and Inflammation: Biological, Diagnostic, and Therapeutic Aspects. ANTIOXIDANTS& REDOX SIGNALING Volume 18, Number 17, 2013 [a] Mary Ann Liebert, Inc. DOI: 10.1089 /ars. 2012.4834.
11. *Bates, J. A. (2004):* Abdominal ultrasound. Churchill Livingstone, Edinburgh, London, 2nd edition; 97-107.
12. *DeFranchis, R.; Eisen, G. M; Laine, L.; et al (2008):* Esophageal Capsule Endoscopy for Screening and Surveillance of Esophageal Varices in Patients with Portal Hypertension. Hepatology;47:1595-1603.
13. Mueller S and Sandrin L. (2010): Liver stiffness: a novel parameter for the diagnosis of liver disease. Hepatic Medicine: Evidence and Research; 2: 49–67.
14. de Lédinghen V, Douvin C, Kettaneh A, Ziol M, Roulot D, et al. (2006) Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. J Acquir Immune DeficSyndr 41: 175–9 .
15. Vallet-Pichard, A.; Mallet, V.; Nalpas, B.; et al. (2007): FIB-4: an inexpensive and accurate marker

of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. Hepatology;46:32–36.
16. Burroughs, A. K.; Triantos, C. K.; O'Beirne, et al. (2009): Predictors of early rebleeding and mortality after acute variceal hemorrhage in patients with cirrhosis. Nat Clin Pract Gastroenterol Hepatol; 6: 72-73.
17. Schepis F, Camma C, Niceforo D, Magnano A, Pallio S, Cinquegrani M, et al.(2001): Which patients with cirrhosis should undergo endoscopic screening for esophageal varices detection? Hepatol; 33: 333-338.
18. Gainnini E, Botta F and Borro P, Risso D, Romagnoli P. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. Gut 2003; 52 (8): 1200-1205.
19. deFranchis, R. (2003): Evaluation and follow-up of patients with cirrhosis and oesophageal varices. J. Hepatol.; 38: 361-3.
20. *Moestrup, S. K and Moller, H. J. (2004):* CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Ann Med; 36: 347–54.
21. *Weaver, L. K.; Hintz-Goldstein, K. A and Pioli, P. A.(2006):* Pivotal advance: activation of cell surface Toll-like receptors causes shedding of the hemoglobin scavenger receptor CD163. J LeukocBiol; 80: 26–35.
22. *Etzerodt, A.; Maniecki, M. B.; Moller, K.; Moller, H. J. and Moestrup, S. K. (2010):* Tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. J LeukocBiol; 88: 1201–5.
23. *Moller, H. J.; de, F. M., Aerts, H.; Hollak, C and Moestrup, S. K.(2004):* Plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher's disease. Eur J Haematol; 72: 135–9.
24. Madhotra, R.; Mulcahy, H. E.; Willner, I.; et al. (2002): Prediction of esophageal varices in patients with cirrhosis. J Clin Gastroenterology; 34:81-5.
25. Vanbiervliet, G.; Barjoan-Marine, E.; Anty, R.; et al. (2005): Serum fibrosis markers can detect large esophageal varices with a high accuracy. European J. of Gastroenterology. Hepatology; 17 (3): 333-338.
26. Chang, M. H.; Sohn, J. H.; Kim, T. Y.; et al. (2007): Non-endoscopic predictors of large esophageal varices in patients with liver cirrhosis. Koream J. Gastroenterology; 49 (6): 376-83.
27. Kazemi, F.; Kettaneh, A.; N'kontchou, G.; et al. (2006): Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophagealvarices. J Hepatology; 45:230-235.
28. *.Agha, A.; Anwar, E.;Bashir, K.; et al. (2009):* External validation of the platelet count/spleen diameter ratio for the diagnosis of esophageal varices in hepatitis C virus-related cirrhosis. Dig Dis Sci.;54(3):654-60.
29. Esmat, S.; Omarn, D. and Rashid, L. (2012): Can we consider the right hepatic lobe size/albumin ratio a noninvasive predictor of oesophagealvarices in hepatitis C virus-related liver cirrhotic Egyptian patients?. European Journal of Internal Medicine; 23:267–272.
30. Michael, A. H.; and James, P. (2006): Thrombocytopenia and platelet function in clinical Hepatology. Textbook. (2end edit). Bruce R. B., John G. O., Adrian M. D and John R. L. (editors) Ch (13): p.198-31.
31. Akyiiz, F.; Yekeler, E.; Kaymakoglu, S.; et al. (2007): The role of thrombopoietin and spleen volume in thrombocytopenia of patients with non cirrhotic and cirrhotic portal hypertension. The Turkish J. of Gastroenterology. 18 (2): 95-99.32.
32. Vilgrain, V.(2001): "Ultrasound of diffuse liver disease and portal hypertension," European Radiology, vol. 11, no. 9, pp. 1563–1577.
33. Sandrin L, Fourquet B, Hasquenoph JM, et al.(2003) Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol; 29: 1705-1713.
34. Castera, L.; Le Bail, B.; Roudot, T. F.; et al., (2009): Early detection in routine clinical practice of cirrhosis and esophageal varices in chronic hepatitis C: comparison of transient elastography (Fibroscan) with standard laboratory tests and non-invasive scores. J Hepatology, 50(1):59-68.
35. Stefanescu, H.; Grigorescu, M.; Maniu, A.; et al. (2011): A New and simple Algorithm for the Noninvasive Assessment of Esophageal Varices in Cirrhotic Patients using serum Fibrosis Markers and transient Elastography. J Gastrointestin Liver Dis. Vol. 20 No 1: 57-64.
36. Sebastiani, G.; Tempesta, D.; Fattovich, G.; et al. (2010): Prediction of oesophagealvarices in hepatic cirrhosis by simple serum non-invasive markers: Results of a multicenter, large-scale study. Journal of Hepatology, vol. 53 630–638.
37. Moller HJ, Gronbeæk H, Schiodt FV *et al*.;(2007): Soluble CD163 from activated macrophages predicts mortality in acute liver failure. *J. Hepatol.*; 47: 671–6.
38. Holland-Fischer P, Gronbeæk H, Sandahi TD *et al*.; (2011): Kupper cells are activated in cirrhotic portal hypertension and not normalized by TIPS. *Gut*;60: 1389–93.