Assessment of the Correlation between Serum Prolidase and Alpha-Fetoprotein Levels in Egyptian Patients with Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) accounts as the sixth most common neoplasm on a global scale and the third most lethal with >600,000 deaths per year worldwide. Despite regular surveillance to detect small HCC, HCC is often diagnosed at advanced stage, after the symptoms related to HCC have appeared, and the 5-year survival rate for patients is only 7%. If HCC could be diagnosed at an early stage, potentially curative option, such as resection, ablation, and transplantation may be considered. Prolidase is an important enzyme that cleaves the bonds of dipeptides containing proline (X-pro), and plays a vital role in collagen turnover, matrix remodeling and cell growth. Metastatic tumor cell produce enhanced number of proteases that enable them to penetrate basement membrane and extracellular matrix. Therefore, tumor progression might depend on the breakdown of collagen and other extracellular matrix proteins.

Aim of the work: Assess the possible diagnostic role of serum prolidase compared to alpha-fetoprotein which is the slandered marker used for diagnosis of HCC.

Methods: The study was conducted upon 90 subjects who were divided into three groups: group I included 40 patients with liver cirrhosis and hepatocellular carcinoma, group II included 40 patients with HCV related liver cirrhosis without HCC, group III had 10 healthy subjects as controls. Plasma prolidase was measured by Enzyme Linked Immunosorbent assay (ELISA) using recombinant human peptidase D/prolidase (PEPD) ELISA kit lot.

Results: In this study, the serum levels of serum prolidase were highest in patients of group I with HCC compared to those with liver cirrhosis and the control groups. Also, prolidase values increased with tumor number, overall size but not with vascular invasion nor with BCLC.

Conclusion: There was a direct significant correlation between serum prolidase level and serum AFP level in HCC patients. Serum prolidase levels directly correlated to the tumor number and overall size so it has a good prognostic value.

Keywords: Serum prolidase; Alpha-fetoprotein; Hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and ranks as the second-leading cause of cancer-induced death in men [1].

AFP is the most commonly used serological marker worldwide to diagnose hepatocellular carcinoma. Despite regular surveillance to detect small HCC, HCC is often diagnosed at advanced stage, after the symptoms related to HCC have appeared, and the 5-year survival rate for patients is only 7% [2]. If HCC could be diagnosed at an early stage, potentially curative options, such as resection, ablation, and transplantation may be considered [3].

In recent years, the development of molecular biology has led to the successful exploration and identification of markers for HCC, which is expected to improve the early diagnostic rate, treatment effect in addition to curative satisfaction [4].

Collagen is the main component of connective tissue, deregulation of tissue collagen metabolism is one of consequences of neoplastic transformation [5]. Metalloproteinase initiate the breakdown of collagen; however, the final step of collagen degradation is mediated by prolidase [6].

Prolidase is an important enzyme that cleaves the bonds of dipeptides containing proline (X-pro), and plays a vital role in collagen turnover, matrix remodeling and cell growth. Metastatic tumor cell produce enhanced number of proteases that enable them to penetrate

basement membrane and extracellular matrix. Therefore, tumor progression might depend on the breakdown of collagen and other extracellular matrix proteins [7].

Aim of the work

Assess the possible diagnostic role of serum prolidase compared to alpha-fetoprotein which is the slandered marker used for diagnosis of HCC.

Patient and Methods

Patients

This study had been carried out on 90 subjects, age range 25-73 year selected from Internal medicine and Hepatology outpatient clinics and inpatient wards at Ain shams university hospitals.

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Subjects were divided as follow

Group I: Include 40 HCC patients diagnosed by imaging and alpha- fetoprotein.

Group II includes 40 matched cirrhotic patients without HCC.

Group III: Include 10 apparently healthy subjects, age and sex matched, having no acute or chronic illness and taking no medications were taken as control group.

Methods

All subjects and controls were subjected to:

Complete history taking and clinical examination.

Laboratory investigations including

- Complete blood count.
- Kidney function tests and electrolytes
- Liver function tests
- Coagulation profile
- Viral markers

Radiological examination including:

- Plain X-ray chest.
- Pelvi-abdominal ultrasound.
- Triphasic CT or MRI abdomen.
- Electrocardiogram (ECG)
- Serum prolidase.
- Serum Alpha-fetoprotein.

Prolidase: Plasma prolidase was measured by Enzyme Linked Immunosorbent assay (ELISA) using recombinant human peptidase D/prolidase (PEPD) ELISA kit lot.

Sample preparation: After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature, this usually take 10 to 20 minutes. Remove the clot by centrifuging at 2000-3000 rpm for 20 minutes. If precipitate appear during reservation, the sample should be centrifuged again.

Statistical calculation of results

Known concentration of human peptidase D /prolidase (PEPD) standard and its corresponding OD is plotted on the log scale (X-axis) and the log axis (Y- axis) respectively, the concentration of human peptidase D/prolidase (PEPD) in sample is determined by plotting the sample OD, on the Y axis the original concentration is calculated by multiplying the dilution factor.

Results

The results obtained from the study were as follow:

As regard prolidase there was a statistical significant difference between the three groups where the highest values were in group I ranging between 11 to 135 ng/ml and mean 79.200 ± 47.800 , as shown in Table 1. There is a statistical difference of p value<0.001 when comparing serum prolidase in groups I and II and I and III while not when comparing groups II and III.

Groups	Prolidase		Anova	
	Range	Mean $\pm$ SD	F	P-value
HCC Patients	11-135	79.200 $\pm$ 47.800	44.743	<0.001*
Cirrhotic Non-HCC	6-50	16.775 $\pm$ 9.011		
Healthy individuals	1.5-10	4.200 $\pm$ 2.720		
Tukey's Test				
I and II		I and III	II and III	
<0.001*		<0.001*	0.522	

Table 1: Comparison between the three groups as regards serum prolidase level. Correlating prolidase level with number of focal lesions shows a significant value of 0.001 as shown in Table 2 where prolidase level is higher as number of HCC lesions increases.

N of F Lesions	Prolidase		Anova	
	Range	Mean ± SD	F	P-value
One	11-135	48.767 ± 41.817	7.668	<0.001*
Two	22.5-135	74.909 ± 45.198		
Three	40-95	75.625 ± 24.356		
Four	95-135	115.000 ± 28.284		
Multiple	135-135	135.000 ± 0.000		
Tukey's Test				
-	One	Two	Three	Four
Two	0.396	-	-	-
Three	0.695	1.000	-	-
Four	0.142	0.622	0.732	-
Multiple	<0.001*	0.010*	0.086	0.958

Table 2: Relation between prolidase and number of HCC lesions. As regards the tumor size positive correlation was also seen between prolidase and size of focal lesion, where prolidase level increase as the tumor size increases with p value less than 0.001* as shown in Figure 1.

Figure 1: Relation between prolidase and tumor size, Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of prolidase and AFP in term of tumor size (diameters; ≤ 3 cm and > 3 cm) the cutoff value of prolidase and AFP for tumor size were: 50 and 1050 respectively.

Correlating prolidase level with number of focal lesions shows a significant value of 0.001 as shown in Table 2 where prolidase level is higher as number of HCC lesions increases. As regards the tumor size positive correlation was also seen between prolidase and size of focal lesion, where prolidase level increase as the tumor size increases with p value less than 0.001* as shown in Figure 1.

Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of prolidase and AFP in term of tumor size (diameters; ≤ 3 cm and > 3 cm). The cut off value of prolidase and AFP for tumor size were: 50 and 1050 respectively.

Prolidase

The positive predictive value 100%, the negative predictive value

56.2% and the accuracy of the test was 93.2% (sensitivity: 74.19%, specificity: 100%).

AFP

The positive predictive value 100%, the negative predictive value 52.9% and the accuracy of the test was 90.7% (sensitivity: 77.42%, specificity: 100%). As shown in Figures 2 and 3.

Another Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of prolidase and AFP in term of number of focal lesions (number: ≤ 1 and ≥ 2). The cut off value of prolidase and AFP were 30 and 1200 respectively.

The positive predictive value 81.8%, the negative predictive value 44.8% and the accuracy of the test was 50.3% (sensitivity: 36%, specificity: 86.67%).

Figure 2: ROC curve of predictive value of prolidase regarding tumor size (diameters; ≤ 3 cm and >3 cm).

Figure 3: ROC curve of predictive value of AFP regarding tumor size (diameters; ≤ 3 cm and >3 cm).

Figure 4: ROC curve of predictive value of prolidase regarding number of focal lesion (number: ≤ 1 and ≥ 2).

Figure 5: ROC curve of predictive value of AFP regarding number of focal lesion (number: ≤ 1 and ≥ 2).

The positive predictive value 72.2%, the negative predictive value 45.5% and the accuracy of the test was 55.7% (sensitivity: 52%, specificity: 66.67%). As shown in Figures 4 and 5.

Discussion

In this study we aimed to assess the correlation between serum prolidase and AFP level in patient with hepatocellular carcinoma and liver cirrhosis secondary to HCV infection and compared with healthy control.

Regarding the serum levels of AFP in the current study, there was a significant difference between patients with HCC and those with liver cirrhosis where the mean was 210.93 ng/ml in patients with HCC and 8.48 ng/ml in patients with liver cirrhosis with a p value <0.001 , this was in agreement with Liu et al. who stated that AFP levels significantly differed in patients with HCC having a mean 250.65 nm/ml and patients with liver cirrhosis with a median 2.32 ng/ml and p value <0.001 .

The relation between AFP and tumor characteristics such as tumor size, tumor number and macro vascular invasion has been evaluated in many studies. In this study we find a significant relation between level of AFP and both tumor size and number with P value = 0.018, 0.024, respectively where AFP level increase when tumor size increase and also show higher level as the tumor number increase.

Concerning the value of prolidase in diagnosing HCC, there was a significant difference in its values in patients with HCC over liver cirrhosis where in HCC the values ranged between 11-135 ng/ml and mean 79.200 ± 47.800 ng/ml compared to 6-50 ng/mL, a mean of 16.775 ± 9.011 in cirrhotic over 1.5-10 ng/mL and a mean 4.200 ± 2.720 ng/ml in healthy controls with a P value <0.001 indicating the highest values in HCC patients. This came into agreement with [8] who found the highest values in HCC with a mean 65.46 ± 27.800 ng/ml compared to 13.79 ± 2.18 ng/ml in cirrhotic and 1.65 ± 0.79 ng/ml in controls with a P value <0.001 .

Concerning correlation between levels of prolidase with different tumor characteristics, in our study, there was a significant positive correlation between prolidase values and tumor number, size with P value less than 0.001 but there was no significant correlation between prolidase level and macro vascular invasion with P value 0.917.

Conclusion

Serum prolidase levels were significantly higher in patients with HCC and mildly elevated with liver cirrhosis. Serum prolidase levels directly correlated to the tumor number and overall size so it has a good prognostic value. There was a direct significant correlation between serum prolidase level and serum AFP level in HCC patients.

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