Role of Serum Annexin A2 As a Diagnostic and Prognostic Biomarker in Hepatocellular Carcinoma in Comparison with Alpha Fetoprotein.

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KEYWORDS
Annexin A2, alpha-fetoprotein, Diagnosis, Treatment monitoring, biomarker, hepatocellular carcinoma.

ABSTRACT
Objectives: This study aimed to evaluate the clinical utility of serum Annexin A2 (ANXA2) in hepatocellular carcinoma (HCC), as a diagnostic and prognostic marker in comparison to alpha-fetoprotein (AFP) in group of Egyptian patients.
Methods: This prospective case-control study involved three groups: HCC patients (n=30), cirrhotic patients (n=30), and a control group (n=30). Participants underwent comprehensive evaluation, including medical history, clinical examinations, and laboratory tests including serum levels of AFP. Serum Annexin A2 levels were measured using ELISA. The HCC group patients were followed up for six months post-treatment to assess changes in serum biomarker levels and tumor progression using imaging techniques.
Results: Serum ANXA2 levels were significantly higher in the HCC group (21.87 ± 10.32 ng/dl) compared to the cirrhotic group (4.46 ± 1.12 ng/dl) (P value=0.0001). AFP levels also showed a significant increase in the HCC group compared to the cirrhotic group (254.48 ± 153.39 ng/dl vs. 10.03 ± 6.97 ng/dl; P= 0.0001). After six months of follow-up, there was a notable decrease in the mean value of ANXA2 post-intervention (P= 0.0001), suggesting its utility as a prognostic marker. ROC analysis determined the optimal cutoff value for serum ANXA2 at 6.3 ng/dl, achieving 100% sensitivity and 98.33% specificity with overall diagnostic accuracy of 98.89%.
Conclusions: The study highlights the potential role of ANXA2 as a useful biomarker for HCC, with higher sensitivity and specificity when compared to AFP. ANXA2 could serve as an effective diagnostic and prognostic biomarker in HCC.

Introduction

Hepatocellular carcinoma (HCC) ranks as the fifth most prevalent cancer globally and the second most common cause of cancer-related mortality [1]. Annually, the incidence and mortality rates are alarmingly close, with approximately 841,000 new cases and 782,000 deaths [2]. HCC typically arises in individuals with a history of cirrhosis, often due to chronic alcohol abuse, non-alcoholic fatty liver disease, or hepatitis C virus (HCV) infection [1]. In Egypt, HCV and HBV account for approximately 45.3% of all new HCC cases, as noted by the National Institute of Cancer in Egypt [3]. The repeated cycles of inflammation and healing in liver cells are believed to be a key factor in HCC development [4]. The concurrent presence of inflammation and cirrhosis, however, significantly hinders the early diagnosis of HCC. Consequently, there is an urgent need for biomarkers that can effectively differentiate HCC from inflammation and cirrhosis, thereby improving patient prognosis [5]. Additionally, identifying such biomarkers could be pivotal in creating new chemo preventive approaches during the ongoing surveillance of cirrhosis patients for HCC [6].
The poor prognosis of HCC is largely due to the absence of specific early-stage symptoms. Consequently, over 60% of HCC patients are diagnosed at a late stage, often after metastasis, resulting in a dismal 5-year survival rate of less than 16% [7]. In stark contrast, early-stage detection offers a much more favorable outlook, with a 5-year survival rate exceeding 70%. Particularly, for those diagnosed at the initial stages (Barcelona Clinic Liver Cancer stages 0 and A), the 5-year survival rate with surgical intervention can surpass 93% [8]. This highlights

the critical role of early detection in enabling effective curative treatments for HCC.

The ideal biomarker for HCC would enable the diagnosis of the disease in asymptomatic individuals and be suitable for widespread screening. Clinically valuable biomarkers should exhibit both high sensitivity and specificity (at least 90%), besides being non-invasive and cost-effective to facilitate broad application [9]. The challenge in combating HCC lies in its often-asymptomatic early stages and inadequate screening methods, leading to over 80% of patients presenting with advanced disease [10]. Notably, 30% of HCC cases are diagnosed before clinical symptoms appear, even with normal serum alpha-fetoprotein (AFP) levels, underscoring the urgent need for more effective early-detection biomarkers [11].

A significant discovery in this field is the annexin family of proteins, known for binding anionic phospholipids in a calcium-dependent fashion. Initially identified in animal cells, annexins are categorized based on their occurrence across various organisms, including vertebrates (ANXA), invertebrates (ANXB), fungi and protozoa (ANXC), plants (ANXD), and protists, such as algae (ANXE) [12,13]. Among these, Annexin A2 (ANXA2) is of particular interest. Predominantly found in human endothelial cells, mononuclear cells, macrophages, marrow cells, and some cancer cells, ANXA2 is a calcium-dependent phospholipid-binding protein. Its overexpression in various human cancers positions it as a potential receptor for increased plasmin generation on cancer cell surfaces [14]. ANXA2's involvement in angiogenesis, cell proliferation, apoptosis, migration, invasion, and adhesion further highlights its multifaceted role in cellular regulation [15,16].

In the context of HCC, ANXA2's expression is negligible in normal liver and chronic hepatitis tissues but significantly elevated in HCC [17]. Elevated serum ANXA2 levels are particularly noted in early-stage, AFP-negative HCC patients [18]. Its role in promoting HCC metastasis and invasion, especially through interaction with HAb18G/CD147, has been documented [19]. However, the precise significance of ANXA2 serum level changes in the early stages of HCC remains to be fully understood. In this study, we aimed to determine the serum levels of ANXA2 in patients with HCC compared to cirrhotic patients and normal people to assess its clinical utility as a diagnostic biomarker. In addition, we aimed to correlate serum levels of ANXA2 with serum levels of AFP before and after treatment to assess the applicability of using ANXA2 as a biomarker for monitoring of treatment in HCC patients.

Methods

Study Design and Settings

This prospective case-control study was conducted at the Endemic Medicine Department, Faculty of Medicine, Cairo University Hospitals, from September 2020 to June 2022. Participants were divided into three groups: the HCC group, cirrhosis group, and control group. Ethical approval was granted by the Ethical Committee of Gastroenterology, Hepatology, and Endemic Medicine at Kasr El Ainy Hospital (Approval No. MD-332-2020). Informed consent was obtained from all participants.

Inclusion and exclusion criteria

The study enrolled participants who were over 18 years of age and provided informed consent for participation. Inclusion criteria for the HCC group mandated a confirmed diagnosis of HCC based on the EASL guidelines [20], specifically those classified under BCLC stages 0, A, or B. These patients were scheduled for treatments such as liver transplantation, surgical resection, transarterial chemoembolization (TACE), or microwave ablation. For the cirrhosis group, inclusion required a confirmed diagnosis of liver cirrhosis, established through ultrasound and transient elastography. The control group comprised individuals with negative serology for HBV and HCV and who had no evidence of fatty liver disease or autoimmune liver pathology.

Participants were excluded across all groups if they declined to participate or refused to sign the informed consent form. Specific exclusion criteria for HCC patients included those with advanced-stage disease, as defined by the BCLC staging system, where the patients were not suitable for intervention. This also included patients with major vascular tumor invasion or metastasis, as confirmed through radiological imaging studies, and those

suspected of having other solid malignancies or metastatic liver tumors.

Sample size calculation

The sample size was calculated using PASS 2008 software based on the diagnostic sensitivity and specificity of ANXA2 reported by Shaker et al. [21]. With a power of 95% and a 5% significance level, 23 patients were required in each group, increased by 20% to 30 to account for potential losses during follow-up.

Data collection

All participants underwent a comprehensive evaluation process. Initially, complete medical records were compiled, encompassing detailed personal and medical illness histories, including the date of HCC diagnosis, any interventions performed, concurrent medications (especially for HBV or HCV treatments), and past medical history. A thorough clinical examination was conducted, focusing on signs of liver failure such as jaundice, ascites, and lower limb edema. The Child-Pugh score and BCLC staging were assessed for HCC patients. Standard laboratory tests included a complete blood count (CBC), liver function tests (AST, ALT, albumin, bilirubin), serum creatinine, International Normalized Ratio (INR) and serum levels of AFP. ANXA2 was measured using the ELISA technique. A six-month post-treatment follow-up for the HCC group included reassessment of serum AFP and ANXA2 levels, along with imaging assessments like abdominal ultrasound and triphasic Computed tomography (CT) of the abdomen to monitor tumor progression, recurrence, ascites, and portal vein involvement.

Sample Collection and Preparation

In this study, five mL venous blood sample were collected from each participant under sterile conditions. From this, 1.8 mL was allocated to sodium citrate tubes for prothrombin time (PT) assays, with the remaining blood deposited into sterile vacutainers containing a clot activator. These samples were left to clot at room temperature for 20 minutes before serum separation via centrifugation at 2000-3000 RPM for 20 minutes. The serum was then divided into two aliquots: one for immediate, routine liver function tests and serum AFP detection, and the other stored at -20 ℃ for future ANXA2 level measurement. Care was taken to avoid hemolysis, repeated freezing, and thawing of samples.

ELISA Assay for ANXA2

The measurement of serum ANXA2 levels was performed using a commercially available ELISA kit (Bioassay Technology Laboratory, China). This kit employs a quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific to ANXA2 is pre-coated onto microplates. During the assay, standards and samples are pipetted into these wells, allowing any present ANXA2 to bind to the immobilized antibody. Following this, unbound substances are washed away, and an enzyme-linked monoclonal antibody specific to ANXA2 is added. After a final wash, a substrate solution is introduced to the wells, producing a color proportional to the amount of ANXA2 initially bound. The reaction is stopped by adding an acidic solution, and the absorbance is measured at 450 nm.

Detailed Assay Procedure

The assay procedure begins with the preparation of all reagents and samples, ensuring they reach room temperature before use. Standard solutions are prepared by diluting a stock solution to create various concentration points. The assay is performed at room temperature. Samples and standards are added to the wells, followed by the addition of the anti-ANXA2 antibody and streptavidin-HRP. After incubation and washing steps, substrate solutions A and B are added, and the plate is incubated in the dark. The stop solution is then added, inducing a color change, and the optical density of each well is measured using a microplate reader set to 450 nm. The entire process includes incubation, washing, addition of substrate and stop solutions, and measurement of the optical density, ensuring accurate determination of serum ANXA2 levels.

Data Analysis and Statistical Methods

To analyze the results, a standard curve was constructed by plotting the average optical density (OD) of each standard against its concentration. The curve was established using computer-based curve-fitting software, with

the best-fit line determined through regression analysis, as advised by Riener et al. (2009). For statistical description, the data were expressed in terms of mean ± standard deviation (SD), median and range for quantitative data and as frequencies and percentages for qualitative data.

The comparison of numerical variables between the study groups was conducted using a one-way analysis of variance (ANOVA) test, complemented by Bonferroni post-hoc tests for multiple two-group comparisons. The paired t-test was employed to compare baseline and follow-up values of ANXA2. For categorical data, the Chi-square test was utilized, with the exact test applied in cases where expected frequencies were below 5. Adjustments for multiple comparisons were made using the Bonferroni method. Sensitivity and specificity were used to represent accuracy, and Receiver Operator Characteristic (ROC) analysis determined the optimal cutoff value for ANXA2 and AFP in diagnosing HCC. All statistical analyses were performed using IBM SPSS version 22 for Windows. A p-value of less than 0.05 was considered statistically significant.

Results

Baseline Characteristics

The study's demographic analysis are summarized in Table 1, indicated that the HCC group had the highest mean age (58.23 ± 7 years), with the cirrhotic group following closely (55.5 ± 10.16 years), however, the age difference between these groups was not statistically significant (P= 0.666). Regarding gender, 67.8% of participants were male, with a higher male presence in the HCC (76.7%) and cirrhotic groups (70%), but these differences were not statistically significant (P= 0.559). Comorbidities such as diabetes and hypertension were observed in 30% and 13.3% of the HCC patients and 33.3% and 30% of the cirrhotic patients, respectively, with no significant differences between the groups (P= 0.781 and 0.117). Additionally, 30% of the HCC group and 20% of the cirrhotic group were smokers, although this difference was not statistically significant (P=0.143). The majority of the study's participants, 81.1% (73 out of 90), were from urban areas in Egypt, while 18.9% (17 patients) were from rural regions. Within the HCC and cirrhotic groups, 90% and 83.3%, respectively, were from urban areas, with no significant statistical difference between these groups (P= 0.462).

Table 1: Demographic characteristics

Variables		HCC group (n= 30)	Cirrhotic group (n =30)	Control group (n= 30)	P-value*
Age (Mean ±SD)		58.23 ± 7	55.5 ± 10.16	33.97 ± 8.35	0.666
Sex	Male	23 (76.7%)	21 (70%)	17 (56.7%)	0.559
	Female	7 (23.3%)	9 (30%)	13 (43.3%)	
Smoking		9 (30%)	6 (20%)	8 (26.7%)	0.143
Diabetes		9 (30%)	10 (33.3%)	0 (0%)	0.781
Hypertension		4 (13.3%)	9 (30%)	0 (0%)	0.117
Residence	Rural	3 (10%)	5 (16.7%)	-	0.462
	Urban	27 (90%)	25 (83.3%)	-	

*Difference between HCC and Cirrhosis groups

Causes of HCC and Cirrhosis

In the HCC group, the primary causes of HCC were as follows: 66.7% had HCV infection, 13.3% were due to HBV infection, 3.3% had combined HBV and HCV infection, and 16.7% were attributed to NASH. All patients in this group had underlying cirrhosis, with none developing de novo HCC. Regarding the cirrhotic group, the causative factors for liver cirrhosis were distributed as follows: 56.7% due to HCV infection, 13.3% from HBV infection, and 30% from other causes such as NASH, autoimmune hepatitis, and Wilson's disease, as shown in

Figure 1.

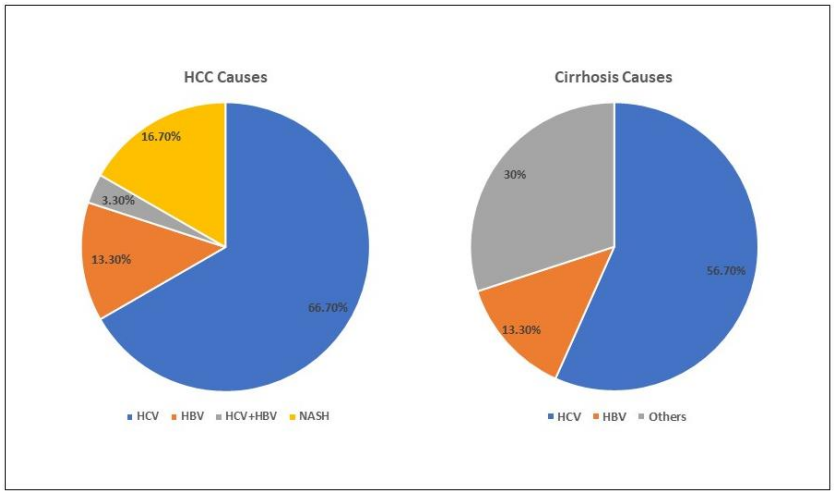

Figure 1: Causes of HCC and Cirrhosis

Previously received treatments

Concerning the treatment for viral infections, among the HCV-infected patients in the HCC group, 65% received direct-acting antivirals (DAAs), while 35% did not undergo treatment. All four patients with HBV infection received antiviral therapy, but the only patient with combined HCV and HBV infection did not receive any treatment. In terms of treatment for HCV and HBV in this group, 64.7% of those with HCV infection received DAAs, while 35.3% were not treated. Similar to the HCC group, all patients with HBV infection in the cirrhotic group were treated with antiviral therapy.

Laboratory and staging parameters

The laboratory parameters for the studied patients, as shown in Table 2, revealed that there were no significant differences between the HCC group and the cirrhotic group in all parameters. In terms of clinical scoring, Figure 2 presents the Child scores for the HCC and cirrhotic patients. Among the HCC patients, 73.3% were classified as Child A and 26.7% as Child B, with none in Child C category, as patients in this category were intentionally excluded from the study. In comparison, 55% of the cirrhotic patients were Child A, 38.3% were Child B, and a minority of 6.7% fell into the Child C category. Additionally, the BCLC staging of the studied HCC patients indicated that 10% were at BCLC stage 0, 60% were at stage A, and 30% were at stage B, outlining the distribution of cancer stages among the participants.

Table 2: Laboratory parameters

Parameters	HCC (n=30)	Cirrhosis (n= 30)	Control (n=30)	P-value*
Hemoglobin (g/dl)	10.95±1.57	11.06±0.82	12.75±1.16	1.00
Platelets (x 103/mm3)	126.27±40.83	112.03±16.22	257.3±51.11	0.481
TLC (x 103/mm3)	4.04±1.6	4.75±0.95	8.04±1.37	0.13
Bilirubin total (mg/dl)	0.99±0.52	0.92±0.28	0.56±0.24	0.485
Albumin (mg/dl)	3.51±0.4	3.05±0.35	4.26±0.42	0.132
AST (IU/ml)	39.37±17.59	41.6±10.52	39.46±22.72	0.553
ALT (IU/ml)	33.47±13.96	38.63±9.99	34.14±15.96	0.105
INR	1.32±0.37	1.58±0.4	1.06±0.08	0.21
Serum Creatinine (mg/dl)	0.92±0.2	0.97±0.37	0.82±0.3	0.404

*Difference between HCC and Cirrhosis groups

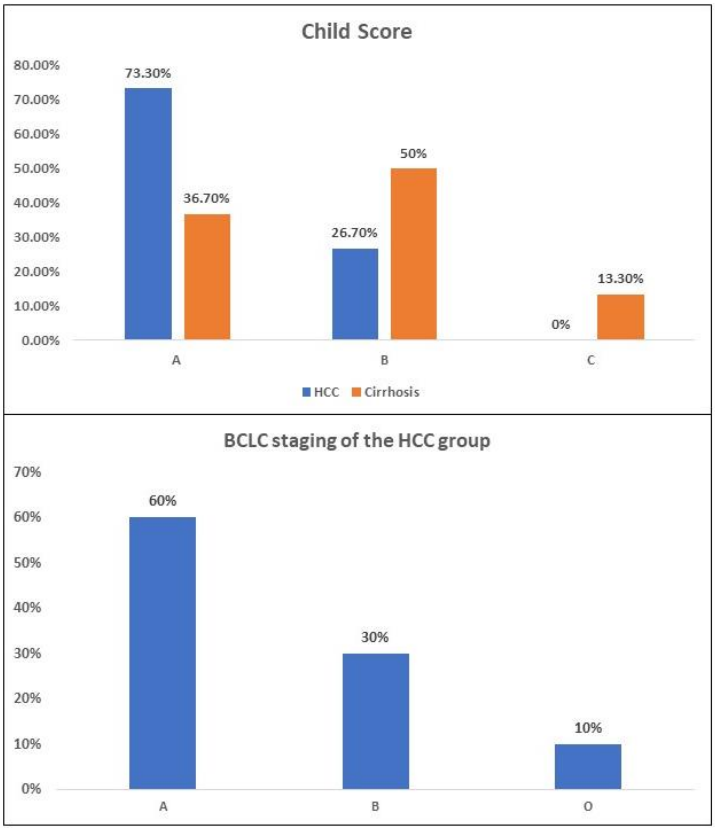

Figure 2: Child Score and HCC Staging

Serum Biomarkers in Studied Groups

Table 3 presents a comparative analysis of serum ANXA2 and AFP levels among the HCC group, cirrhotic group, and control group. The mean serum ANXA2 level in the HCC group (21.87 ± 10.32 ng/dl) was significantly higher compared to the cirrhotic group (4.46 ± 1.12 ng/dl) and the control group (2.12 ± 1.88 ng/dl), with the differences being statistically significant (P= 0.0001). Similarly, serum AFP levels were markedly elevated in the HCC group, averaging 254.48 ± 153.39 ng/dl, in contrast to the cirrhotic group (10.03 ± 6.97 ng/dl) and the control group (3.49 ± 1.87 ng/dl), again showing significant statistical differences (P= 0.0001).

Table 3: The levels of ANXA2 and AFP in the HCC, cirrhotic, and control groups

Biomarker	HCC	Cirrhosis	Control	P1	P2	P3
ANXA2 (ng/dl)	21.87± 10.32	4.46± 1.12	2.12± 1.88	0.0001	0.0001	0.42
AFP (ng/dl)	254.48 ± 153.39	10.03 ± 6.97	3.49 ± 1.87	0.0001	0.0001	1

P1: Comparison between HCC and Cirrhosis
P2: Comparison between HCC and Control
P3: Comparison between Cirrhosis and Control

Hepatic Assessment of HCC Patients

In the hepatic assessment of HCC patients, detailed in Table 4, all patients (100%) exhibited liver cirrhosis. The mean size of focal hepatic lesions was 3.19 cm. Ninety percent of the patients (27/30), had lesions located in the right lobe of the liver, while only 10% (3/30) had lesions in the left lobe. None of the participants had bi-lobar lesions. Regarding the number of focal lesions, 76.7% (23/30) of the patients had a single lesion, and 23.3% (7/30) had two lesions. Additionally, a minority of the patients, 13.3% (4/30), presented with ascites.

Table 4: Hepatic assessment of the studied group of HCC patients

Radiographic findings		N (%)
Liver cirrhosis		30 (100.00%)
Size of the focal lesion, cm (Mean ± SD)		3.19 ± 2
Site of focal lesion	Right lobe	27 (90.00%)
	Left lobe	3 (10.00%)
Number of lesions	One	23 (76.70%)
	Two	7 (23.30%)
Ascites	Yes	4 (13.30%)
	No	26 (86.70%)

Interventions for HCC Patients

Following the baseline assessment of ANXA2, various interventions were undertaken for the HCC patients. Liver transplantation was performed in 6.7% of the patients, while the most common intervention, microwave ablation (MWA) of the hepatic focal lesion, was done in 50% of the cases. Surgical liver resection, such as segmentectomy, was conducted in 6.7% of the patients. A combined procedure of TACE and MWA was performed in one patient (3.3%), and TACE alone was performed in 33.3% of the patients, highlighting the diverse therapeutic approaches undertaken in this study.

Follow-up Outcomes on ANXA2 and AFP in HCC Patients

Post-intervention follow-up at 6 months for patients with HCC revealed significant changes in serum ANXA2 levels. Of the 30 patients, 26 were available for follow-up; the remaining four were lost to follow-up due to mortality or non-compliance. There was a notable decrease in the mean serum ANXA2 levels from an initial 21.87 ± 10.32 ng/dl to 5.485 ± 1.87 ng/dl post-intervention (P= 0.0001). Similarly, a significant reduction was observed in the levels of AFP post-intervention. Figure 3 compares the baseline and follow-up levels of AFP among these patients, showing a decrease in the mean AFP level from 221.86 ± 112.32 ng/dl to 48.3 ± 22.15 ng/dl (P= 0.0001). This reduction in AFP levels further supports the therapeutic impact on HCC patients.

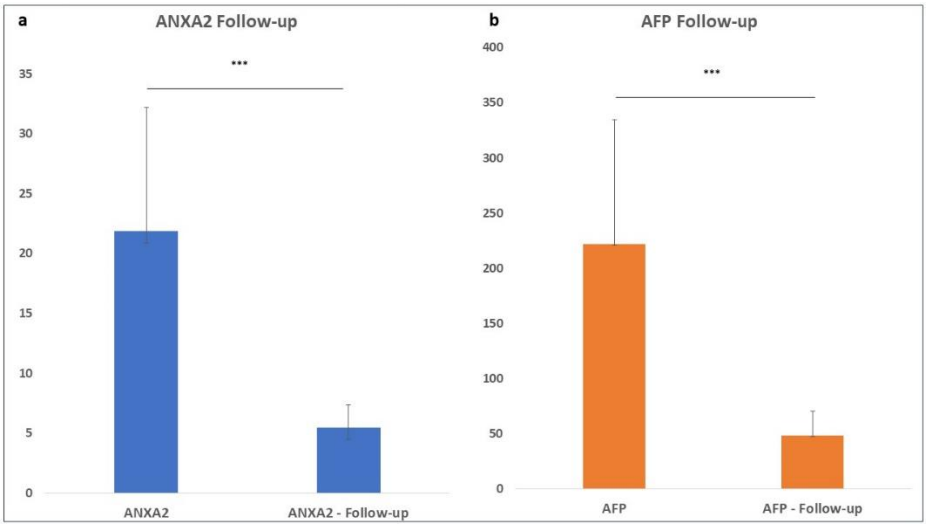

Figure 3: Follow-up Biomarkers in HCC group; a) ANXA2, b) AFP

Comparative Analysis and ROC Curve Findings

Table 5 compares the levels of ANXA2 and AFP between the HCC, cirrhotic, and control groups. Both ANXA2 and AFP levels were significantly higher in the HCC group compared to the cirrhotic and control groups (P= 0.0001). However, the difference in these biomarkers between the cirrhotic and control groups was not statistically significant. ROC analysis was conducted to determine the optimal cut-off value of serum ANXA2 for diagnosing HCC (Figure 4). The best cut-off was identified at 6.3 ng/dl, achieving 100% sensitivity and 98.3% specificity, with a PPV of 96.77% and an NPV of 100%, indicating a high diagnostic accuracy of 98.89%. In contrast, an AFP value of 10.25 ng/dl showed 90% sensitivity and 81.7% specificity, while a value of 24 ng/dl exhibited 86.7% sensitivity and 98.3% specificity, delineating the diagnostic efficacy of these biomarkers in HCC. Additionally, a significant positive correlation was found between the baseline and post-intervention levels of ANXA2 (r= 0.514, P= 0.007).

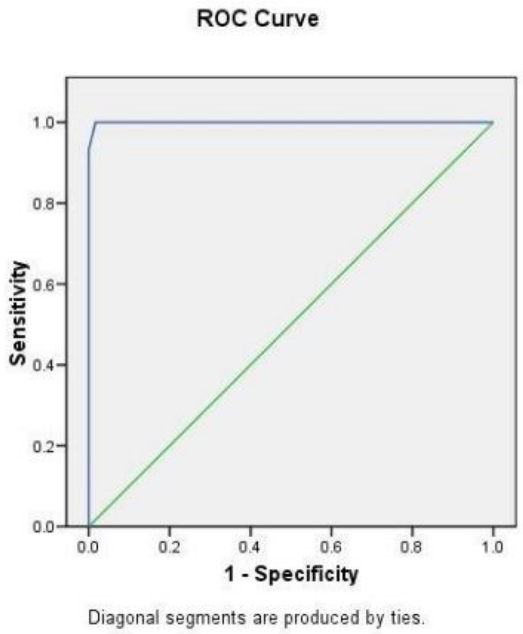

Figure 4: ROC curve of ANXA2

Table 5: Diagnostics value of ANXA2 and AFP

	Cut-off Value	Sensitivity	Specificity	PVP	PVN	Diagnostic Accuracy
ANXA2	6.3 ng/dl	100 %	98.33 %	96.77 %	100 %	98.89 %
AFP	10.25 ng/dl	90%	81.7%	-	-	-
	24 ng/dl	86.7%	98.3%	-	-	-

Discussion

In this case-control study, our findings showed a higher prevalence of HCC and cirrhosis in male patients compared to females. These findings align with previous research, where the study by Liu et al., [22], highlighted a higher susceptibility to liver cirrhosis and HCC in men compared to women Research by Akinyemiju et al., indicated that HCC occurs three times more frequently in males than females [23]. The higher incidence of HCV in the older male population in Egypt, a leading cause of liver cirrhosis and HCC, is a contributing factor to this trend [24]. Prior to the widespread use of antiretroviral therapy, HCV was a major cause of these liver conditions. Furthermore, this study highlighted the increased cumulative impact of factors like HCV, alcohol use, and smoking on the development of HCC with advancing age, supporting the findings of Makarova-Rusher et al. [25]. This context establishes a comprehensive understanding of the risk factors and gender disparities associated with HCC and cirrhosis in the Egyptian population.

In this research, diabetes was noted in 30% of HCC and 33% of cirrhotic patients, suggesting its potential as a risk factor for both diseases, though no significant statistical difference was observed between the groups, corroborating Bakir and Ali-Eldin's findings [26]. However, a Korean study by Sang-Wook et al., highlighted a positive association between glucose intolerance severity and HCC risk, suggesting diabetes is an independent risk factor for HCC [27]. The study also pointed out the connection between diabetes and liver cirrhosis, with cirrhosis leading to insulin resistance, as evidenced by impaired glucose tolerance in 60% of cirrhotic patients. Regarding smoking, it was prevalent in 30% of HCC patients, aligning with El-Serag et al.'s identification of smoking as an independent HCC risk factor [28]. This is further supported by Bakir and Ali-Eldin's study, which found a higher smoking rate among HCC patients compared to cirrhotic and controls [26], and by Niu et al.'s study linking regular and passive smoking to HCC, especially in the context of existing liver disease [29].

This investigation highlighted that most HCC patients resided in urban areas of Egypt. This finding resonates with the observations of Thomas et al., who noted a higher incidence of HCC in densely populated metropolitan areas, possibly due to better access to healthcare services for cancer screening, such as CT and MRI scans [30]. Furthermore, urban living and higher income levels may contribute to risk factors like obesity and hazardous alcohol use, which are associated with HCC. The study also aligned with the conclusions of Fattovich et al. [31] and Sangiovanni et al. [32], affirming that HCC predominantly develops in cirrhotic livers, with a yearly incidence of 1.5% among cirrhotic patients and one-third of them likely to develop HCC in their lifetime. In our study, all HCC patients developed the disease following liver cirrhosis, with the leading causes being post-HCV infection, NASH, and HBV infection. The high incidence of HCC following HCV infection in Egypt could be attributed to the enhancement of screening programs and diagnostic tools, as described by Ibrahim et al. [33], and the national HCV screening campaign initiated by the Egyptian Ministry of Health in 2018, as detailed by Elsharkawy et al. [34].

Regarding the treatment of HCV, 65% of HCC patients in our study were post-DAA treatment, reflecting El-Akel et al. [35] and Reig et al. [36] findings about the potential role of DAAs in HCC recurrence. This notion was further supported by Conti et al. [37], who observed early HCC occurrence in patients receiving DAAs for HCV. However, this view is contested by studies like the one by Telep et al. [38], which suggest that the observed increase in HCC incidence post-DAA treatment may be linked to improved screening and higher rates of follow-up rather than DAAs themselves. Wei et al. [39] and Li et al. [40] also highlighted the effectiveness and safety of DAAs in eradicating HCV, with Li et al. [40] found no association between DAAs treatment and increased HCC occurrence compared to interferon treatment, in a large retrospective cohort study comparing different treatment groups. We also observed that NASH was a significant contributor to HCC progression, aligning with Torres and Harrison's findings [41]. Additionally, HBV, the least common cause of HCC in our cohort, aligns

with Ismail et al. [42] and Elbahrawy et al. [43], reflecting the lower infection rates and decline in prevalence in Egypt due to national immunization efforts.

The cirrhotic group in our study exhibited slightly elevated ALT and AST levels, indicative of early hepatocellular damage, as described by Iluz-Freundlich et al. [44]. Radiologically, most HCC patients (76.7%) had a single focal lesion predominantly in the right hepatic lobe, aligning with studies from India and Japan [45], suggesting a correlation with better prognosis due to the lobe's larger size and blood supply. In terms of treatment, half of our HCC patients opted for MWA, and a third for TACE, reflecting the current preference for these methods in managing non-resectable HCC, as reported by Pleguezuelo et al. [46] and supported by Hinshaw et al. [47], regarding the efficacy of the latest MWA systems.

In our study, the baseline serum ANXA2 levels averaged 21.87 ng/dl, were significantly higher in HCC patients than in cirrhotic patients with average serum levels of 4.46 ng/dl, aligning with Sharma, who found elevated ANXA2 levels in HCC compared to liver cirrhosis and other liver conditions [48]. This study agrees with Zhang et al. [49] and Mohammad et al. [50], both of whom noted that ANXA2; which is typically low in normal and chronic hepatitis liver tissues, is upregulated in HCC, potentially contributing to metastasis and invasion via interaction with HAb18G/CD147. Regarding AFP, our findings showed its limited utility in differentiating HCC from other liver disorders due to its high false-positive and false-negative rates, as discussed by Hanif et al. [51] and Zhou et al. [52]. AFP levels, while slightly higher in cirrhotic patients, were significantly elevated in the HCC group, supporting Zhou et al.'s observation about the variable sensitivity and specificity of AFP depending on the cutoff value used [52].

A follow-up after 6 months revealed a notable decrease in ANXA2 levels post-intervention in HCC patients, suggesting its potential utility as a biomarker for monitoring treatment and in detecting HCC recurrence. This finding is supported by Mohammad et al. [50] and Zhang et al. [49], who highlighted the diagnostic and prognostic significance of ANXA2 in cancer, particularly its association with metastases and recurrence risk. Our study's ROC analysis determined an optimal cutoff value for serum Annexin A2 at 6.3 ng/dl, offering 100% sensitivity and 98.33% specificity, outperforming other studies such as those by Zhang et al. [49] and Shaker et al. [21], which suggested higher cutoff values but with lower sensitivity and specificity. The difference in cutoff values could be attributed to our study focusing on early-stage HCC patients fit for intervention, unlike other studies that included advanced-stage HCC patients.

One of the primary limitations of this study is its relatively small sample size, which may affect the generalizability of the findings to the broader population of HCC patients. Additionally, the absence of Child C cirrhotic patients potentially limits the applicability of the results to all stages of liver cirrhosis, potentially skewing the understanding of ANXA2's effectiveness across the full spectrum of the disease. The study's focus on a specific Egyptian patient population also raises questions about its applicability to diverse ethnic and geographical groups, considering the variations in HCC etiology and progression globally. Furthermore, the study's short-term follow-up period of six months may not fully capture the long-term prognostic value of ANXA2, particularly in terms of recurrence and survival rates. Finally, the reliance on a single biomarker measurement technique (ELISA) for ANXA2 might limit the robustness of the findings, as the incorporation of additional detection methods could provide a more comprehensive evaluation.

Conclusions

This study highlights serum ANXA2 as a valuable biomarker with comparable sensitivity and specificity to AFP, thus offering a potential tool for both diagnosis and monitoring of treatment in HCC. Despite AFP's limitations, such as its low sensitivity at a 200 ng/dl cutoff and its ineffectiveness in early-stage or small HCCs, ANXA2 emerges as a cost-effective alternative. The fluctuating cutoff value of AFP across different ethnic groups due to varying epidemiological factors further underscores the need for alternative markers like ANXA2. While AFP remains a key screening tool, its limited capacity to differentiate HCC from benign liver disorders necessitates further research on ANXA2, especially in more diverse patient groups, including those with Child C cirrhosis and in larger cohorts. Additionally, ongoing studies are exploring the potential of ANXA2 as an anti-cancer therapy, potentially enhancing its significance in future cancer treatments.

References

[1] Asafo-Agyei KO, Samant H. Hepatocellular Carcinoma. 2023.

[2] Abudeif A. Epidemiology and Risk Factors of Hepatocellular Carcinoma in Egypt. Sohag Medical Journal. 2019 Oct 1;23(3):8–12.

[3] Ezzat R, Eltabbakh M, El Kassas M. Unique situation of hepatocellular carcinoma in Egypt: A review of epidemiology and control measures. World J Gastrointest Oncol. 2021 Dec 15;13(12):1919–38.

[4] Chen HJ, Hu MH, Xu FG, Xu HJ, She JJ, Xia HP. Understanding the inflammation-cancer transformation in the development of primary liver cancer. Hepatoma Res. 2018 Jul 5;4(7):29.

[5] Tsuchiya N. Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2015;21(37):10573.

[6] Schütte K. Current biomarkers for hepatocellular carcinoma: Surveillance, diagnosis and prediction of prognosis. World J Hepatol. 2014;7(2):139.

[7] Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular Carcinoma Incidence, Mortality, and Survival Trends in the United States From 1975 to 2005. Journal of Clinical Oncology. 2009 Mar 20;27(9):1485–91.

[8] Takayama T, Makuuchi M, Kojiro M, Lauwers GY, Adams RB, Wilson SR, et al. Early Hepatocellular Carcinoma: Pathology, Imaging, and Therapy. Ann Surg Oncol. 2008 Apr 31;15(4):972–8.

[9] Xing M, Wang X, Kirken R, He L, Zhang JY. Immunodiagnostic Biomarkers for Hepatocellular Carcinoma (HCC): The First Step in Detection and Treatment. Int J Mol Sci. 2021 Jun 7;22(11):6139.

[10] El-Serag HB. Hepatocellular Carcinoma. New England Journal of Medicine. 2011 Sep 22;365(12):1118–27.

[11] Malaguarnera M, Vacante M, Fichera R, Cappellani A, Cristaldi E, Motta M. Chromogranin A (CgA) serum level as a marker of progression in hepatocellular carcinoma (HCC) of elderly patients. Arch Gerontol Geriatr. 2010 Jul;51(1):81–5.

[12] Lokman NA, Ween MP, Oehler MK, Ricciardelli C. The Role of Annexin A2 in Tumorigenesis

and Cancer Progression. Cancer Microenvironment. 2011 Aug 5;4(2):199–208.

[13]Bharadwaj A, Bydoun M, Holloway R, Waisman D. Annexin A2 Heterotetramer: Structure and Function. Int J Mol Sci. 2013 Mar 19;14(3):6259–305.

[14]Zhang Q, Ye Z, Yang Q, He X, Wang H, Zhao Z. Upregulated expression of Annexin II is a prognostic marker for patients with gastric cancer. World J Surg Oncol. 2012 Dec 8;10(1):103.

[15]Yang SL, Chou YT, Wu CN, Ho MS. Annexin II Binds to Capsid Protein VP1 of Enterovirus 71 and Enhances Viral Infectivity. J Virol. 2011 Nov 15;85(22):11809–20.

[16]Sun Y, Gao G, Cai J, Wang Y, Qu X, He L, et al. Annexin A2 is a discriminative serological candidate in early hepatocellular carcinoma. Carcinogenesis. 2013 Mar;34(3):595–604.

[17]Song. Evaluation of annexin II as a potential serum marker for hepatocellular carcinoma using a developed sandwich ELISA method. Int J Mol Med. 2009 Oct 16;24(06).

[18]Forner A, Reig ME, Rodriguez de Lope C, Bruix J. Current Strategy for Staging and Treatment: The BCLC Update and Future Prospects. Semin Liver Dis. 2010 Feb 19;30(01):061–74.

[19]Zhang HJ. Expression characteristics and diagnostic value of annexin A2 in hepatocellular carcinoma. World J Gastroenterol. 2012;18(41):5897.

[20]Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul JL, et al. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018 Jul;69(1):182–236.

[21]Shaker MK, Abdel Fattah HI, Sabbour GS, Montasser IF, Abdelhakam SM, El Hadidy E, et al. Annexin A2 as a biomarker for hepatocellular carcinoma in Egyptian patients. World J Hepatol. 2017;9(9):469.

[22]Liu P, Xie SH, Hu S, Cheng X, Gao T, Zhang C, et al. Age-specific sex difference in the incidence of hepatocellular carcinoma in the United States. Oncotarget. 2017 Sep 15;8(40):68131–7.

[23]Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level. JAMA Oncol. 2017 Dec 1;3(12):1683.

[24]Economic and Social Justice Unit. HCV Treatment in Egypt Why cost remains a challenge? [Internet]. 2014. Available from: https://eipr.org/en/publications/hepatitis-c-treatment-egypt-why-cost-remains-challenge

[25]Makarova-Rusher O V., Altekruse SF, McNeel TS, Ulahannan S, Duffy AG, Graubard BI, et al. Population attributable fractions of risk factors for hepatocellular carcinoma in the United States. Cancer. 2016 Jun 21;122(11):1757–65.

[26]Bakir ASM, Ali-Eldin ZA. Is diabetes mellitus a risk factor for hepatocellular carcinoma in Egyptian patients. In 2012. Available from: https://api.semanticscholar.org/CorpusID:4820182

[27]Yi S, Choi J, Yi J, Lee Y, Han KJ. Risk factors for hepatocellular carcinoma by age, sex, and liver disorder status: A prospective cohort study in Korea. Cancer. 2018 Jul 18;124(13):2748–57.

[28]El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and Treatment of Hepatocellular Carcinoma. Gastroenterology. 2008 May;134(6):1752–63.

[29]Niu Y, Yang X, Chen Y, Jin X, Xie Y, Tang Y, et al. Distinct prognostic values of Annexin family members expression in acute myeloid leukemia. Clinical and Translational Oncology. 2019 Sep 29;21(9):1186–96.

[30]Thomas JA, Kendall BJ, Dalais C, Macdonald GA, Thrift AP. Hepatocellular and extrahepatic cancers in non-alcoholic fatty liver disease: A systematic review and meta-analysis. Eur J Cancer. 2022 Sep;173:250–62.

[31]Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: Incidence and risk factors. Gastroenterology. 2004 Nov;127(5):S35–50.

[32]Sangiovanni A, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, et al. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. Hepatology. 2006 Jun;43(6):1303–10.

[33]Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program. J Cancer Epidemiol. 2014;2014:1–18.

[34]Elsharkawy A, El-Raziky M, El-Akel W, El-Saeed K, Eletreby R, Hassany M, et al. Planning and prioritizing direct-acting antivirals treatment for HCV patients in countries with limited resources: Lessons from the Egyptian experience. J Hepatol. 2018 Apr;68(4):691–8.

[35]El-Akel W, El-Sayed MH, El Kassas M, El-Serafy M, Khairy M, Elsaeed K, et al. National treatment programme of hepatitis C in Egypt: Hepatitis C virus model of care. J Viral Hepat. 2017 Apr;24(4):262–7.

[36]Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol. 2016 Oct;65(4):719–26.

[37]Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016 Oct;65(4):727–33.

[38]Telep LE, Muramoto D, Osinusi A, Brainard DM, Reddy KR, Singer AW, et al. No increased risk of hepatocellular carcinoma recurrence in patients following interferon-free, direct acting anti-viral treatment for hepatitis C virus: a cohort study using large-scale administrative medical claims data. J Hepatol. 2017;66(1):S533.

[39]Wei L, Wang X, Lv L, Liu J, Xing H, Song Y, et al. The emerging role of microRNAs and long noncoding RNAs in drug resistance of hepatocellular carcinoma. Mol Cancer. 2019 Dec 25;18(1):147.

[40]Li DK, Ren Y, Fierer DS, Rutledge S, Shaikh OS, Lo Re V, et al. The short-term incidence of hepatocellular carcinoma is not increased after hepatitis C treatment with direct-acting antivirals: An ERCHIVES study. Hepatology. 2018 Jun 19;67(6):2244–53.

[41]Torres DM, Williams CD, Harrison SA. Features, Diagnosis, and Treatment of Nonalcoholic Fatty Liver Disease. Clinical Gastroenterology and Hepatology. 2012 Aug;10(8):837–58.

[42]Ismail SA, Cuadros DF, Benova L. Hepatitis B in Egypt: A cross-sectional analysis of prevalence and risk factors for active infection from a nationwide survey. Liver International. 2017 Dec 7;37(12):1814–22.

[43]Elbahrawy A, Ibrahim MK, Eliwa A, Alboraie M, Madian A, Aly HH. Current situation of viral hepatitis in Egypt. Microbiol Immunol. 2021 Sep 21;65(9):352–72.

[44]Iluz-Freundlich D, Zhang M, Uhanova J, Minuk GY. The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. Ann Hepatol. 2020 Mar;19(2):204–8.

[45]Kudo M, Kawamura Y, Hasegawa K, Tateishi R, Kariyama K, Shiina S, et al. Management of Hepatocellular Carcinoma in Japan: JSH Consensus Statements and Recommendations 2021 Update. Liver Cancer. 2021;10(3):181–223.

[46]Pleguezuelo M, Marelli L, Misseri M, Germani G, Calvaruso V, Xiruochakis E, et al. TACE versus TAE as therapy for hepatocellular carcinoma. Expert Rev Anticancer Ther. 2008 Oct 10;8(10):1623–41.

[47]Hinshaw JL, Lubner MG, Ziemlewicz TJ, Lee FT, Brace CL. Percutaneous Tumor Ablation Tools: Microwave, Radiofrequency, or Cryoablation—What Should You Use and Why? RadioGraphics. 2014 Sep;34(5):1344–62.

[48]Sharma MC. Annexin A2 (ANX A2): An emerging biomarker and potential therapeutic target for aggressive cancers. Int J Cancer. 2019 May 31;144(9):2074–81.

[49]Zhang HJ. Expression characteristics and diagnostic value of annexin A2 in hepatocellular carcinoma. World J Gastroenterol. 2012;18(41):5897.

[50]Mohammad HS, Kurokohchi K, Yoneyama H, Tokuda M, Morishita A, Jian G, et al. Annexin A2 expression and phosphorylation are up-regulated in hepatocellular carcinoma. Int J Oncol. 2008 Dec;33(6):1157–63.

[51]Hanif H, Ali MJ, Susheela AT, Khan IW, Luna-Cuadros MA, Khan MM, et al. Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. World J Gastroenterol. 2022 Jan 14;28(2):216–29.

[52]Zhou L. Serum tumor markers for detection of hepatocellular carcinoma. World J Gastroenterol. 2006;12(8):1175..