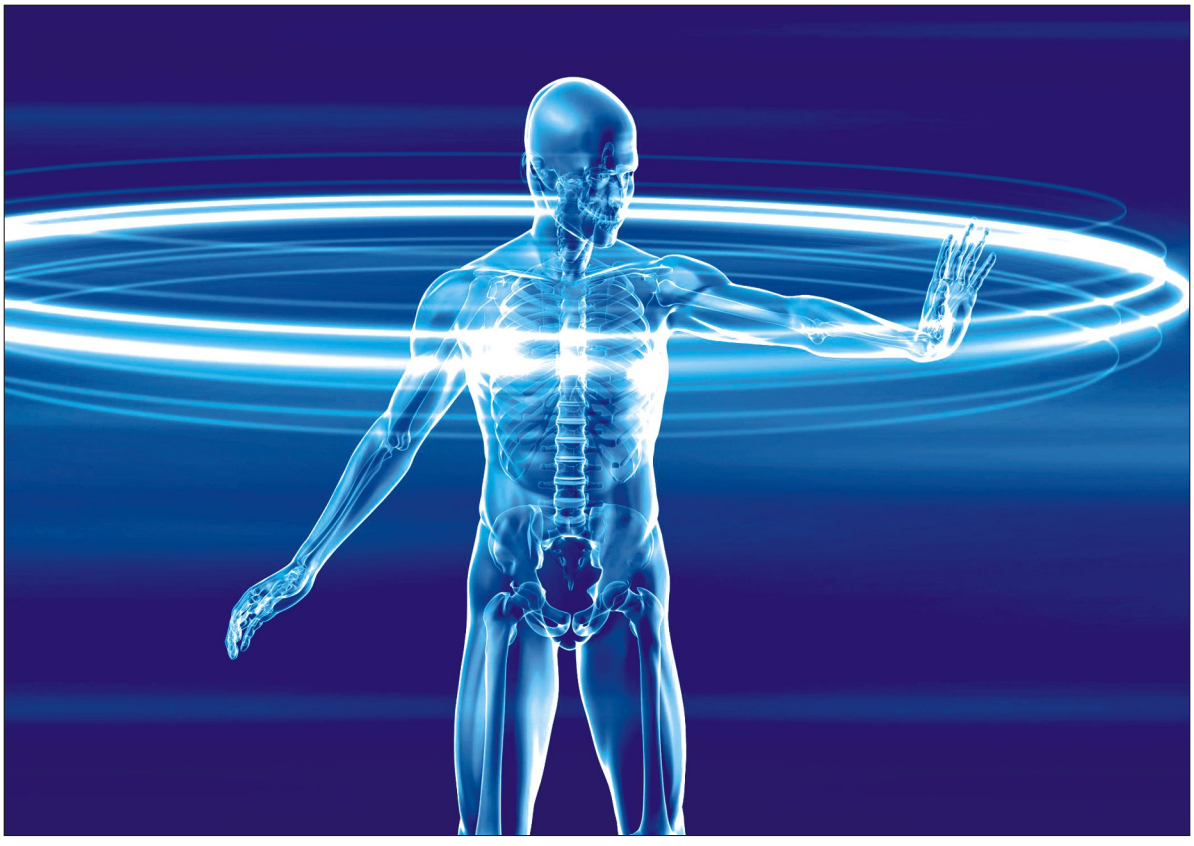

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Research Article
Serum Endoglin and IL-6 Levels as Complementary Diagnostic Biomarkers for Hepatocellular Carcinoma in Egyptian Liver Cirrhosis Patients

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Abstract

Background and Objective: Hepatocellular carcinoma is one of the most common fatal diseases in the Egyptian population. Alpha-fetoprotein is widely used for hepatocellular carcinoma screening and diagnosis. Now-a-days, it couldn't be considered as an effective diagnostic tool due to its low sensitivity and specificity. So, the aim of this prospective study was to evaluate the diagnostic value of serum endoglin and IL-6 as complementary biomarkers in hepatocellular carcinoma patients with underlying cirrhosis compared to alpha-fetoprotein. **Materials and Methods:** There were 70 individuals included and divided into three main groups, group I, 30 liver cirrhosis patients, group II, 30 liver cirrhosis patients with associated hepatocellular carcinoma and group III, 10 matched healthy volunteers as a control group. Serum IL-6 and endoglin levels were estimated in all subjects using the ELISA technique. **Results:** In group II patients, the sensitivity of endoglin and IL-6 was 93.3 and 83.3%, respectively versus 60% for α-fetoprotein. The combined use of endoglin and α-fetoprotein improved the sensitivity to 97% while the combined use of IL-6 and α-fetoprotein improved the sensitivity to 94%. **Conclusion:** Based on the results reported in this study, it was found that endoglin and IL-6 are very useful tools for the diagnosis of hepatocellular carcinoma in Egyptian cirrhotic patients. They were found more specific and sensitive than AFP. Both serum markers showed significant higher levels in hepatocellular carcinoma patients compared to the liver cirrhosis patients and the control group. Endoglin and IL-6 were found to be more sensitive, specific and reliable serum markers than α-fetoprotein for diagnosis and early detection of liver cirrhosis associated hepatocellular carcinoma. The combined use of each one of them with α-fetoprotein can improve the sensitivity and the specificity in the diagnosis of hepatocellular carcinoma. So, they could be useful complementary biomarkers in the diagnosis of liver cirrhosis associated hepatocellular carcinoma.

Key words: Hepatocellular carcinoma, liver cirrhosis, serum α-fetoprotein, serum endoglin, serum IL-6

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most commonly encountered primary malignant tumor affecting the liver with high morbidity and mortality rates. Although, it is not so common in Western countries, it is considered as one of the most common cancers in Africa and Far East because hepatitis is endemic in such areas[1].

Liver cirrhosis is one of the risk factors for development of HCC. It was reported that 80% of HCC patients had had preexisting liver cirrhosis[2].

Alpha-fetoprotein (AFP) is the primary and the most widely used marker for HCC. The AFP levels combined with abdominal ultrasonography were considered the standard measures for HCC screening in patients with liver cirrhosis. However, AFP is increased in only 40-75% of HCC patients[3] and about 40% of patients with early HCC show normal levels of AFP[4].

In addition, AFP can be elevated in other hepatic lesions such as, viral hepatitis, intrahepatic cholangiocarcinoma and in cases of cancer colon with liver metastasis. Thus, AFP is considered as an unsatisfactory diagnostic tool because of its low sensitivity and specificity. Moreover, it cannot be used alone as a screening test for HCC[5].

Therefore, the search for new biomarkers that could be used as an adjuvant to AFP to improve its sensitivity and specificity for HCC detection becomes mandatory[6].

Endoglin (CD105) is a cell-membrane glycoprotein weakly expressed in normal endothelial cells but over-expressed in endothelia of vessels in several human solid malignancies. It is considered as a marker associated with endothelial cells proliferation. It is a part of transforming growth factor-β (TGF-β) receptor complex as it attaches to TGF-β1 and TGF-β3 with high affinity[7,8].

Endoglin expression is mainly tissue specific, especially in vascular endothelial cells of tissues with active angiogenesis, such as inflamed tissue and tumor stroma[9].

Therefore, endoglin is suggested to be a useful marker not only in diagnosis of HCC but also in its follow up[10,11].

Interleukin-6 (IL-6) is a pleiotropic cytokine secreted by T-cells and can stimulate B-cells proliferation, differentiation and antibody production[12]. The IL-6 may also act as a growth factor as it regulates cell growth in different malignancies and is probably responsible for paraneoplastic symptoms like fever, weight loss and night sweating[13].

It was noticed that the increased production of IL-6 has been associated with different human diseases, such as autoimmune disease, chronic inflammatory disease and many types of cancer. In the last century, elevation in serum IL-6 levels was observed in patients with primary liver cancer[14]. The HCC patients have markedly elevated levels of IL-6 than the healthy controls[15].

So, the aim of this study was to evaluate the possible role of both endoglin and IL-6 as complementary biomarkers that could be used in the screening and diagnosis of HCC in cirrhotic patients and to compare their diagnostic performance as possible tumor markers with that of serum AFP.

MATERIALS AND METHODS

This study was a prospective longitudinal case-control study. It was performed at Tanta University Hospitals between December, 2014-2015 on patients admitted to Tropical Medicine Department. Written informed consent was obtained from all participants. Ethical approval for this study was provided by ethics and research committee of our institute.

All participants were subjected to full history taking, complete clinical examination, abdominal ultrasound, laboratory investigations including: Serum AFP, serum endoglin and serum IL-6.

Studied individuals were classified into 3 groups:

- **Group I:** Included 30 liver cirrhosis patients without HCC
- **Group II:** Included 30 liver cirrhosis associated HCC patients

This group was subdivided into 2 subgroups according to serum level of AFP suggested by Peng *et al.*[16]:

  - Liver cirrhosis associated HCC patients with serum AFP level >400 ng mL^{-1}. This subgroup included 20 patients
  - Liver cirrhosis associated HCC patients with serum AFP level <400 ng mL^{-1}. This subgroup included 10 patients

- **Group III:** Included 10 apparently healthy volunteers with matched age and sex having no acute or chronic illness, with normal liver functions as controls

All patients who had chronic inflammatory diseases, hematological malignancies and/or cancer of any organs other than the liver were excluded from the study.

At least, one of the following criteria was a must for the diagnosis of HCC according to the guidelines of clinical diagnosis and staging for hepatocellular carcinoma[17]:

- Hepatic space occupying lesion with a serum AFP level ≥400 ng mL^{-1}
- Hepatic space occupying lesion with arterial phase enhancement and rapid washout in portovenous phase in triphasic CT

Sampling and procedure: Five milliliters venous blood was collected from each subject under complete aseptic technique. The blood was left to clot, then the serum was separated by centrifugation at 1000×g for 15 min. The separated serum was stored at -20°C for estimation of AFP, endoglin and IL-6 levels.

The AFP was measured by quantitative ELISA technique supplied by Phoenix Pharmaceutical Inc., USA. Serum endoglin was measured by quantitative ELISA technique supplied by Diagnostic Automation Inc., USA. Detection and quantitative measurement of IL-6 in serum were done using AviBion human IL-6 ELISA kit (Orgenium Laboratories, Finland) according to manufacturer's instruction.

Statistical analysis: Results were collected, tabulated and statistically analyzed using statistical package SPSS version 10 (Chicago, USA). Quantitative data are presented as Mean±SD. A student t-test was used for comparison of means of 2 groups. One-way ANOVA test was used to compare more than 2 groups. Post ANOVA comparisons were done using Tukey test. Pearson's correlation was used for detection of the relation between 2 variables. A $p<0.05$ was considered statistically significant.

RESULTS

Comparison of Mean±SD of tested markers between diseased groups and control group was demonstrated in (Table 1). It shows that, the level of serum AFP in patients with liver cirrhosis associated HCC was significantly higher than those of healthy controls and liver cirrhosis.

It also demonstrates that the levels of endoglin and serum IL-6 in patients with liver cirrhosis were significantly higher than those of healthy controls. Levels in patients with liver cirrhosis associated HCC were significantly higher than those of healthy controls and liver cirrhosis.

The correlation between serum AFP, endoglin and IL-6 in groups I and II was demonstrated in Table 2. The table showed positive correlation between serum AFP, endoglin and IL-6 in the studied groups.

There was no statistically significant difference between endoglin and IL-6 serum levels in the liver cirrhosis associated HCC patients with AFP <400 ng mL^{-1}, compared to the liver cirrhosis associated HCC patients with AFP >400 ng mL^{-1} (Table 3).

Sensitivity, specificity, positive predictive and negative predictive values and accuracy of serum AFP, serum endoglin and serum IL-6 in diagnosis of liver cirrhosis associated

Table 1: Comparison of Mean±SD of different biochemical and immunological parameters between the diseased groups and the control group (n = 70)

	Groups			ANOVA		Tukey test		
	Cirrhosis (n = 30)	HCC (n = 30)	Control (n = 10)	F-value	p-value	Cirrhosis and HCC	Cirrhosis and control	HCC and control
AFP (ng mL^{-1})								
Range	3.0- 35.00	10.00-6415.00	2.1-24.00	36.46	<0.001*	<0.001*	0.899	<0.001*
Mean±SD	14.12±8.51	2377.02±2144.66	10.99±6.75					
Endoglin (ng mL^{-1})								
Range	3.20-10.30	4.60-16.75	2.90-6.95	43.34	<0.001*	<0.001*	<0.001*	<0.001*
Mean±SD	7.35±2.23	10.35±3.83	4.03±1.06					
IL-6 (pg mL^{-1})								
Range	10.40-50.20	26.00-177.80	4.11-9.25	83.74	<0.001*	<0.001*	<0.001*	<0.001*
Mean±SD	32.01±10.99	91.18±43.61	6.59±1.57					

*Significant ($p<0.05$)

Table 2: Correlation between serum AFP, endoglin and IL-6 of the studied liver cirrhosis and liver cirrhosis associated HCC patients (n = 60)

		AFP (ng mL^{-1})		Endoglin (ng mL^{-1})	
Groups		r	p-value	r	p-value
Liver cirrhosis (n = 30)	Endoglin (ng mL^{-1})	0.859	<0.001*		
	IL-6 (pg mL^{-1})	0.706	<0.001*	0.780	<0.001*
HCC (n = 30)	Endoglin (ng mL^{-1})	0.864	<0.001*		
	IL-6 (pg mL^{-1})	0.809	<0.001*	0.840	<0.001*

*Significant ($p<0.05$), r: Pearson correlation coefficient

Table 3: Comparison between endoglin and IL-6 in the liver cirrhosis associated HCC patients with AFP <400 ng mL^{-1} (n = 10) and in the liver cirrhosis associated HCC patients with AFP >400 ng mL^{-1} (n = 20)

	Mean±SD		t-test	
HCC (n = 30)	Serum AFP <400 ng mL^{-1} (n = 10)	Serum AFP >400 ng mL^{-1} (n = 20)	t	p-value
Endoglin (ng mL^{-1})	13.960±2.002	15.612±2.981	1.679	0.104
IL-6 (pg mL^{-1})	150.106±24.820	173.897±41.341	1.785	0.085

Table 4: Sensitivity, specificity, positive predictive and negative predictive values and accuracy of serum AFP, serum endoglin and serum IL-6 in diagnosis of liver cirrhosis associated HCC

Marker	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Nagitive predictive value (%)	Accuracy (%)
Serum AFP (Cut-off value at >35 ng mL^{-1})	60.0	91.0	89.0	76.9	85.9
Serum endoglin (Cut-off value at >9.11 ng mL^{-1})	93.3	92.5	90.5	94.9	95.1
Serum IL-6 (Cut-off value at >47.98 pg mL^{-1})	83.3	91.5	90.0	88.6	94.5
Combined endoglin (>9.11 ng mL^{-1}) and AFP (>35 ng mL^{-1})	97.0	98.0	96.15	100	98.67
Combined IL-6 (>47.98 pg mL^{-1}) and AFP (>35 ng mL^{-1})	94.0	93.0	91.15	95	94.53

HCC patients were shown in Table 4. The best results were obtained by the combination of serum endoglin and serum AFP at cut-off values >9.11 and >35 ng mL^{-1}, respectively.

DISCUSSION

In the present study, AFP was statistically higher in HCC cirrhotic liver patients in comparison with cirrhotic patients without HCC and control group ($p<0.001$).

These findings are in accordance with Peng *et al.*[16] who reported that AFP is often significantly elevated in HCC patients when compared with cirrhotic patients.

Moreover, Wang *et al.*[18] demonstrated that almost all HCCs detected had high level of AFP and a significant difference was observed when compared with the control group.

On the other hand, Cedrone *et al.*[19] found that AFP levels were normal in the majority of patients with HCC. Also, Tariq[20] demonstrated that AFP serum concentrations are normal in up to 40% of HCCs.

In the present study, it was found that the sensitivity, specificity, positive predictive, negative predictive values and accuracy of serum AFP in diagnosis of liver cirrhosis associated HCC patients among the studied groups at cut-off value >35 ng mL^{-1} were 60, 91, 89, 76.9 and 85.9%, respectively .

Our results were to a great extent similar to the results of Soresi *et al.*[21] who showed that the best cut-off value of AFP has been reported to be 30 IU mL^{-1} with 65% sensitivity and 89% specificity.

Gomaa *et al.*[22] revealed that AFP value above 400 IU mL^{-1} has been considered to be diagnostic for HCC in patients with cirrhosis. Zhou *et al.*[23] reported that some investigations have shown that the cut-off value is fluctuant in different ethnic groups and one of the possible reasons for this difference is the diverse living circumstance which has a great influence on epidemiology.

Lau and Lai[24] stated that the specificity of AFP is very high when the levels are above 400 IU mL^{-1}. Lok *et al.*[25] demonstrated that AFP levels are increased progressively from normal healthy subjects and compensated cirrhosis to histologically proven HCC.

In the present study, there was a significant increase in the level of serum endoglin in the liver cirrhosis patients compared with the control group and in liver cirrhosis associated HCC patients compared with the other groups.

These findings are in accordance with studies of Reda *et al.*[26], Selim and Ahmed[27] and Elnemr *et al.*[28] who found that serum endoglin was significantly increased in liver cirrhosis compared with the control group and in HCC compared with the other groups.

In the present study it was found that the sensitivity, specificity, positive predictive and negative predictive values and accuracy of serum endoglin in diagnosis of liver cirrhosis associated HCC patients among the studied groups at cut-off value >9.11 ng mL^{-1} were 93.3, 92.5, 90.5, 94.9 and 95.1%, respectively.

Yagmur *et al.*[2] revealed that at cut-off value 6.9 ng mL^{-1}, the sensitivity of endoglin was 57.8%, the specificity was 78.9%.

Selim and Ahmed[27] showed that, the best cut-off value for endoglin to differentiate HCC and liver cirrhosis groups was 6.9 ng mL^{-1} and the diagnostic sensitivity was 72%, specificity was 80%, positive predictive value was 78.26%, negative predictive value was 74.07% and diagnostic accuracy was 76%.

In the present study, there was a significant increase in the level of serum IL-6 in the liver cirrhosis patients compared with the control group and there was a significant increase in

the level of serum IL-6 in the liver cirrhosis associated HCC patients compared with the other groups.

These findings are in agreement with Song *et al.*[29] who have confirmed that serum IL-6 level was increased in patients with established HCC.

Also, Soresi *et al.*[21] found that the median IL-6 levels in cirrhosis associated HCC patients were higher than those in cirrhotic patients and the controls. Cirrhotic patients also had higher median IL-6 values than controls. The IL-6 values significantly increased as the disease worsened, indicating that neoplastic degeneration even in its initial stages, causes variations in IL-6 levels, which could enable us to discriminate cirrhotic from HCC patients.

Othman *et al.*[30] showed that serum levels of IL-6 were significantly higher in all patients groups compared with the control group and they found significantly higher circulating IL-6 titers in HCC than in the cirrhotic group.

On the other hand, Zekri *et al.*[31] and Tovey *et al.*[32] showed that IL-6 was apparently normal in both HCC and patients with chronic liver disease.

On contrary to our results, Metwaly *et al.*[33] found a significant decrease in serum IL-6 concentration in HCC patients as compared with patients with liver cirrhosis.

In the present study, it was found that the sensitivity, specificity, positive predictive and negative predictive values and accuracy of serum IL-6 in diagnosis of liver cirrhosis associated HCC patients among the studied groups at cut-off value >47.98 pg mL^{-1} were 83.3, 91.5, 90, 88.6 and 94.5%, respectively .

A study of Porta *et al.*[15] reported that at cut-off value 12 pg mL^{-1}, IL-6 sensitivity was 73% and specificity was 87%.

Othman *et al.*[30] reported that at cut-off value of IL-6 (8.6 pg mL^{-1}), the sensitivity was 90% and specificity was 86.67% and accuracy 87.5%.

In the present study, it was found that the combined use of serum endoglin and serum AFP at cut-off values >9.11 and >35 ng mL^{-1}, respectively significantly increased sensitivity and specificity for diagnosis of HCC among the studied groups to 97 and 98%, respectively.

These findings are in accordance with Elnemr *et al.*[28] who reported that the combination of both markers improved the overall sensitivity from 70-85%.

Reda *et al.*[26] showed that the combined use of AFP and endoglin led to an increase in the sensitivity of AFP from 43.3-93.3%.

Selim and Ahmed[27] showed that the combination of both AFP and endoglin improves overall accuracy (79%), sensitivity (89%), specificity (85%), PPV (84%) and NPV (77%) in prediction of HCC.

In the present study, it was found that the combined use of serum IL-6 and serum AFP at cut-off values >47.98 pg mL^{-1} and >35 ng mL^{-1}, respectively significantly increased the sensitivity and specificity for the diagnosis of HCC among the studied groups to 94 and 93%, respectively.

These findings were in accordance with Porta *et al.*[15] who found that IL-6 could be considered as a promising tumor marker for HCC. In particular, the diagnostic value of the test is significantly increased when it is associated with AFP. Combining the two markers provides a new perspective in the diagnosis of HCC.

Wong *et al.*[34] showed that when AFP and IL-6 criteria were combined, the sensitivity of diagnosing HCC increased to 83%. Also, El-Folly *et al.*[35] and Haque *et al.*[36] found that combination of IL-6 and AFP improved the sensitivity in diagnosing HCC and predicting future HCC development.

In the present study there was a positive correlation between serum AFP, serum endoglin and serum IL-6 of the studied cirrhotic and cirrhosis associated HCC patients. El-Folly *et al.*[35] and Haque *et al.*[36], found similar results and showed a significant positive correlation between mean IL-6 and AFP levels in HCC patients. Abdu Allah *et al.*[37] found a positive correlation between endoglin and AFP.

Reda *et al.*[26] showed that there was a significant positive correlation between serum endoglin and serum AFP in chronic liver diseases and a highly significant positive correlation between them in the HCC group.

In the present study there was no significant difference between endoglin and IL-6 in liver cirrhosis associated HCC patients with serum AFP level <400 ng mL^{-1} and in liver cirrhosis associated HCC patients with serum AFP level >400 ng mL^{-1}.

Hsia *et al.*[38] found that IL-6 is helpful to identify a subset of HCC patients with low AFP level and may serve as complementary tumor marker with AFP in diagnosis of HCC.

Abdu Allah *et al.*[37] showed that there was no statistically significant difference between both HCC subgroups (with high AFP versus normal AFP) as regard endoglin level. Endoglin level in HCC cirrhotic patients with normal AFP is as high as those with high AFP. Thus, endoglin could be used as a useful possible diagnostic marker in HCC patients with normal AFP.

Othman *et al.*[30] found that high levels of IL-6 are observed in HCC patients and may be helpful to identify a subset of HCC patients with low AFP level.

CONCLUSION

The results of the present study suggest that endoglin and IL-6 were more sensitive, specific and reliable serum

markers than AFP for diagnosis and early detection of liver cirrhosis associated HCC. The combined use of each of them with AFP can improve the sensitivity and the specificity in the diagnosis of HCC. Also, endoglin and IL-6 are helpful to identify a subset of cirrhosis associated HCC patients with low serum AFP level.

So, they could be useful complementary biomarkers in the diagnosis of liver cirrhosis associated HCC.

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