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Endoglin: A Novel Biomarker in Assessment of Liver Fibrosis

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ABSTRACT

Endoglin (CD105 – cluster of differentiation 105) is a type I homodimeric cell membrane protein that is composed of two disulfide-linked subunits. It is primarily expressed on endothelial cells especially after activation. In addition, it is found on the surface of several other cell types, e.g. hematopoietic progenitor cells, bone marrow stromal fibroblasts, activated monocytes, differentiated macrophages, melanocytes, and syncytiotrophoblasts of placenta. To assess serum soluble endoglin (CD105) in patients with liver fibrosis and hepatocellular carcinoma (HCC) comparing with normal controls. The study was conducted on 45 patients; 15 of them were with liver fibrosis (group I), 30 were with HCC(group II) and 15 healthy volunteers with matched age and gender as a control group (group III). All individuals were subjected to the following: full history taking, complete clinical examination, abdominal ultrasound and laboratory investigations included; Liver function tests, Serum alpha fetoprotein and serum soluble endoglin. Statistical significant difference between group I,II and III as regard ALT ,AST, ALP,ALB,TB,DB,GGT and prothrombin time and concentration. A highly statistical significant increase in endoglin serum level in both liver fibrosis and HCC groups compared to control group. On the other side there was no statistical significant difference between HCC and fibrotic group regarding endoglin level. There was no statistical significant difference between HCC patients with high alpha fetoprotein and those with normal alpha fetoprotein as regard endoglin level. There were positive correlations between endoglin and the serum level of AST,ALT,ALP,TB,DB,GGT, prothrombin time and AFP, while negative correlations between serum endoglin and albumin ,TP and prothrombin conc. Endoglin has a potential role to be complementary biomarker in pathogenesis of liver fibrosis and can be used as a non invasive technique for diagnosis of liver fibrosis.

Keywords: CD105, Serum alphafetoprotien, Liver fibrosis,Hepatocellular carcinoma.

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INTRODUCTION

Chronic hepatitis C (CHC) is a worldwide healthcare problem (prevalence is around 200 million, with a high prevalence in Egypt). CHC is characterized by hepatic fibrosis which may progress to cirrhosis and hepatocellular carcinoma (HCC)[1]. HCC may complicate liver cirrhosis. In Egypt , HCC represents 7% of all cancer cases and shows a male : female ratio of 3:1. Liver injury induce tissue hypoxia that aggravate cell damage and inflammation with consequence stimulation of angiogenesis, fibrogenesis and liver carcinogenesis[2].

Serum markers may allow non- invasive and cost effective method for assessment and monitoring of hepatic fibrosis in CHC patients instead of liver biopsy that has many limitations[3].

Emerging evidence indicates that endoglin a 180 kD transmembrane glycoprotein constitutively phosphorylated with a marked tissue-specificity may play an important role in liver fibrosis through acting as TGF-β co-receptor [4]Transforming growth factor (TGF)-β represents an important pro-fibrogenic factor and aberrant TGF-β action has been implicated in many disease processes of the liver fibrosis. Endoglin has been shown to differentially regulates TGF-β signal transduction by inhibiting ALK5-Smad2/3 signaling and augmenting ALK1-Smad1/5 signaling[5].

Morris and colleagues, 2011 showed that endoglin overexpression in hepatic stellate cells is associated with enhanced TGF-β-driven Smad1/5 phosphorylation and α-smooth muscle actin production without altering Smad2/3 signaling. These findings suggest that endoglin may play an important role in hepatic fibrosis by altering the balance of TGF-β signaling via the ALK1-Smad1/5 and ALK-Smad2/3 pathways[6].

Endoglin (CD105) is expressed predominantly on vascular endothelial cells and its promoter is strongly and selectively active in endothelial cells consistently. High CD105 expression is associated with immature vessels of tumor, inflamed or damaged tissue[7][6]. High CD105 expression correlates with poor prognosis in more than 10 solid tumor types. CD105, but not other angiogenesis markers such as VEGFR-2, may have prognostic significance that can be useful for patient management[8].

Because of its apparent specificity for tumor-associated blood vessels, CD105 is also of clinical interest as a therapeutic target, with monoclonal antibody therapy in early stage clinical trials[9].

As HCC is mainly angiogenesis dependent so serum CD105 may be a useful marker for follow up. Antiangiogenic therapy represents one of the most promising modalities for this cancer treatment[10].

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AIM OF THE WORK :

The aim of this work was to assess serum soluble endoglin (CD105) in patients with liver fibrosis and hepatocellular carcinoma comparing with normal controls .

SUBJECTS AND METHODS

Informed written consent from each patient was obtained before starting the data collection. Patients were obtained from National Cancer Institute (NCI), Cairo university, Egypt. With respect to patients' confidentiality, patients were represented in the study by code numbers. All personal data was concealed. The study included 45 patients with liver fibrosis and HCC, 15 of them were with liver fibrosis and the other 30 were HCC. Fifteen healthy volunteers with matched age and gender were en rolled in the study as a control group.

The subjects were classified into 3 groups according to clinical findings, abdominal ultrasound, laboratory findings, and alpha fetoprotein as follows: Group 1; included 15 patients with liver fibrosis. Diagnosis was confirmed by abdominal ultrasound, serological tests and liver biopsy. Group II; included 30 patients with hepatocellular carcinoma. They were sub classified into 2 subgroups (Group IIa and Group IIb) according to level of AFP , 15 HCC patients with high level of AFP and 15 HCC patients with normal level of AFP. Those patients were diagnosed by abdominal ultrasonography hepatic focal lesion and/ or portal vein thrombosis, AFP level, liver biopsy and triphasic scan to liver. Group III (control) included 15 healthy volunteers with matched age and gender. Exclusion criteria: the cases with chronic inflammatory diseases, hematological malignancy and cancer of any organ other than the liver were excluded from the study.

All individuals were subjected to the following: full history taking, complete clinical examination, abdominal ultrasound and laboratory investigations included; Liver function tests and Serum alpha fetoprotein. The serum soluble endoglin level was measured in all patients by ELISA technique using commercial kits (R&D systems). It is a 4.5 h solid phase ELISA test which employs the quantitative sandwich enzyme immunoassay technique.

Sampling:

10 mL venous blood samples were taken by sterile venipuncture, were distributed as follows: 2 mL of venous blood was delivered in a graduated vacutainer plastic tube containing 3.2% sodium citrate for prothrombin time and concentration and was estimated at the same time. 8 mL of venous blood was delivered in a vacutainer plain test tube. Blood was left for a sufficient time to clot, serum was then separated after centrifugation at 3000 rpm for 10 minutes, then liver function tests and AFP level were done and the rest of the serum was stored at -20 ºC to be tested for endoglin.

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STATISTICAL ANALYSIS:

Results were collected, tabulated, statistically analyzed by personal computer and statistical package SPSS version 10. Two types of statistics were done:Descriptive statistics: e.g. mean (x) and standard deviation (SD) and Analytic statistics. Student t-test: is a test of significance used for comparison of the means between two groups having quantitative variables & Mann-Whitney test (nonparametric test): is a test of significance used for comparison between two groups not normally distributed having quantitative variables. Also, Spearman correlation coefficient (r) (nonparametric test): is a test used to measure the association between two quantitative variables. the Level of significance was set as P-value <0.05 .

RESULTS

Table 1: Comparison between Group I ,II and III regarding serum biochemical markers.

Variable	control (n = 15) Mean±SD:	Liver fibrosis (n = 15) Mean±SD:	HCC (n = 30) Mean±SD:	P value
AST (IU/L)	19.29±5.43	61.25±30.95	189.95±280.93	P1<0.001 P2<0.001
ALT (U/L):	16.78±6.22	35.71±19.57	89.27±147.19	<0.01 <0.001
ALP (U/L)	60.40±12.42	109.12±72.22	122.13±76.89	<0.01 <0.001
TB(mg/dl)	0.50±0.20	5.45±6.16	76.72±41.50	<0.001 <0.001
GGT(IU/L)	13.28±4.94	35.64±25.84	4.53 ± 5.05	<0.001 <0.001
DB(mg/dl)	0.25±0.14	3.42±5.04	2.85±3.71	<0.001 <0.001
Albumin(g/dl)	4.21±0.54	2.17±0.51	2.20±0.55	<0.001 <0.001
T. protein (g/dl)	7.28±0.44	6.65±1.43	6.17±0.93	>0.05 <0.001
Prothrombin time	12.39±0.18	20.39±6.00	19.81±5.71	<0.001 <0.001
Prothrombinconc%	98.54±2.78	48.72±18.81	50.80±17.04	<0.001 <0.001
AFPng/ml	0.98±1.21	5.88±8.15	13999±2868	<0.001
Endoglinng/ml	4.02±0.84	6.17±1.80	6.44±1.67	P1<0.05P2<0.05 P3>0.05

P.value< 0.001 is considered very highly significant. P. value < 0.01 is considered highly significant. P.value< 0.05 is considered significant.P.value>0.05 is considered nonsignificant.

Table 2: Comparison between group IIa and group IIb as regard serum level of endoglin.

Variable	HCC with normal AFP n = 15 Mean ±SD	HCC with abnormal AFP n = 15 Mean ±SD	P-value
Endoglin (ng/ml)	6.20±1.78	6.68±1.57	>0.05 NS

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Table 3: Spearman's correlation coefficient of endoglin with different liver functions and measures in liver fibrosis & hepatocellular carcinoma groups.

Items	HCC& fibrosis patient groups	
	R	P value
AST(IU/L)	0.334	<0.01
ALT(IU/L)	0.159	>0.05
Alkaline phosphatase(IU/L)	0.399	<0.001
Total bilirubin(mg/dl)	0.281	<0.05
Direct bilirubin(mg/dl)	0.258	<0.05
Albumin (g/dl)	- 0.235	<0.05
Total protein(g/dl)	- 0.179	>0.05
GGT(IU/L)	0.497	<0.001
Prothrombin time	0.296	<0.05
Prothrombin conc.%	- 0.288	<0.05
Alpha fetoprotein(ng/ml)	0.429	<0.001

r= Spearman Correlation coefficient test

Table 4: Sensitivity, specificity, positive predictive value and negative predictive value of alpha fetoprotein and endoglin in fibrosis, hepatocellular carcinoma and control groups.

	Sensitivity	Specificity	PP value	NP value	Accuracy
AFP (ng/ml) Cut level 1.6	84 %	81 %	93.5 %	58%	83%
Endoglin (ng/ml) Cut level 4.8	80%	88 %	95.5%	53%	82 %

DISCUSSION

The present study showed statistical significant difference between group I,II and III as regard ALT ,AST, ALP,ALB,TB,DB,GGT and prothrombin time and concentration . These records could be related to the fact that aminotrasferases are typically elevated in all liver disorders, appearing to be more sensitive and specific tests for acute than chronic hepatocellular damage, with a more frequent usage in epidemiologic studies to document the incidence of viral hepatitis. Increased release, decreased clearance and/or impaired synthesis are all incriminated in their fluctuating levels[11]. As fibrosis progresses, bilirubin increasesas a result of reduced hepatic excretion and less enterohepatic circulation attributable to portal systemic shunt[12]. Also hypoalbuminaemia is more common among individuals with chronic liver disease reflecting both severe liver damage and decreased albumin synthesis[13]. The decrease of prothrombin concentration in advancing liver fibrosis indicates a damage of liver parenchyma resulting in decreased production of coagulation proteins with increased risk of bleeding tendencies[14].

The present study showed a highly statistical significant increase in serum endoglin in patients with liver fibrosis compared to control group .

These findings were in accordance with Yagmura et al, (2007), who found that serum CD105 is significantly elevated in cirrhotic patients compared with control[15]. During the inflammatory disease, as liver fibrosis, endoglin expression in endothelial cells is strongly up regulated and is consistently associated with an infiltrate of inflammatory cells[16].Clemente et al ., 2006 reported that activated hepatic stellate cells as well as portal and septal myofibroblasts expressed endoglin and circulating CD105 levels are associated with progressive hepatic fibrosis in chronic hepatitis C infection[17].

Also a highly statistical significant increase in serum endoglin in HCC group in comparison to control group. These findings is in agreement with the results reported by Yagmura et al., (2007) who found that HCC patients showed the highest endoglin concentrations comparison to healthy controls[15]. This could be explained by the prevention of inhibition of endothelial proliferation through TGF-b in liver cirrhosis due to CD105 overexpression as the development of an angiogenic response may depend on the balance between TGF-b and CD105 expression[18]. CD105 is weakly expressed in normal tissues, but it is strongly expressed in tumor endothelial cells. Recent studies have suggested that CD105 is a proliferation-associated marker of endothelial cells, and that its expression correlates strongly with cell proliferation markers in tumor endothelial cells[19].

The exact mechanisms for CD105 regulation of vascular development have not been fully elucidated. One hypothesis for high endoglin levels in such patients might be that CD105 antagonizes the inhibitory effects of TGF-b1 on human vascular endothelial cells and that CD105 is required for the formation of new blood vessels like that in tumor development . Therefore, high levels of serum CD105 found in HCC patient with liver fibrosis may reflect the rise of TGF-β in liver fibrosis owing to endothelial cell injury[20][5].

In the present study we found no statistical significant difference between HCC group and fibrotic group as regard serum endoglin level . An explanation for this is that the liver cell damage might induce hypoxia condition in liver tissues with subsequent angiogenesis and precancerous changes. During liver fibrosis and cirrhosis, fibrogenesis induces intrahepatic shunts and the barrier between the sinusoids and the hepatocytes . Fibrous pseudo lobes form as discrete hypoxia unit to induce angiogenesis. Furthermore, hepatitis B virus X protein increases the transcriptional activity and protein level of HIF-1alpha, and thereby promote angiogenesis during hepatocarcinogenesis . Therefore cells in cirrhotic liver are under a sustained, mechanically reduced blood flow, which induces angiogenesis in fibrotic tissues[21][22] .

No statistical significant difference was found between both HCC subgroups (with high alpha fetoprotein versus normal alpha fetoprotein) as regard endoglin level .

There were positive correlation between endoglin and AST, ALT, ALP, TB, DB, GGT, prothrombin time and AFP. While, negative correlations were found between serum endoglin and albumin ,TP and prothrombin conc.

The obtained results showed that the best cut off value for endoglin in serum in HCC and liver fibrotic patient groups was 5.1 ng/ml, as the diagnostic sensitivity was 80%, specificity of 88 %, positive predictive value was 95.5 %, negative predictive value was 53% and diagnostic accuracy was 82 %.

These findings were in accordance with the results reported by Yagmura et al, (2007) and Salem D et al., (2012) [15][19] who found the specificities of endoglin in HCC and in liver fibrosis and cirrhosis populations varied between 77.8 and 91.4%, whereas the sensitivities were found between 57.8 and 84.4% and they also confirmed the correlations obtained by our study.

It could be concluded that, endoglin has potential to be a novel complementary biomarker in pathogenesis of liver fibrosis and can be used as a non invasive technique for diagnosis of liver fibrosis. Meanwhile, endoglin cannot differentiate between fibrosis and HCC. Fortunately, its level in patients with HCC having normal AFP is as high as those with abnormal AFP, make it a possible marker for follow up of treatment. The cost benefit should be put in consideration to determine how far using of this marker together with AFP is useful for diagnosis of suspected patients with HCC specially with normal level of AFP.

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