Assessment of Cancer Antigen 125 in Post-HCV Chronic Liver Disease and Hepatocellular Carcinoma Patients

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Short title: *serum CA125 and CLD patients with or without HCC.*

Background and study aim: Most of the morbidity and mortality of chronic liver diseases is due to its progression and complication of cirrhosis as ascites. The cancer antigen (CA) -125 is a high molecular mass glycoprotein produced both by ovarian cancer cells as well as by normal cells derived from coelomic epithelium. This study was conducted to assess the serum and/or ascitic fluid level of CA125 in HCV cirrhotic patients (decompensated or not) with or without Hepatocellular Carcinoma (HCC).

Patients and Methods: Group I: 60 post HCV liver cirrhoses cases; (A) 30 cases without ascites, (B) 30 cases with ascites. Group II: 60 HCC of post HCV liver cirrhosis cases; (C) 30 cases without ascites, (D) 30 cases with ascites. Serum and ascetic fluid level of CA-125 as well as serum AFP were assessed in all cases.

Results: Serum level of CA-125 between group A&B being higher in group B ($P<0.01$), however between groups C&D being higher in group D ($P<0.01$). There was a positive correlation between serum level of CA-125 and AFP level in group A and C ($P>0.05$). Regarding Group B and D, there was a positive correlation between serum level of CA-125 and each of AFP level ($P>0.05$) and ascitic level of CA-125 ($P<0.01$). Concerning the ascitic level of Ca 125, being higher in group D than B ($P<0.01$). Only AFP had a significant diagnostic performance ($P<0.01$) in differentiating HCC groups from non-HCC groups.

Conclusion: Elevated serum and ascetic level of CA125 in decompensated cirrhosis with or without HCC. AFP had a diagnostic performance in HCC diagnosis.

INTRODUCTION

Chronic liver diseases and their complications constitute a major health problem all over the world and especially in our country **[1]**. Most of the morbidity and mortality of chronic liver diseases is due to its progression and complications of cirrhosis **[2]**. Ascites is a frequent complication of advanced liver cirrhosis. Over 50% of cirrhotic patients develop ascites due to increased sodium retention in the kidneys, leading to expansion of extracellular volume and accumulation of fluid in the peritoneum **[3]**. The cancer antigen (CA) -125 is a high molecular mass glycoprotein produced both by ovarian cancer epithelial cells and mesothelial cells **[4]**.

Serum CA-125 levels are used as a marker of tumour activity in patients known to have ovarian carcinoma **[5]**, the severity of liver disease evaluated by MELD score and the presence of ascites were significantly correlated with the elevation of CA-125 level **[6]**. Serum CA-125 levels are generally found to be higher in malignant conditions compared to benign conditions. Levels exceeding 1000 U/ml have been described in benign conditions associated with massive pleural effusion and ascites **[7]**.

Hepatocellular carcinoma (HCC) is a global problem worldwide. Egypt is the sixth country in the Middle East and Arab world and the third one in Africa, with a high prevalence of HCC, the major risk factor for HCC is HCV infection which mainly burden

in Egypt **[8]**. Alpha fetoprotein (AFP) is a fetal specific glycoprotein; more than 70% of HCC patients have a high serum concentration of AFP because of the tumor excretion **[9]**. AFP serves as an important tool in screening of HCC patients; however patients with acute exacerbation of viral hepatitis and not associated with HCC may also have markedly increased AFP levels **[10]**. Together, AFP (cut-off: 200 ng/mL) and CA 125 have been reported to give a combined sensitivity of 96%. The use of AFP (sensitivity 58.8%, specificity of 97.4%) and CA125 (sensitivity of 92%, specificity 48.5%) together as screening blood markers for HCC has been suggested, a negative result for both markers would most likely rule out HCC **[11]**.

As no enough Egyptian studies were done on the validity of CA-125 and chronic liver diseases or HCC patients, so we aimed to assess the serum and/or ascitic fluid level of CA125 in post HCV cirrhotic patients (decompensated or not) with or without HCC.

PATIENTS AND METHODS

Study design: A cross sectional observational study

Patients: patients were recruited from those attending Tropical Medicine Department-Ain Shams University and from Professor Doctor Yassin Abdel Gaffar Charity Center for Liver Disease and Research. The study included number of (120) cases and divided into two groups: Group I; Formed of 60 post HCV liver cirrhosis cases; (A) 30 cases without ascites, (B) 30 cases with ascites. Group II; Formed of 60 HCC on top of post HCV liver cirrhosis cases; (C) 30 cases without ascites, (D) 30 cases with ascites. Inclusion Criteria: Male Patients, Clinical, laboratory and ultrasonographic criteria suggestive of chronic liver disease post HCV liver cirrhosis, HCC cases were diagnosed by; High AFP > cut-off limit (>200ng), Characteristic features of HCC by triphasic spiral abdominal CT **[12]**. Exclusion Criteria: Patients with other causes of liver disease, extrahepatic metastasis or other simultaneous malignancies, Patients with advanced systemic disease and other causes of ascites or serous sac effusion.

Serum and ascetic fluid level of CA-125 were assessed and measured: Specimen collection and preparation: 5ml of venous blood as well as an ascitic sample were taken under complete aseptic conditions. Instrument: The Roche diagnostics cobas e 411 analyzer was used. Steps of test: Sandwich principle, total duration of assay: 18 minutes. Normal range of CA125 serum level: up to 21 u/ml, Ascitic fluid level: up to 35 u/ml [13].

Statistical analysis

IBM SPSS statistics (V. 19.0, IBM Corp., USA, 2010) was used for data analysis. Data were expressed as Mean ± SD for quantitative parametric measures in addition to Median Percentiles for quantitative non-parametric measures and both number and percentage for categorized data.

The following tests were done: (1) Comparison between two independent groups for non-parametric data using Wilcoxon Rank Sum test. (2) Ranked Sperman correlation test to study the possible association between each two variables among each group for non-parameteric data. (3) Chi-square test to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered sig., while at 0.01 was considered highly sig. (4) The ROC was constructed to evaluate the most discriminating markers between the compared groups, AUC can also be calculated.

RESULTS

In order to fulfill our aim, (120) cases were included and divided into two groups; Group I: included 60 post HCV liver cirrhoses cases; (A) (30) patients with compensated cirrhoses. (B) (30) patients with decompensated cirrhosis. Group II: included 60 HCC of post HCV liver cirrhosis cases; (C) 30 cases with compensated cirrhoses, (D) 30 cases with decompensated cirrhosis.

Concerning serum level of CA-125; there was a significant difference between groups (A) & (B) being higher in group (B) ($P<0.01$), also a significant difference between groups (C) & (D) being higher in group (D) ($P<0.01$) as in Table (1).

In Table (2) and (3): There were a positive correlation between serum level of CA-125 and AFP in group (A) and (C) ($P>0.05$). Regarding Group (B) and (D), there was a positive correlation between serum level of CA-125 and each of AFP level ($P>0.05$) and ascitic level of CA-125 ($P<0.01$). Table (4) shows that there was a significant difference between groups B (decompensated non HCC) & D (decompensated HCC) concerning

ascitic level of Ca 125 being higher in group D (P<0.01).

Regarding the diagnostic performance of AFP and serum CA125 in differentiating compensated HCC group (C) from non-HCC group (A), as in Fig. (1); Only AFP had significant diagnostic performance (P<0.01) in differentiating compensated HCC group (C) from non-HCC group (A). However, diagnostic performance of AFP, serum CA125 and ascetic fluid CA125 in differentiating decompensated HCC (D) group from non-HCC (B) group as in Figure (2); Only AFP had significant diagnostic performance in differentiating decompensated HCC (D) group from non-HCC (B).

Table (1): Comparison between studied groups concerning serum levels of CA-125

Variables	Measures	Group A (N=15)	Group B (N=15)	P
Serum CA125	Median (IQR)	15.7 (9.7–44.0)	229.3 (116.9–524.3)	¤<0.001*
	Range	3.5–179.0	32.0–2412.7	
Variables	**Measures**	**Group C (N=15)**	**Group D (N=15)**	**P**
Serum CA125	Median (IQR)	44.4 (9.8–73.0)	224.6 (191.6–464.4)	¤<0.001*
	Range	1.0–164.0	62.8–819.0	

IQR: 1st – 3rd interquartiles, ¤Mann Whitney test, ^Independent t-test, *Significant
Decompensated HCC (D) had significantly higher serum CA125 and decompensated non-HCC (B) had significantly higher serum CA125.

Table (2): Correlation between CA-125 serum level and other parameters in group A and B

Variables		Mean ± SD	CA-125 34.77 ± 44.78		
			R	P	Sig.
Group (A)	AFP (Up to 10ng/ml)	9.867 ± 5.8	0.145	0.444	NS
Group (B)	AFP (Up to 10ng/ml)	9.93 ± 10.18	0.023	0.903	NS
	Ascitic CA-125	**505.46 ± 438.6**	**0.15**	**0.005**	**HS**
	Ascitic PMN	116 ± 120	0.13	0.492	NS

Ranked Sperman Correlation Test
NB: Highly significant; HS< 0.01, Significant; S <0.05, Non-significant; NS >0.05
PMN: polymorphonuclear leukocytes.

Table (3): Correlation between CA-125 serum levels and other parameters in group C and D

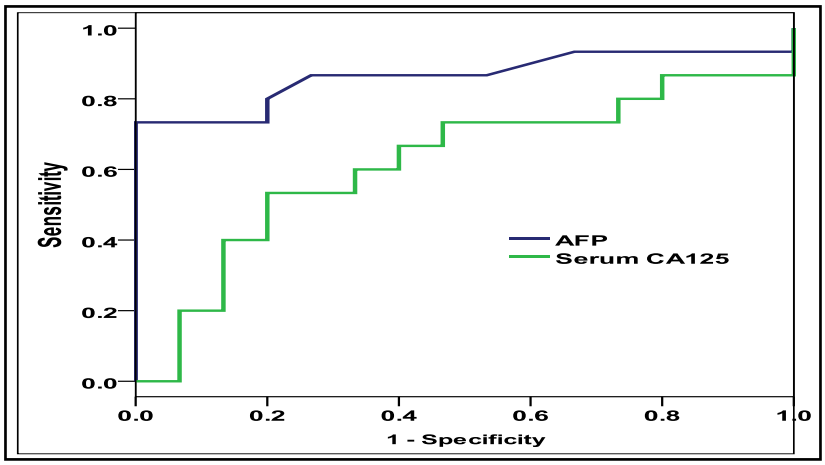
Variables		Mean ± SD	CA-125 34.77 ± 44.78		
			R	P	Sig.
Group (C)	AFP (Up to 10 ng/ml)	282 ± 400.3	-0.146	0.44	NS
Group (D)	AFP (Up to 10 ng/ml)	990.9 ± 614	-0.052	0.786	NS
	Ascitic CA-125	**734.013 ± 391.3**	**0.786**	**0**	**HS**
	Ascitic PMN	**97 ± 53.6**	**0.506**	**0.004**	**HS**

Ranked Sperman Correlation Test
NB: Highly significant; HS< 0.01, Significant; S <0.05, Non-significant; NS >0.05
PMN: polymorphonuclear leukocytes.

Table (4): Comparison between group B and group D concerning ascitic levels of CA-125

	Group B		Group D		z-value	P-value	Sig.
	Mean	±SD	Mean	±SD			
Ascitic CA-125 Normal value up to 35 u/ml	505.4	438.6	734.01	391.3	-2.958	0.003	HS

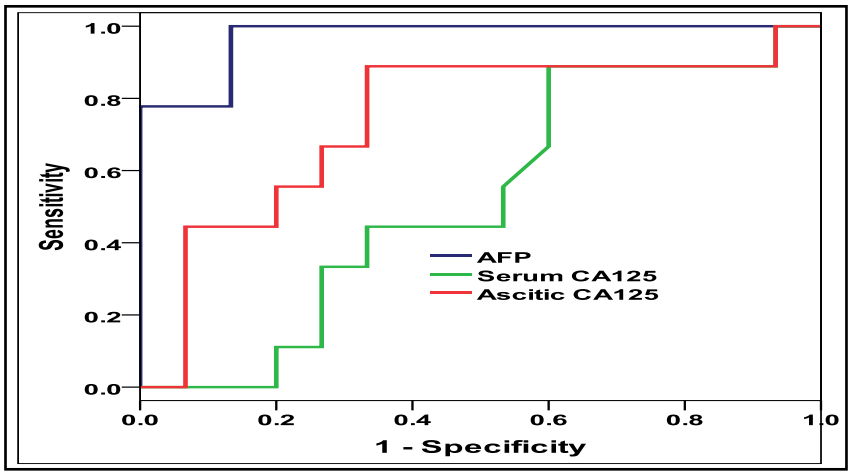
Wilcoxon Rank Sum Test



Lab	AUC	SE	P	95% CI	Cutoff
AFP	0.864	0.076	<0.001*	0.696–1.000	≥26.0
Serum CA125	0.618	0.107	0.272	0.408–0.828	--

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *Significant

Figure (1): ROC curve for AFP and serum CA125 in differentiating compensated HCC group (C) and non-HCC group (A).



Lab	AUC	SE	P	95% CI	Cutoff
AFP	**0.970**	**0.029**	**<0.001***	**0.000–1.000**	**≥11.5**
Serum CA125	**0.522**	**0.121**	**0.858**	**0.286–0.759**	**--**
Ascitic CA125	**0.741**	**0.112**	**0.053**	**0.521–0.961**	**--**

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *Significant

Figure (2): ROC curve for AFP and serum CA125 in differentiating decompensated HCC (D) group from non-HCC (B) group

DISCUSSION

It may be one of the first Egyptian studies that assessed the correlation between CA-125 and compensated and decompensated CLD with or without HCC.

Regarding the serum level of CA125, there was a significant elevation in both compensated group (A) and decompensated group (B) post HCV cirrhotic cases but more higher in decompensated group (B), this in agreement with each of; Piekarska et al. **[14],** who stated that the mean serum levels of CA-125 in patients with cirrhosis (with and without ascites) were elevated above the normal level, Xiao and Liu **[15]** who found the elevation of CA-125 in sera of cirrhotic patients without ascites and Assmar et al. **[16]** who reported that CA-125 was not a tissue or tumor specific antigen, elevated level could be detected in patient without malignant transformation and indicate presence of cirrhosis in benign liver disease cases.

There was a significant positive correlation between serum level and ascetic level of CA125 in both non HCC (B) and HCC (D) decompensated groups and this was in a harmony with Hussain and Camilleri **[17]** who suggested that simultaneous assay of CA-125 in serum and body fluid and/or with a panel of other tumor marker could be accurate in diagnosing malignant and non malignant cases, also significant elevation of CA-125 could occur in HCC cases. Also Tuzun et al. **[18]** reported a positive correlation between serum and ascitic fluid levels of CA-125, however ascitic fluid levels were higher than serum levels, which explained that the antigen is mesothelial rather than tumoral origin. However our results reported that the ascitic level of Ca 125 was elevated in decompensated HCC group (D) than non HCC group (B), this supported that the antigen origin may be from both mesothelial and tumoral cells.

Regarding the correlation between AFP and CA125 serum and ascitic level; Only AFP had significant diagnostic performance in differentiating compensated and decompensated HCC groups (C) & (D) from non-HCC groups (A) &(B). However Lopez et al. **[19]** who stated that AFP-CA-125 combination had the highest sensitivity to diagnose 96% of the HCC patients, also both could be combined for HCC screening due to their excellent sensitivity and specificity, respectively: a negative result for both, or even CA 125 alone, would discourage HCC diagnosis, while positive results of both would make HCC presence highly probable. A positive CA 125 and negative AFP would be equivocal for HCC. Also Elias and Kew **[20]** reported that CA 125 has a highly sensitivity but lacks specificity for HCC diagnosis.

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Conflicts of interest: None.

Ethical approval: Approved ;written consents have been taken from all included patients.

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