Tumor necrosis factor-alpha and alpha-fetoprotein as biomarkers for diagnosis and follow-up of hepatocellular carcinoma before and after interventional therapy

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Introduction
Up to 90% of the hepatocellular carcinoma (HCC) cases in Egypt were attributable to hepatitis C virus (HCV) infection. The absolute positive and negative markers for HCC are still deficient. Alpha-fetoprotein (AFP), the most widely used biomarker for early detection and clinical follow-up of patients with HCC, has a sensitivity and a specificity of 41–65% and 80–94%, respectively, even with low cutoff value at 20 ng/ml. High plasma levels of tumor necrosis factor-alpha (TNF-α) are associated with some cancers, and it has an important central role in hepatocarcinogenesis and involved in cancer invasion with or without metastasis.
Aim
To evaluate the diagnostic accuracy of TNF-α versus AFP as biomarkers for detection of HCC on top of HCV-related cirrhosis and to assess treatment response by using TNF-α and AFP after locoregional intervention of HCC.
Patients and methods
A total of 27 normal control, 51 cirrhotic patients, and 69 cirrhotic patients with HCC were studied in two phases. Radiofrequency ablation and transarterial chemoembolization were done, and patients were followed up for response and tumor marker values.
Results
TNF-α in the diagnosis of Egyptian patients with HCC related to HCV cirrhosis had a sensitivity of 100% and a specificity of 94.1% at a cutoff value of more than or equal to 30 pg/ml. Moreover, more than or equal to 15.2% decrement is a good predictor of complete ablation versus partially or failed ablation with a sensitivity of 78.6%, a specificity of 83.3%, and overall accuracy of 80.77%.
Conclusion
Combined use of TNF-α in addition to AFP increases sensitivity and specificity for early diagnosis of HCC rather than the use of each tumor marker alone. Moreover, TNF-α could be a better noninvasive tumor marker than AFP for assessment of response after locoregional therapy of HCC.

Keywords:
alpha-fetoprotein, hepatocellular carcinoma, hepatitis C virus, tumor necrosis factor-alpha

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, with more than 1 000 000 new cases worldwide annually [1]. Globally, it is the second leading cause of cancer-related death.

Up to 90% of the HCC cases in Egypt were attributable to hepatitis C virus (HCV) infection [2]. This high figure was explained by the fact that Egypt has the highest rate of HCV in the world, with estimated range from 6 to 28% [3].

The absolute positive and negative markers for HCC are still deficient, and even those characterized by very high sensitivity and specificity do not have a widespread diagnostic practicality [4].

Alpha-fetoprotein (AFP) is the most widely used biomarker for early detection and clinical follow-up of patients with HCC [5] and has a sensitivity and a specificity of 41–65% and 80–94%, respectively, with a low cutoff value at 20 ng/ml [6].

Internationally, AFP at a cutoff level of 200 ng/ml is indicative of HCC [7]. However, it does not satisfy results in early detection of HCC, limiting its application owing to its low positive rate and high false-positive and false-negative results [8]. Acute and

chronic viral hepatitis as well as patients with HCV-related cirrhosis may be associated with slightly high AFP values [5].

Tumor necrosis factor-alpha (TNF-α) is one of the cytokines produced by macrophage in several physiological, inflammatory, and malignant conditions. It has multiple opposing tumorigenic effects and is considered a key mediator of cancer-related chronic inflammation in some malignant human cancers [9].

Higher plasma levels of TNF-α are associated with disease progress in some cancers and known as an important mediator of cancer cachexia [10].

It has an important central role in hepatocarcinogenesis and is involved in cancer invasion with or without metastasis [11].

Moreover, it is overexpressed specifically in larger-sized, multinodular, massive-type or metastatic HCCs. However, its potential role in HCC diagnosis and therapy is still unclear [12,13].

The aim of this study is to evaluate the diagnostic accuracy of TNF-α versus AFP as biomarkers for detection of HCC on top of HCV-related cirrhosis and to assess treatment response by using TNF-α and AFP after locoregional intervention of HCC.

Patients and methods

This is a case–control (phase I) and follow-up prospective (cohort) (phase II) study that was performed on 120 patients (51 cirrhotic without HCC and 69 cirrhotic with HCC) selected from the Outpatient Hepatology Clinics and HCC Early Detection Clinic, Specialized Medical Hospital, Mansoura University, Egypt, from March 2015 to March 2017.

Inclusion criteria

The first phase was conducted on 120 patients who were divided into three groups (control vs. cirrhotic groups with or without HCC).

Group 1 included 27 normal individuals as control group.
Group 2 included 51 cirrhotic patients without HCC diagnosed clinically, laboratory, and radiologically. This group was further subdivided according to Child–Turcotte–Pugh score into classes A, B, and C [14,15] and Model for End-Stage Liver Disease score [16].
Group 3 included 69 cirrhotic patients with HCC proved radiologically by abdominal ultrasound and triphasic abdominal computed tomography (CT) and subdivided according to Barcelona Clinic Liver Cancer (BCLC) scoring [17] to classify HCC cases.

Exclusion criteria

Patients with other malignancies such as gastric and breast cancer and multiple myeloma; severe comorbidity such as uncontrolled diabetes mellitus, hypertemsion, and heart failure; morbid obesity (BMI $\geq$40 kg/m^2); severe psychosis; autoimmune diseases such as systemic lupus erythemaosus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and autoimune hepatitis syndrome (AIH); other causes of liver cirrhosis (e.g., hepatitis B virus and Wilson disease); patients with inflammation such as spontaneous bacterial peritonitis; or those with chest infection were excluded.

Methods

Phase I study:

(1) Thorough history taking and clinical examination were done.
(2) Laboratory tests included biochemical tests, such as serum albumin and bilirubin; liver enzymes such as aspartate transaminase and alanine transaminase, and international normalized ratio; serum creatinine; complete blood count; erythrocyte sedimentation rate; antinuclear antibody; glycated hemoglobin; HCV; and hepatitis B virus. Tumor biomarkers included serum AFP and TNF-α levels.
(3) Imaging studies were done to assess liver state (cirrhosis or HCC on top of cirrhosis) including abdominal ultrasound (liver, spleen, portal vein, or ascites) and triphasic CT abdomen (for HCC group diagnosis).

Phase II study:

(4) Interventional management of some patients was done: management of patients with HCC was done according to BCLC staging system [18] with radiofrequency ablation, transarterial chemoembolization, or supportive treatment.
(5) Radiological assessment of treatment response was done:
Assessing treatment response after 1 month of radiofrequency ablation or after 3 months of transarterial chemoembolization by new triphasic CT according to EASL guideline [19].
Complete response represented disappearance of intratumoral arterial enhancement, reflecting complete necrosis.

Partial response represented more than or equal to 50% decrease of enhanced areas.
Progressive disease represented more than or equal to 25% increase in the size of one measurable lesion or appearance of a new lesion.
Stable disease represented any tumor response between partial response and progressive disease.

(6) Postinterventional assessment of tumor biomarkers was done at the time of radiological assessment.
(7) Assessment of percent changes in AFP and TNF-α after intervention for predicting response was done. Pre-interventional tumor marker level and post-interventional level.

All patients signed a written informed consent form to participate in the study. This study was approved by Mansoura Institutional Ethics and Investigation Committees.

Data were analyzed using SPSS (USA), version 21. Analyzed data were represented as mean±SD and frequency (number-percent). For analysis of quantitative data, *t* test was used for comparison of two groups, and to compare more than two groups, one-way analysis of variance test was used. To compare qualitative data, χ^2 test was used. *P* value less than 0.05 was considered significant at 95% confidence interval.

Discussion

The incidence of HCC is increasing in highly endemic areas of HCV infection [1] and up to 90% of patients with HCC develop cirrhosis [20,21]. In Egypt, it ranks second and sixth most common cancer among men and women, respectively, owing to high incidence of viral hepatitis [22].

Goldman *et al.* [2] stated that 90% of HCC cases in Egypt were attributable to HCV infection.

The increasing prevalence of HCC and high incidence of HCV infection in Egypt make screening programs and surveillance of HCV patients as important tools for early detection of small HCCs [23].

The best known and widely used current serum biomarker for HCC is AFP [6,24]. However, its level may increase in chronic liver disease, but it remains low in most patients with cirrhosis in the absence of HCC [24]. It showed also high false-positive and negative results [8].

The standard surveillance protocol depends on two nonideal tools, ultrasound and AFP, which enable early HCC detection in only 30% of high-risk patients [25].

AFP has low sensitivity of 64% at a cutoff level of 20 ng/ml, 22.4% at a cutoff level of more than or equal to 200 ng/ml, and of 17.1% at a cutoff level of more than or equal to 400 ng/ml [26,27].

The current study confirmed the inaccuracy of AFP with sensitivity of 13% at a cutoff level more than or equal to 200 ng/ml, 42.02% at a cutoff level more than or equal to 20, and 92.8% at the study cutoff level more than or equal to 2.62 ng/ml.

On the contrary, AFP has a specificity of 91% at a cutoff level of 20 ng/ml [27] and nearly 99% at a cutoff level of more than or equal to 200 ng/ml [28].

This is the same as our study which also confirmed this high specificity (98%) at cutoff more than or equal to 200 ng/ml which decreased to 70.58% at cutoff level more than or equal to 20 and 35.3% at the study cutoff level. This clarifies the difficulty of adopting a particular cutoff value because as the value goes up, specificity goes up, whereas sensitivity goes down [29] (Table 1).

Table 1 Clinicodemographic and laboratory data of hepatitis C virus cirrhotic groups

Variables	Cirrhotic without HCC (*N*=51)	Cirrhotic with HCC (*N*=69)	Statistic *P**
Sex			
Male	26 (51)	57 (82.6)	0.001
Female	25 (49)	12 (17.4)	
Age	54.84±7.72	58.54±7.89	0.021
AST (U/l)	72.27±53	83.71±73.68	0.127
ALT (U/l)	52.92±35.09	68.16±53.66	0.018
Serum creatinine (mg/dl)	1.06±0.5	0.94±0.29	0.272
Hemoglobin (g/dl)	11.18±2.12	11.95±2.15	0.092
WBCs (/cmm^3)	6.01±2.76	5.19±3.22	0.034
Platelet count (/cmm^3)	108.17±54.07	119.29±65.24	0.506
Serum albumin (g/ml)	2.99±0.79	3.32±0.69	0.008
Serum bilirubin (mg/dl)	3.61±5.44	2.22±3.62	0.004
INR	1.42±0.34	1.22±0.19	0.001
CTP score	8.29±2.71	6.78±1.85	0.002
MELD	12.79±6.79	8.86±4.50	0.001

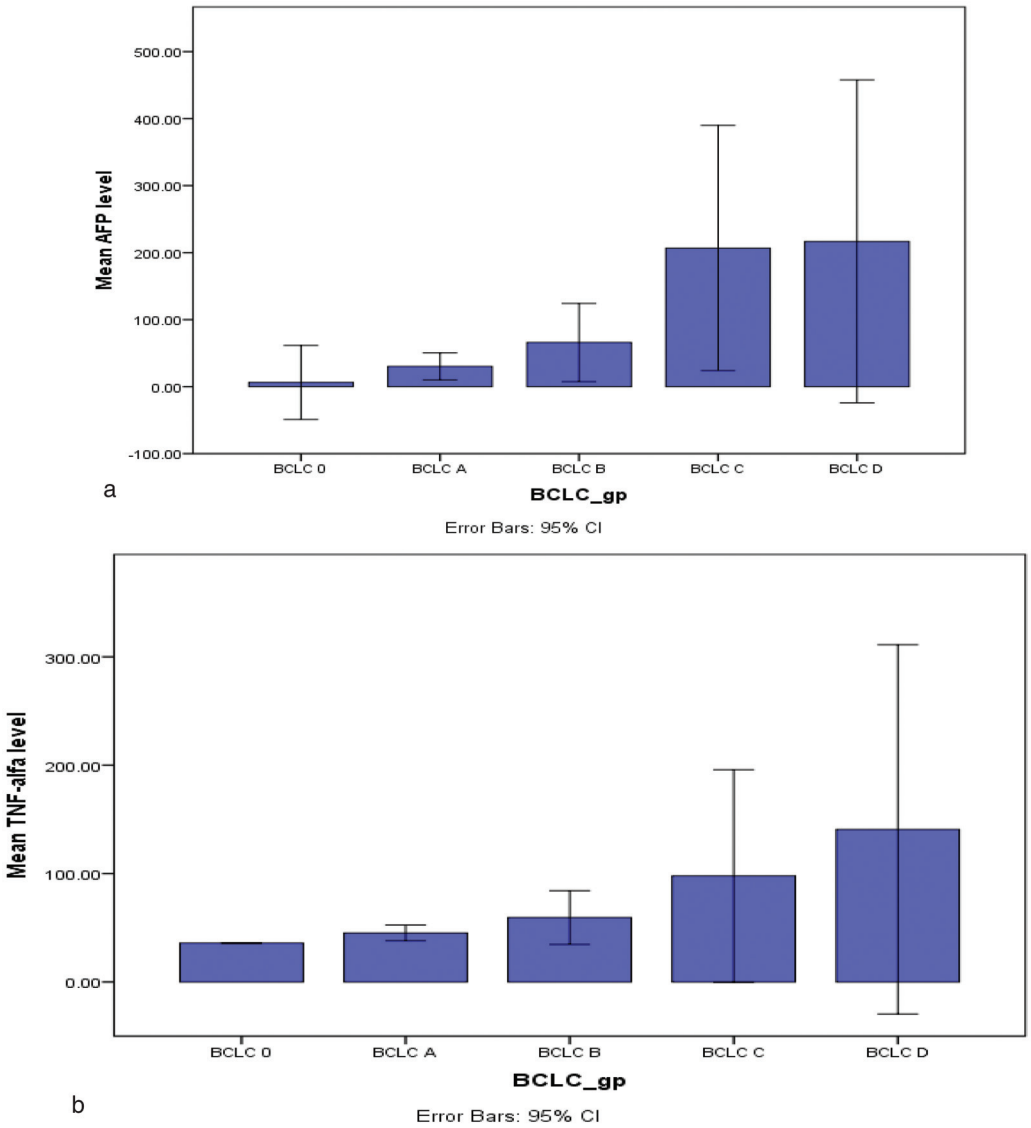
Data are presented as mean±SD and *n* (%). All patients were positive for hepatitis C virus antibodies, and those with HCC were significantly male (82.6%, *P*=0.001), with higher age (*P*=0.021). ALT, alanine transaminase; AST, aspartate transaminase; CTP, Child–Turcotte–Pugh; HCC, hepatocellular carcinoma; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; WBC, white blood cell.

Table 2 Comparison of alpha-fetoprotein and tumor necrosis factor-alpha in all study groups and subanalysis between different groups

Parameters	Groups			*P** (Kruskal-Wallis)
	Control	Cirrhotic without HCC Mean±SD (95% CI)	Cirrhotic with HCC	
AFP (ng/ml)	1.59±0.38 (1.45–1.75)	32.08±92.04 (6.19–57.96)	121.43±276.18 (55.09–187.78)	<**0.0005**
TNF-α (pg/ml)	16.29±4.53 (14.49–18.08)	22.41±5.89 (20.76–24.07)	80.08±160.24 (41.59–118.58)	<**0.0005**
Each 2 groups		*P*** (pairwise comparisons of groups)		
		AFP	TNF-α	
Control vs. cirrhotic without HCC		<**0.0005**	0.076	
Control vs. HCC		<**0.0005**	<**0.0005**	
Cirrhotic without HCC vs. HCC		**0.005**	<**0.0005**	

Highly statistically significant differences in the levels of AFP, and TNF-α among all three groups. On subanalysis, there were significant differences in the level of both AFP and TNF-α in comparing all groups except for TNF, which is not significantly increased in control versus patients without HCC. AFP, alpha-fetoprotein; CI, confidence interval; HCC, hepatocellular carcinoma; TNF-α, tumor necrosis factor-alpha. *Significant. **High significance. The bold valued very high significant *P*** very high significance.

Figure 1



(a) Mean AFP level in different BCLC classes. It showed that there was a statistically nonsignificant increase in AFP level through different BCLC classes. (b) Mean TNF-α level in different BCLC classes. It showed that there was a statistically significant increase in TNF-α level through different BCLC classes. AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; TNF-α, tumor necrosis factor-alpha.

A statistically highly significant difference in AFP level among all three study groups ($P<0.0005$) was noted (Table 2). This is in agreement with the results of Elbedewy *et al.* [30], Zakhary *et al.* [31], and Gopal *et al* [32].

TNF-α level also showed a statistically highly significant difference among all three study groups ($P<0.0005$). This is in accordance with several studies[31,23,30].

Post-hoc analysis revealed that the significant difference in AFP level exists between each pair of the three groups (HCC>cirrhosis>control), going hand in hand with Elbedewy *et al.* [30]. However, for TNF-α, the significant difference exists between HCC group and control group (HCC>control), which is in agreement with many studies [33–35].

In our study, TNF-α showed statistically significant increase as the tumor progresses through BCLC staging system, especially classes (C and D) ($P=0.032$), which is in agreement with results of Elbedewy *et al.* [30]. Moreover, it showed statistically significant difference between early and late HCC BCLC stages (Fig. 1).

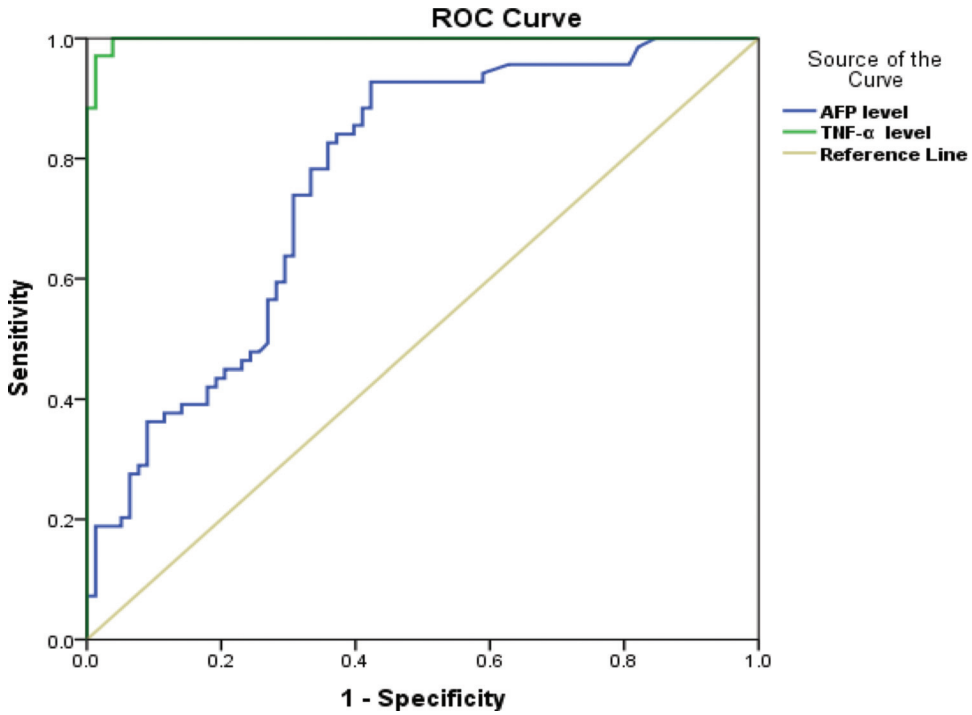
TNF-α was one of the most sensitive cytokines used as markers for disease progression in HCV-infected patients [36] and serum level of TNF-α revealed significant rise with the progression of the disease [37]. This could be explained by the vital role of TNF-α in HCC advancing process [11,37] and can be considered a marker of hepatocyte damage [38] with increasing secretion of TNF-α with higher stages of inflammation and fibrosis [37].

Our study confirmed high diagnostic accuracy of TNF-α in the diagnosis of Egyptian patients with HCC related to HCV cirrhosis, with sensitivity of 100% and a specificity of 94.1% at a cutoff value of more than or equal to 30 pg/ml. So, TNF-α was found to be a better tumor marker for diagnosis of HCC than AFP.

Overall accuracy (area under the receiver operating characteristic) of TNF-α and AFP were 0.998 and 0.764, respectively, and ROC curves showed that AUC for TNF-α was significantly higher than that for AFP ($P<0.0005$).

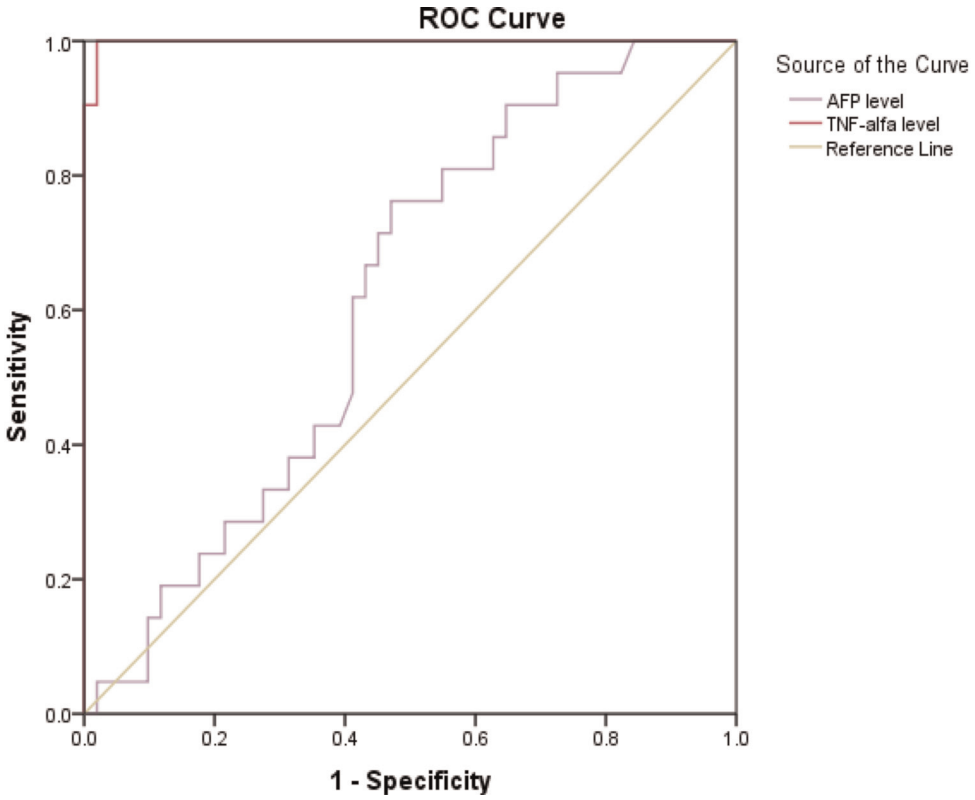
These results go hand in hand with several studies with sample size smaller than our study and lower diagnostic accuracy (Figs 2–4).

Figure 2



Receiver operating characteristics (ROC) curves of TNF-α and AFP for diagnosis of HCC. Tumor marker levels are plotted for their ability to predict HCC, showing that overall accuracy (area under the receiver operating characteristic) of TNF-α and AFP was 0.998, and 0.764, respectively. TNF-α at cutoff value of more than or equal to 30 pg/ml was diagnostic for HCC, with sensitivity of 100%, specificity of 94.1%, and overall accuracy of 97.5%. However, sensitivity and specificity of AFP were 92.8 and 35.3%, respectively, at the current study cutoff of more than or equal to 2.62. Pairwise comparisons of ROC curves showed that AUC for TNF-α was significantly higher than that for AFP ($P<0.0005$). AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; TNF-α, tumor necrosis factor-alpha.

Figure 3



Receiver operating characteristics (ROC) curves of TNF-α and AFP for diagnosis of early stages of HCC. Tumor marker levels are plotted for their ability to predict very early and early stages of HCC. Overall accuracy (area under the receiver operating characteristic) of TNF-α and AFP was 0.998, and 0.617, respectively. TNF-α at cutoff value of more than or equal to 32.35 pg/ml was diagnostic for detection of early-stage HCC (BCLC 0-A) versus cirrhotic patients without HCC, with sensitivity of 100% and specificity of 98%. Sensitivity and specificity of AFP were 76 and 53%, respectively, at a cutoff of more than or equal to 6.71 ng/ml. Pairwise comparisons of ROC curves showed that AUC for TNF-α was significantly higher than that for AFP ($P<0.0005$). AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; TNF-α, tumor necrosis factor-alpha.

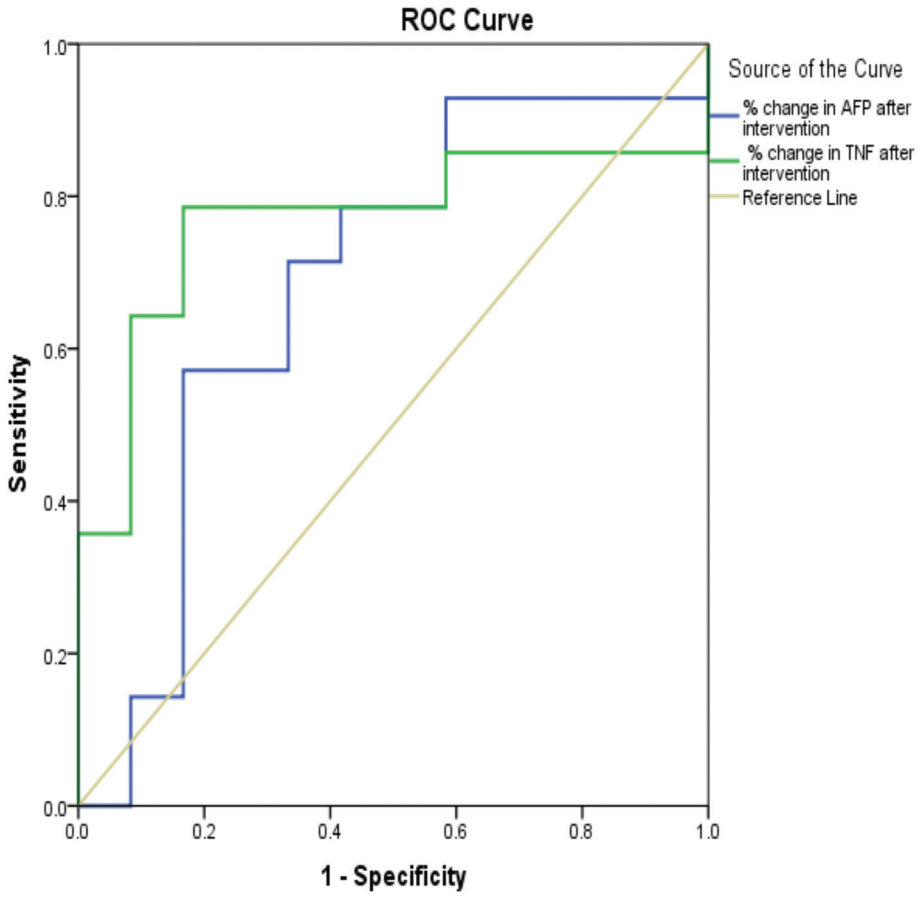
On assessment of the role of these tumor markers to detect early HCC among HCV cirrhotic patients (Fig. 5), our study showed that TNF-α not AFP was diagnostic for detection of early stage of HCC at cutoff level of more than or equal to 32.35 pg/ml with sensitivity and specificity of 100 and 98%, respectively.Our study showed that the low specificity of AFP at cutoff level more than or equal to 20 ng/ml for diagnosis of HCC (70.58%) can be augmented to 98.08% by adding TNF-α cutoff value of more than or equal to 30 pg/ml in patients with AFP values between levels of 20 and 200 ng/ml, and this is the same as reported by Zakhary *et al* [31], who recommend combined use of tumor markers to improve sensitivity and specificity for HCC diagnosis (Tables 3 and 4).

In assessment of the response to locoregional therapy, our study showed that TNF-α level was significantly decreased after locoregional therapy of HCC (P=0.006). This significance was preserved in patients with complete ablation (P=0.035) and lost in patients with partial or failed ablation because of remnants or persistence of tumor tissue. In contrast, level of AFP was not statistically significantly decreased after locoregional therapy, regardless of response category.

Measuring degree of TNF-α decrement after locoregional therapy of HCC to assess response was done by TNF-α percent change. Our results showed that more than or equal to 15.2% decrement is a good predictor of complete ablation versus partially or failed ablation with sensitivity of 78.6%, specificity of 83.3%, and overall accuracy of 80.77% (P=0.021).

This could be in concordance with El-Serag and Rudolph [39] who confirmed the diagnostic and prognostic value of TNF-α when their study showed a high serum level of several cytokines including TFN-α in HCC group in comparison with normal controls before HCC surgical resection. This level decreased in

Figure 4



Receiver operating characteristics (ROC) curves of % change in TNF-α and AFP after intervention for prediction of response. Simple logistic regression analysis was run to test the value of TNF-α percent change in predicting complete ablation; it was shown that % decrease in TNF-α after intervention by more than or equal to 15.2 was a statistically significant predictor of complete ablation (*B*=–2.909, Wald=8.26, *P*=0.004, and odds ratio=0.055). AFP, alpha-fetoprotein; TNF-α, tumor necrosis factor-alpha.

Table 3 Combination of both tumor markers (tumor necrosis factor-alpha and alpha-fetoprotein) for hepatocellular carcinoma diagnosis

	TNF-α (pg/ml)	AFP (ng/ml)			Combined two biomarkers	
Level of biomarker	≥30	≥2.62	≥20	≥200	TNF-α≥30 and AFP ≥20	TNF-α≥30 and AFP ≥200
Sensitivity (%)	100	92.8	42.02	13	42.03	13
Specificity (%)	94.1	35.3	**70.58**	98	**98.08**	98
PPV (%)	95.8	66	65.91	90	96.67	90
NPV (%)	100	78.3	47.37	45.45	55.56	45.45
Overall accuracy	97.5	68.3	54.2	49.2	65.8	49.2

Using TNF-α at cutoff value more than or equal to 30 pg/ml adjuvant to AFP value between levels 20 and 200 ng/ml can improve specificity of AFP in the diagnosis of hepatocellular carcinoma from 70.58 to 98.04%. On assessment of treatment response for the 26 patients in phase II after locoregional treatment, half of them (14) showed complete response, no cases showed progressive disease, and the others partial or failed grouped in one sector (12 cases). AFP, alpha-fetoprotein; NPV, negative predictive value; PPV, positive predictive value; TNF-α, tumor necrosis factor-alpha.

patients without recurrence and increased again in patients with recurrence.

Conclusion

In conclusion, combined use of TNF-α and AFP rises sensitivity and specificity at different cutoff values.

Moreover, TNF-α could be a better noninvasive tumor marker than AFP for assessment of response after locoregional therapy of HCC.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

Table 4 Alpha-fetoprotein and tumor necrosis factor-alpha before and after intervention

	AFP (ng/ml)			TNF-α (pg/ml)		
	Before intervention	After intervention	*P** value	Before intervention	After intervention	*P** value
Intervention group (26)	98.18±244.66	72.71±235.63	0.211	43.67±10.2	35.88±14.94	**0.006**
RFA (4)	9.33±6.59	5.11±3.49	0.464	37.9±4.66	30.75±2.52	0.068
TACE (22)	114.34±263.58	85.01±255.08	0.217	44.72±10.64	36.81±16.09	**0.02**
Complete ablation (14)	53.06±101.04	20.61±40.63	0.05	42.74±9.01	31.41±15.71	**0.035**
Partial or failed ablation (12)	150.82±344.03	133.50±341.68	1	44.77±11.76	41.1±12.65	0.158

AFP, alpha-fetoprotein; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TNF-α, tumor necrosis factor-alpha. There was an overall postinterventional statistically significant decrease in serum TNF-α level in patients with hepatocellular carcinoma who undergo locoregional therapy (P=0.006) in comparison with AFP. TACE group showed significant decrease in TNF-α level (P=0.02), whereas RFA group did not achieve this decline (P=0.068). Patients with hepatocellular carcinoma with postinterventional radiologically proved complete ablation showed also statistically significant decrease in TNF-α level (P=0.035). However, we found there was no statistically significant difference in postinterventional serum AFP level in patients with hepatocellular carcinoma who undergo locoregional therapy (P=0.211). Neither intervention type nor response showed any statistical significant decrement effect in the level of AFP level. *Significance (Wilcoxon signed ranks test). 0.006 high significance. 0.02 significant. 0.035 significant.

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