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Diagnostic Accuracy of α-1-Acid Glycoprotein in Diagnosis of Hepatocellular Carcinoma

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Abstract

Background: This study aimed to evaluate the diagnostic value of α-1-acid glycoprotein (AGP) to improve the diagnosis of hepatocellular carcinoma (HCC). Methods: AGP was measured in serum of 53 HCC patients and 20 liver cirrhosis (LC) patients, in addition to 15 healthy individuals. The diagnostic performances of AGP was determined and compared with alpha fetoprotein (AFP) for the diagnosis of HCC using area under the ROC curve (AUC). Results: In the patients with HCC, the mean serum concentration of AGP was 1.38 ± 0.4 ng ml^{-1}. As expected, it was significantly higher than that of the LC group (1.12 ± 0.4 ng ml^{-1}, $P< 0.05$). The detection of HCC using AGP produced better AUC (0.74), sensitivity (74%) and specificity (75%) compared to AFP at cutoff 400 (U L^{-1}) had AUC (0.70), sensitivity (40%) and specificity (95%). The combination of AFP and AGP had AUC (0.84), sensitivity 74%, specificity 80 %.

Keywords: Liver cancer, Diagnosis, Blood markers

Introduction

Hepatocellular carcinoma (HCC) is the seventh most common cancer worldwide, and the third most common cause of cancer-related mortality [1]. HCV infection is a major risk for the development of HCC [2] which leads to cirrhosis in about 10 to 20 percent of patients [3]. In Egypt, there is a growing incidence of HCC (10–120/100,000), which represent the leading cause of death from all other cancer sites [4]. The early detection of HCC and opportunity to select appropriate treatment are important benefits of HCC screening [5]. AFP is a serological marker currently available for the detection of HCC [6], but AFP level is limited by its low sensitivity [7].

Several biomarkers, such as des-gamma carboxyprothrombin, glycosylated AFP, glypican-3, human hepatocyte growth factor, and insulin-like growth factor-1, are promising, but none of these markers has been validated for clinical use [8]. There is increasing evidence that the inflammatory process is inherently associated with many different cancer types, including HCC

[9]. α-1-acid glycoprotein (AGP) is a member of the lipocalins [10] and is an acute phase protein, synthesized predominantly in the liver as a single polypeptide of 41–43 kDa, made up of 183 amino acids, with a hydrophobic prosthetic group, and a high content of sialic acid [11]. Cytokines can cause AGP level to increase as part of an inflammatory response [12]. The level of AGP has been suggested to be a potential marker for diagnosing HCC [13]. The aim of the present work is to compare the diagnostic value of AGP with the traditionally used marker (AFP) in patients with HCC associated with hepatitis C infection.

Patients and Methods

Patients

A total of 88 consecutive Egyptian individuals (62 males and 26 females aged 25-70 years) attending the Tropical Medicine Department, Mansoura University hospitals, Mansoura, Egypt were enrolled in this study .They were classified into 3 groups.

The first group included 53 patients with hepatocellular carcinoma (HCC), their ages ranged from 46-70 years (mean age ± SD, 57 ±7 years). The diagnosis of HCC was done according to American Association for the Study of Liver Diseases (AASLD) Practice Guidelines [14]. The second group included 20 patients with liver cirrhosis their ages ranged from 42-66 years (mean age ± SD, 53 ± 7 years). A third group of 15 apparently healthy subjects serving as control group were also included in this study their ages ranged from 25-65 years (mean age ± SD, 55 ± 6 years), they were clinically free with normal laboratory findings and negative viral hepatitis markers and normal abdominal ultrasonographic findings. An informed consent was obtained from each individual participated in the present study and all were fully informed concerning the nature of the disease and the diagnostic procedures involved.

All patients were negative test for anti-HIV antibodies and none of the patients had history of habitual alcohol consumption. Patients with heart failure, kidney failure, rheumatoid arthritis, autoimmune liver diseases, hepatitis B virus, metabolic disorders or other malignancies were excluded. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Blood samples

Blood samples collected from all patients by vein-puncture at the time of diagnosis, before any treatment and a part of the blood was treated immediately with EDTA-K_3 for complete blood count ,and another portion was treated with a sodium citrate solution to perform prothrombin time. Serum was immediately separated by centrifugation at 3800 rpm for 10 min and divided into two aliquots. The first aliquot was used for routine investigations. The second aliquot was stored at −20 °C and thawed only at the time of the assay of AGP.

Laboratory tests

All patients with liver cirrhosis and hepatocellular carcinoma were positive for anti-HCV antibody. The HCV infection was diagnosed based on biochemical and serologic test. All patients and control were subjected to Liver function tests included alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin and alkaline phosphatase (ALP) measured on an automated biochemistry analyzer (Hitachi 917; Roche Diagnostics, Mannheim, Germany). Complete blood pictures were determined by KX-21 Sysmex automated hematology analyzer (Sysmex Corporation, Hyogo, Japan). Prothrombin time, concentration and prothrombin- INR (international normalized ratio) were determined by (Coatron M1, TECO, Neufahrn, Germany) and using the reagent (Diamed GmbH, Ottobrunn, Germany). The level of serum Alpha fetoprotein (AFP) was estimated by chemiluminescence, with Immulite (1000) AFP kit (Diagnostic Products Corporation; Los Angeles, CA, USA).

Detection of AGP using ELISA

Serum AGP was determined using AssayMax Human AGP ELISA kit (Assaypro, St. Charles, MO) according to manufacture procedure.

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 15.0 (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean ± SD. Differences in continuous variables were performed using

analysis of variance (ANOVA) and X^2 test for categorical variables. All tests were two-tailed and statistical significance was performed at the 0.05 level. The correlation was evaluated by Spearman correlation coefficient. The diagnostic value of each serum marker was performed by the area under the ROC curves (AUC). The AUC can be statistically interpreted as the probability of the test to correctly distinguish the patients with HCC from LC. The turning point of the curve was determined to the best cut-off value for the diagnosis, and it was also a maximal value at the sum of the sensitivity and specificity. Sensitivity, specificity, predictive value (PPV) and negative predictive value (NPV) and odds ratios (with 95% confidence intervals) were calculated by standard formulae [15].

Results

Patient clinical characteristics

A series of patients with HCC included 53 patients was compared with two different groups: 20 patients with liver cirrhosis (LC), and 15 Healthy individuals. The laboratory background of HCC, liver cirrhosis patients, and healthy individuals in this study are summarized in Table 1. Patients with HCC were associated with lower mean platelet count, albumin levels, higher mean ALT, AST, prothrombin - INR, total bilirubin, alpha fetoprotein and alpha-1-acid glycoprotein. There was non significant difference in alkaline phosphatase. The ANOVA test showed that there was significant difference in AST, ALT , albumin, total bilirubin, prothrombin – INR, platelet count, alpha fetoprotein and alpha-1-acid glycoprotein ($P<0.05$). Univariate analysis of all variables tested in the present study revealed that AGP differed significantly ($p<0.0001$) between patients with HCC and patients with liver cirrhosis.

Diagnostic performance of AGP and AFP using areas under the ROC curves

The blood markers AGP and AFP distribution for patients in the study groups were depicted in Fig. 1a-b. The AUC of candidate HCC markers and (p value) were in a decreasing rank AGP (0.74, 95% CI: 0.61-0.87 (0.001) and AFP (0.70, 95% CI: 0.58- 0.82) (< 0.009) at the cutoff value of 400 U L^{-1} respectively; Fig. 2 a-b. Taking both sensitivity and specificity into account, the selection of the cutoff point was according to maximum number of sensitivity and specificity. Based on diagnostic accuracy of AGP and AFP; AGP at cutoff 1.3 ng ml^{-1} produced better sensitivity (74%) higher than sensitivity of AFP at the cutoff 400 (U L^{-1}).

Table 1. Laboratory data of healthy individuals and patients with liver cirrhosis and hepatocellular carcinoma (HCC).

Variable	Healthy(n=15)	Cirrhosis(n=20)	HCC(n=53)	P value
ALT (U L^{-1})	19 ± 3	39 ± 30	55 ± 33	0.058
AST(U L^{-1})	14 ± 2	59 ± 40	93 ± 50	0.01
ALP(U L^{-1})	68 ±11	140 ± 53	176 ± 166	0.401
AFP (U L^{-1})	1.9 ± 1.1	13.4 ± 20.2	731 ± 214	<0.0001
Albumin(g L^{-1})	47 ± 2	38 ± 7	32 ± 5	<0.0001
Total bilirubin (mg dL^{-1})	0.7 ± 0.1	1.7 ± 1.0	2.2 ± 1.3	0.110
Platelet count (×109 L^{-1})	331 ± 159	139 ± 82	102 ± 73	0.068
PT - INR	1.06 ± 0.04	1.4 ± 0.2	1.48 ± 0.3	0.204
AGP (ng mL^{-1})	0.8 ± 0 .1	1.12 ± 0.4	1.38 ± 0.4	<0.0001

Continuous variables were expressed as mean ± SD

References values: Alanine aminotransferase (ALT) up to 45 U L^{-1}; Aspartate aminotransferase (AST) up to 40 U L^{-1}; Albumin 38–54 g L^{-1}; Total Bilirubin up to 1 mg dL^{-1} ; Alkaline phosphatase (ALP) 20–190 U L^{-1}; international normalised ratio (INR) 1; Platelet count 150–400 (×109 L^{-1});Alpha Fetoprotein (AFP) up to 10 (U L^{-1}); $p>0.05$ is considered non significant; $p<0.05$ is considered significant. p value < 0.01 is considered highly significant, $p < 0.001$ is considered very significant and $p < 0.0001$ is considered extremely significant.

The significance was performed using (ANOVA) test.

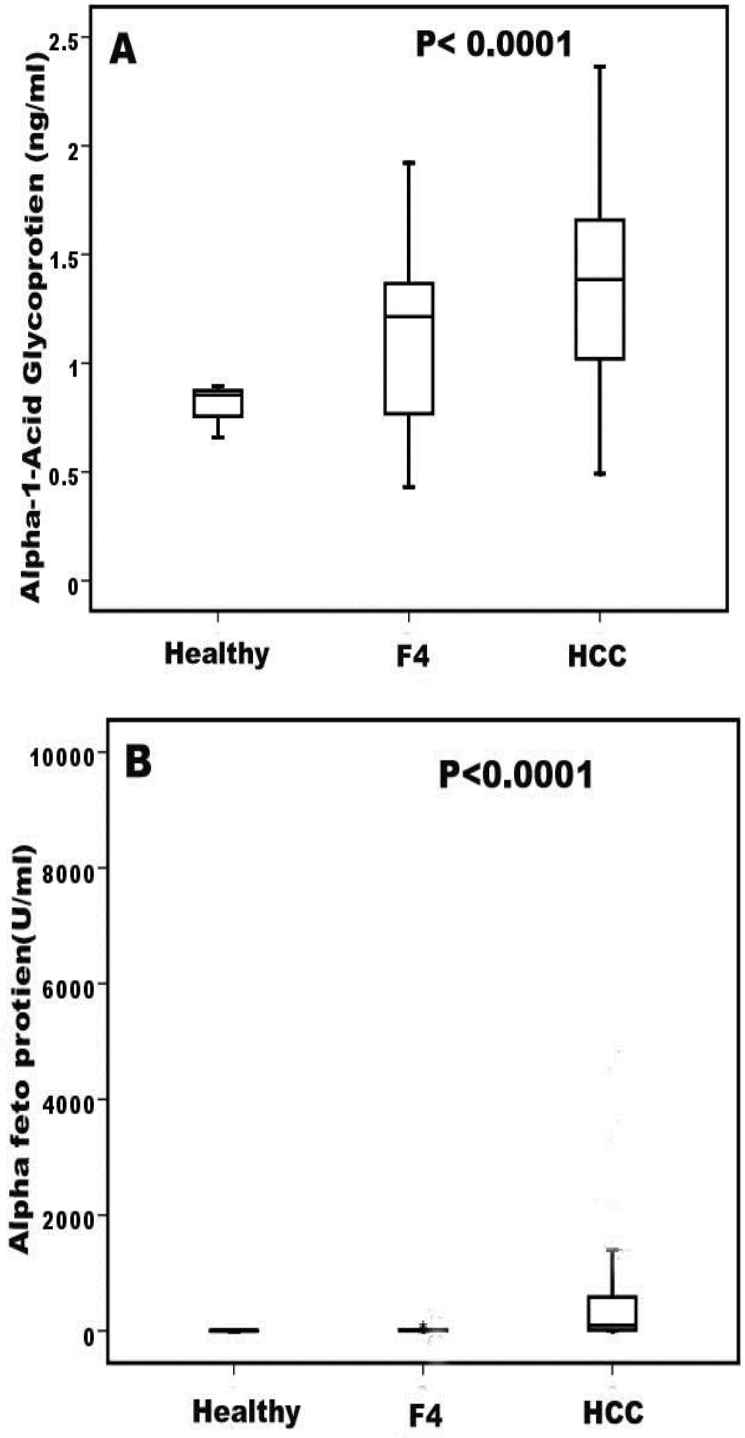

Fig. 1 Box plots for of candidate blood markers AGP and AFP for discriminate HCC from healthy individuals and liver cirrhosis patients. **A**. Box plots of AGP **B**. Box plots of total AFP. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences between healthy individuals, liver cirrhosis and hepatocellular carcinoma patients was determined by ANOVA test ($p < 0.0001$).

However, AGP produced specificity (75%) which was less specific than AFP (95%) in relation to LC. Also, AGP had a higher accuracy (74%) compared to AFP (55%) and negative predictive value (NPV) for AGP (52%) was higher than NPV for AFP (37%).

The relative risk [odds ratio, (OR)] of having HCC using the cutoff values determined by ROC curve analyses were 6.5 (2.1-20.2) for AGP and 12.5 (1.6-100.3) for AFP and 11.2 (3.1-39) for AGP and AFP. The area under the curve (AUC) of the combination between AGP and AFP was 0.84 ($p < 0.0001$) for diagnosing patients with HCC from liver cirrhosis Fig. 1c. This combination yielded a sensitivity of 74%, specificity of 80% and accuracy of 75% for identifying HCC with (OD) 11.2.

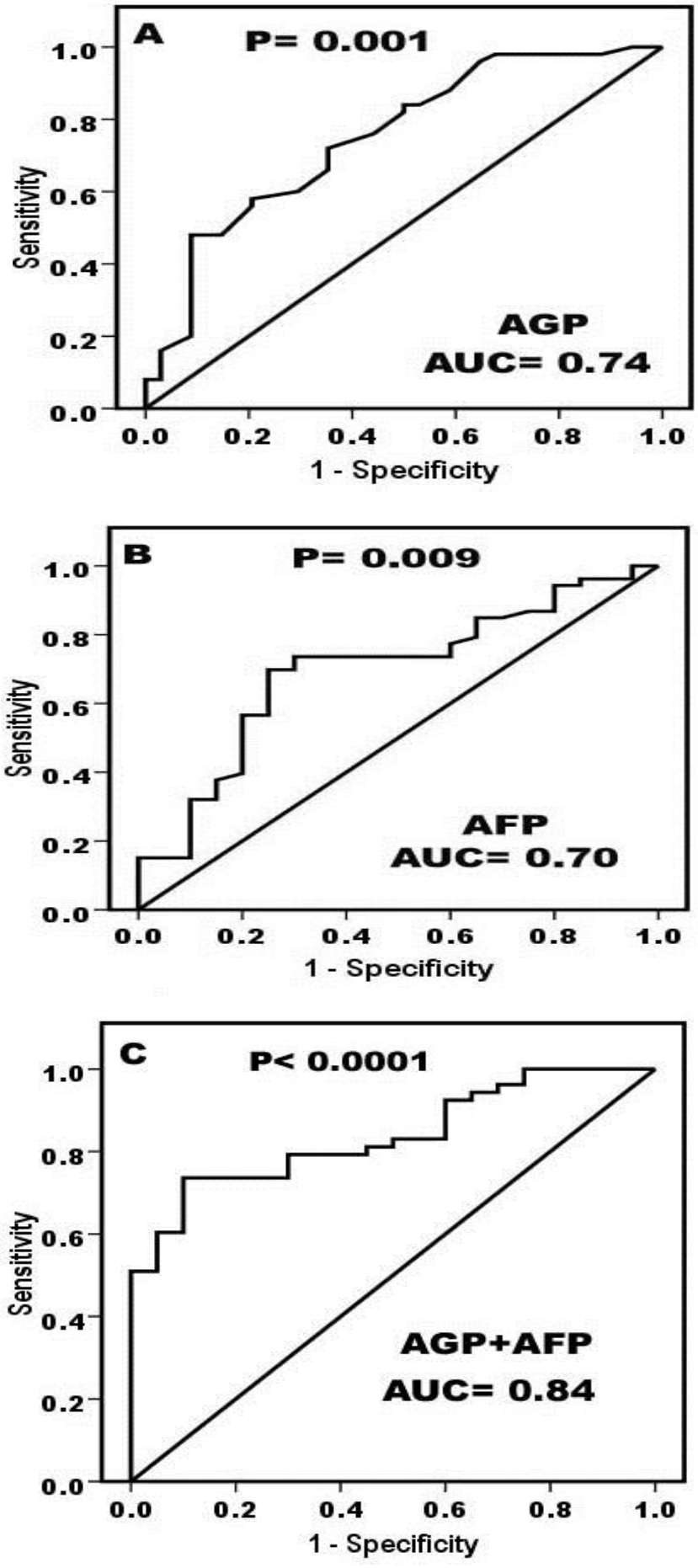

Fig. 2 ROC curves for candidates markers for discriminating patients with HCC from LC. **A**. ROC curve of AGP. **B**. ROC curve of AFP. **C**. ROC curve of AGP and AFP. The true positive rate (sensitivity) is plotted as a function of the false rate (1−specificity). Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. AUC (area under curve) value represents the combined effects of both sensitivity and specificity of single or combined marker in diagnosing patients with HCC.

Discussion

AGP is a protein synthesized in the liver, easily measurable and requires only a microplate colorimetric reader. From normal control, liver cirrhosis to HCC patients, the AGP level in serum increase with increase the diseases severity. The hepatoma cell could produce and secrete abnormally much more AGP into serum that may be used as an indicator of HCC [16]. In the present study, AGP at cutoff 1.3 ng ml^{-1} was found to have sensitivity 74% and accuracy 73% for diagnosing HCC from liver cirrhosis with AUC of 0.74.

This disagrees with the study of Bachtiar et al [17] who reported that AGP had AUC of 0.91 with similar sensitivity 77% and higher accuracy 83%, disagrees with the study of Bachtiar et al [18] who reported that AGP had AUC of 0.94 with similar sensitivity 71% and higher accuracy 82% and Kang et al who reported that AGP had AUC of 0.83. This difference might be due to ethnic differences [19]; genetic variations [20], the difference cutoff value of AGP in Egyptian HCC patients than studies in other countries [18].

It is also possible that the etiology of liver disease (HBV and HCV) can alter the AGP level [18] and HCV genotype. In this study, HCV was the underlying etiology in all of our patients with liver cirrhosis and patients with HCC. More than 70% of HCC patients have high serum concentrations of AFP because of tumor excretion in HCC patients due to the re-expression of the related gene, which is usually repressed in adult subjects [21].

In the present study, the sensitivity and specificity of AFP "the gold standard marker" at cutoff 400 U L^{-1} were 40% and 95 %. This agrees with the study of Sanai et al. [22] who reported that AFP cut-off levels of 100, 200, and 400 U L^{-1} showed similar sensitivity (40%, 36%, and 32%) and specificity (96%, 99%, and 100% respectively). Bessa et al. [23] had similar results (AUC 0.71) to our results (AUC 0.70) for predicting HCC using AFP in Egyptian patients with HCV related HCC. Thus there is a need for the enhancement of the detection of HCC using AFP.

Conclusions

We concluded that, the combination of AGP and AFP improved sensitivity (74%), specificity (80%) and efficiency (75%) with AUC (0.84). AGP is more sensitive than AFP that could improve the accuracy of HCC diagnosis from liver cirrhosis patients.

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الملخص العربى

الدقة التشخيصية للبروتين السكري الحامضى ألفا في تشخيص سرطان الكبد

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هذه الدراسة تهدف إلى تقييم القدرة التشخيصية للبروتين السكري الحامضى ألفا لتحسين تشخيص سرطان الكبد حيث تم قياس البروتين السكري الحامضى ألفا في مصل الدم من 53 مريضا بسرطان الكبد و 20 مريضا بتشمع الكبد، بالإضافة إلى 15 شخص من الأفراد الأصحاء وقد تم تعيين المساحة تحتي منحنى الروك والحساسية والتخصصية والكفاءة لهذا الدليل ومقارنتها بدليل الألفا فيتو بروتين. وبناء عليه فقد وجد أن هذا الدليل يزداد مستواه فى أمصال مرضى سرطان الكبد عن المصابين بتشمع الكبد وأن الكشف عن سرطان الكبد باستخدام البروتين السكري الحامضى ألفا يعطى قيمة للمساحة تحت المنحنى الروك (0.74) وحساسية بنسبة 74% وتخصصية بنسبة 75٪ مقارنة بالألفا فيتو بروتين .وكانت المساحة تحت المنحنى الروك (0.70) وحساسية 40٪ وتخصصية 95٪. وبالجمع بين البروتين السكري الحامضى ألفا و الألفا فيتو بروتين يعطى مساحه تحت المنحنى الروك (0.84) و حساسية بنسبة 74% وتخصصية بنسبة 80% وبالتالي فإن البروتين السكري الحامضى ألفا يمكن استخدامه في تحسين دقة التشخيص لسرطان الكبد