Significance of Angiopoietin-2 as a Serum Marker for Hepatocellular Carcinoma

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Abstract: Background and study aims: Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and one of the major causes of death. The aim of this study was to investigate the potential role of Angiopoietin-2 as a non-invasive marker for HCC. Patients and Methods: This study was conducted on 30 patients with documented HCC and 30 cirrhotic patients with no evidence of HCC; as well as 30 healthy subjects who served as control group. The levels of alfa fetoprotein (AFP) and angiopoietin-2 (Ang-2) were measured for all cases together with full clinical assessment, liver biochemical profile, viral markers, ultrasound, abdominal triphasic computerized tomography (CT) scan and guided liver biopsy for HCC cases with atypical triphasic CT pattern. Results: There was a statistically highly significant elevation ($p< 0.001$) in the mean serum AFP in HCC group (155.5 ± 271.5 ng/ml) when compared with the control group (6.3 ± 2.4 ng/ml) and also a highly significant elevation ($p<0.01$) when compared to the cirrhosis group (29.3 ± 31.2 ng/ml). There was a statistically highly significant elevation ($p< 0.001$) in the mean serum Ang-2 in HCC group (10855 ± 5321.92 pg/ml) when compared with both the control (480.67 ± 202.3 pg/ml) and cirrhosis (5578.33 ± 2928.21 pg/ml) groups. The diagnostic sensitivity of AFP at a cutoff of 200 ng/ml was 24% and the specificity was 100%. The cutoff level of Ang-2 for diagnosis of HCC in this study was 8100 pg/ml, with a sensitivity and specificity of 70% and 80% respectively. Serum Ang-2 was significantly elevated in HCC patients with portal vein thrombosis than those without. There was a significant positive correlation between the number of hepatic focal lesions and the serum level of Ang-2. The combined use of the two markers (AFP and Ang-2) led to an increase in the sensitivity of AFP from 53.3% to 83.3%. Conclusion: Serum Ang-2 is elevated in patients with cirrhosis and further elevated in patients with HCC, so its use as an independent tumor marker in the diagnosis of HCC is to be considered. Simultaneous measurement of serum AFP and Ang-2 may enhance the sensitivity of HCC detection.

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1. Introduction:

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver *(El Serag & Rudolph, 2007).* Over a decade (1993-2002), there was nearly a twofold increase of the proportion of HCC among chronic liver disease (CLD) patients in Egypt with a significant decline of hepatitis B virus (HBV) and slight increase of hepatitis C virus (HCV) as risk factors *(El-Zayadi et al., 2005)*. Alfa-feto protein (AFP) is an inadequate screening test for HCC *(Sherman, 2001; Bruix & Sherman, 2005),* but still has a role in the diagnosis, since in cirrhotic patients with a mass in the liver an AFP greater than 200ng/mL has a very high positive predictive value for HCC *(Trevisani et al., 2001).* Angiopoeitin (Ang)-2 is a 66 kDa protein consisting of 496 amino acid residues which was detected by homology screening as a naturally occurring antagonist for both Ang-1 and the Tie-2 receptor. In adult mice and humans, they found that Ang-2 is expressed only at the sites of vascular remodeling (*Maisonpierre et al., 1997).* As HCCs are hypervascularized tumors the generation of new arterial vessels is a prerequisite for their survival. The induction of neoangiogenesis is mainly driven by hypoxia, leading to activation of several angiogenic pathways, including the angiopoietin/Tie-2 pathway. In this context, Ang-2 [in presence of vascular endothelial growth factor (VEGF)] allows remodeling of arterial vessels not stabilized by the effects of Ang-1 *(Holash et al., 1999).* Ang-2 is overexpressed in HCC-as measured by immunohistochemistry-especially of the highly vascular type *(Sugimachi et al., 2003).* Its expression is associated with portal infiltration, microvessel density, recurrence of HCC and decreased survival *(Wada et al., 2006).*

Recent studies reported high serum Ang-2 values in patients with HCC suggesting that it might represent a useful marker for HCC and a complementary diagnostic tool *(Scholz et al., 2007).*

The aim of this work was to investigate the potential role of Ang-2 as a diagnostic serum marker for HCC in patients with liver cirrhosis and to assess its sensitivity and specificity as compared to AFP and to assess whether the combined use of both markers can improve the diagnostic power of HCC.

2. Materials and methods

Study groups:

This study was conducted on 60 patients admitted to the Hepatology and Gastroenterology Department, Theodor Bilharz Research Institute (TBRI) in the period between November 2008 and June 2009. In addition, 30 apparently healthy individuals served as a control group.

They were divided into three main groups:

Group I (HCC group):

Included 30 cirrhotic patients with HCC, 19 of them were males (63.3%) and 11 females (36.7%). Five patients with focal hepatic lesions did not show the typical HCC pattern on triphasic CT scan: these lesions were biopsied guided by ultrasound for histopathological assessment and proved to be HCC.

Group II (liver cirrhosis group):

Included 30 patients with post hepatitic liver cirrhosis; 17 of them were males (56.7%) and 13 females (43.3%).

Group III (control group):

Included 30 apparently healthy individuals, 9 of them were males (30%) and 21 females (70%). They were completely free clinically, with normal laboratory findings, negative viral hepatitis markers and normal abdominal ultrasonographic findings.

Exclusion criteria:

Inflammatory or septic conditions as spontaneous bacterial peritonitis
(SBP).

Focal hepatic lesions other than HCC (cholangiocarcinoma, hemangioma, hepatoblastoma, metastatic focal lesions…etc).

Carcinoma elsewhere.

Methodology:

Full history taking and clinical examination were done to all patients and the following routine laboratory investigations:

- Complete blood picture and erythrocyte sedimentation rate (ESR).
- Liver function tests: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), serum bilirubin (direct and indirect), serum proteins and serum albumin, prothromhin time and concentration (PT and PC) and international normalized ratio (INR).
- Renal function tests: serum urea and creatinine.
- Hepatitis markers: hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (HBcAb) total, antibody to hepatitis B surface antigen (HBsAb) and anti-HCV antibody.
- Fasting and 2 hours post-prandial blood sugar.

Evaluation of the severity of liver cirrhosis was assessed in each cirrhotic patient with the Modified Child score (Pugh et al., 1973).

Imaging studies:

Abdominal ultrasonography:

Abdominal ultrasonography was performed for all groups. Patients were examined using a real time machine (Hitachi, EUB-515A).

Liver was assessed for size, smoothness of the surface, texture, portal vein diameter, and thickening of portal tracts as an indicator for schistosomal hepatic fibrosis (Abdel-Wahab et al., 1992). The presence of focal lesions and their detailed description as regards number, size, site, echogenicity was reported. Doppler ultrasound was used to assess the patency of the portal vein so as to detect the presence of malignant thrombi and assess their extension. It was also used to detect any Doppler signals inside and around the lesions as the presence of intra-lesional arterial signals is highly suggestive of malignancy (Maruyama et al., 2008). A complete abdominal scanning was done to detect any other abnormality including the presence of ascites, lymph nodes or abdominal masses.

Triphasic abdominal CT scanning:

Spiral triphasic CT abdomen was done to all patients in HCC group for the diagnosis of hepatic focal lesions with specific features of HCC as previously described (Van Leeuwen et al., 1996) (Paley and Ros, 1998) (Hoon Ji et al., 2001).

Ultrasonographic-guided liver biopsy:

Biopsy was done to 5 patients with focal lesions using Trucut needles under ultrasound guidance after careful explanation of the procedure to the patient, with informed written consent to the procedure and after fulfillment of the following criteria:

1. Acceptable prothrombin time (<17 sec) and concentration (>60%), INR <2 and platelet count > 75.000/cc.
2. No ascites at the time of biopsy.
3. No other obstacles to liver biopsy (e.g. inaccessible lesion or lesion adjacent to vital structures like blood vessels).

All biopsies were histopathologically graded by Steiner-Edmondson grading system (Edmondson and Steiner, 1954).

Tumor markers:

A 15 ml blood sample was drawn from each subject immediately after diagnosis. Blood samples were centrifuged and serum aliquoted and stored at –70°C until tested for AFP and Ang-2.

1-Measurement of serum AFP (ng/dl)

AFP assay:

Serum AFP was measured by enzyme-linked immunosorbent assay (ELISA) technique using commercially available immunometric assays utilizing enhanced chemiluminescence (EQUIPAR Diagnostics, France) with cutoff 20 ng/dl as a maximum level of normal.

2- Measurement of serum Ang-2

Ang-2 assay characteristics:

Sensitivity:

The minimum detectable dose of the human Ang-2 is <6 pg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentrations.

Specificity:

Buffered solutions of a panel of substances ranging in concentrations from 10.000 to 50.000 pg/mL were assayed with the Human Ang-2 kit and found to have no cross-reactivity: Human Ang-1, Ang-4, granulocyte monocyte colony stimulating factor (GM-CSF), interferon (IFN)γ, interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and tumor necrosis factor (TNF)-α. Random normal serum samples from various species were also evaluated with the Human Ang-2 kit. No crossreactivity was observed with the goat, hamster, mouse and rat serum samples. There was moderate cross-reactivity with rabbit and swine, and significant cross-reactivity with fetal bovine, calf, horse and monkey serum samples.

Principle of the test:

The Human Ang-2 kit (Biosource International, USA) is a solid phase sandwich ELISA. A monoclonal antibody specific to human Ang-2 has been coated onto the wells of the microtiter strips provided. During the first incubation, standards of known human Ang-2 content, controls, and unknown samples are pipetted into the coated wells, followed by the addition of biotinylated second anti-Ang-2 antibody. After washing, streptavidin-peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of human Ang-2 present in the original specimen.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Differences between groups were analyzed either by using the Chi square test or student's t test and non-parametric (Mann Whitney test) for comparison between two groups or ANOVA test for multiple group comparison. Spearman rank correlation coefficient was used to determine significant correlations among different parameters. The analysis was performed using Statistical Analysis System, version 6.03, on an IBM at personal computer.

3. Results

The mean age ± SD within the HCC group was (50.6 ± 6.7) compared to the cirrhosis group (50.7± 6.0) and the control group (32.2± 7.2). The number of males within HCC, cirrhosis and control groups were 19/30 (63%), 17/30 (57%) and 9/30 (30%) respectively. There was no statistically significant difference in age and sex between the three studied groups. Table (1) showed the results of the laboratory liver profile of the studied groups.

Table (1): Liver profile of the studied groups

Parameters	HCC (n= 30)	Cirrhosis (n= 30)	Control (n= 30)	p value
Total bilirubin (up to 1mg/dl)	2.54 ± 2.06 a	2.67 ± 2.35 a	0.88 ± 0.22	a p< 0.01 vs. control HS
Direct bilirubin (up to 0.25mg/dl)	1.42 ± 1.45 a	1.56 ± 1.67 a	0.25 ± 0.05	a p< 0.01 vs. control HS
ALT (30-65 U/l)	56.5 ± 34.2 a	44.03 ± 27.58 a	19.37 ± 8.5	a p< 0.01 vs. control HS
AST (15-37 U/l)	60.57± 36.62 a	49.87 ± 31.04 a	24.77 ± 10.89	a p< 0.01 vs. control HS
ALP (61-171 U/l)	195.8 ± 99.07 a, b	148.5 ± 115.66 a	70.1 ± 33.1	a p< 0.01 vs. control HS b p< 0.05 vs. cirrhosis S

S= significant P< 0.05 HS= highly significant P< 0.01

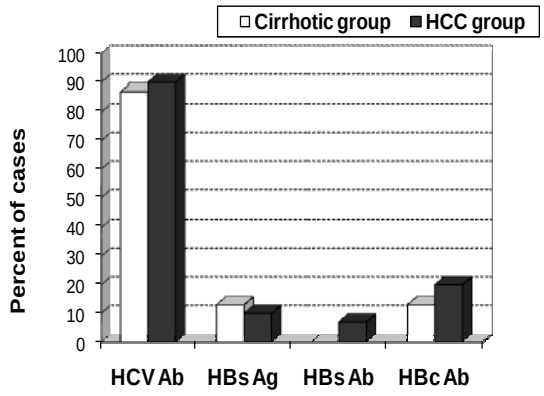


Figure (1): Viral markers of the studied groups

Among the cirrhosis group, 26 patients (86.7%) were positive for HCV antibody, while 4 patients (13.3%) had positive HBsAg and HBcAb and no patient was positive for HBsAb. Among the HCC group, 27 patients (90%) were positive for HCV antibody, 2 of those patients with HCV had previous exposure to HBV and were immune (HBsAb and HBcAb positive) and 3 patients (10%) were positive to HBsAg and HBcAb showing no statistically significant difference between both groups (Figure 1).

Five patients (17%) in the cirrhosis group were Child class A compared to 9 patients (30%) in the HCC group. Nine patients (30%) in the cirrhosis group and 10 patients (33%) of the HCC group were Child class B, while 16 patients (53%) in the cirrhosis group were Child class C as compared to 11 patients (37%) in the HCC group with no statistically significant difference between both groups.

The ultrasonographic features of the focal hepatic lesions in HCC patients showed that 19 patients (63%) had single focal lesion, 6 patients (20%) had 2 focal lesions, and 5 patients (17%) had multiple focal lesions. Four patients (13%) had focal lesions ≤ 2cms in diameter while 26 patients (87%) had focal lesions > 2cms in diameter. The focal lesions were located in the right hepatic lobe in 19 patients (63%), in the left hepatic lobe in 7 patients (23%), while 4 patients (13%) had focal lesions detected in both lobes. Twenty one lesions (70%) were hypo-echoic, 1 lesion (3.3%) was hyper-echoic and 8 lesions (26.7%) were iso-echoic. There were 5 cases (17%) of portal vein thrombosis in HCC group and none in liver cirrhosis group (0%).

As regards CT pattern of HCCs in triphasic CT scan: 25 lesions (83%) showed typical enhancement features of HCC (typical specific pattern of arterial uptake followed by venous washout in the delayed portal/venous phase), while 5 lesions (17%) showed atypical enhancement pattern on different CT scan phases and were biopsied under ultrasound guidance and examined histopathologically: 2 lesions (40%) were grade I, while the other 3 lesions were grade II (60%).

There was a statistically highly significant elevation ($p< 0.001$) in the mean serum AFP in HCC group (155.5 ± 271.5 ng/ml) when compared with the control group (6.3 ± 2.4 ng/ml) and also a highly significant elevation ($p<0.01$) when compared to the cirrhosis group (29.3 ± 31.2 ng/ml) (Table 2).

There was a statistically highly significant elevation ($p< 0.001$) in the mean serum Ang-2 in HCC group (10855 ± 5321.92 pg/ml) when compared with both the control (480.67 ± 202.3 pg/ml) and cirrhosis (5578.33 ± 2928.21 pg/ml) groups (Table 2)

Table (2): Mean levels of AFP and Ang-2 in studied groups

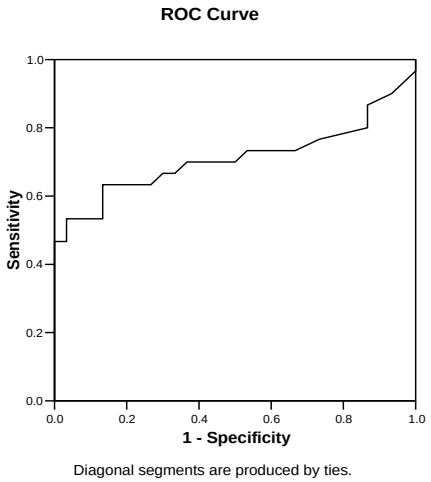
	HCC (n= 30)	Cirrhosis (n= 30)	Control (n= 30)	P value
AFP	*155.5±271.5* [a, b]	*29.3 ± 31.2*	*6.3± 2.4*	[a] *p< 0.001 vs. control* HS [b] *p< 0.01 vs. cirrhosis* HS
Ang-2	*10855.0 ± 5321.92* [ab]	*5578.33 ± 2928.21* [a]	*480.67 ± 202.30*	[a] *p< 0.001 vs. control* HS [b] *p< 0.001 vs. cirrhosis* HS

HS= highly significant P< 0.01

Specificity and sensitivity of AFP:

When using the receiver operator characterizing (ROC) curve, to improve the specificity and sensitivity of AFP in the differentiation between HCC and cirrhosis, the cutoff value of 75.5 ng/ml yielded a sensitivity and specificity of 53.3% and 86.7%, respectively (best cutoff). When the cutoff of AFP was increased to 200 ng/m1 the sensitivity dropped to 24% and specificity was 100% (Figure 2).

Figure (2): ROC curve for AFP



Specificity and sensitivity of Ang-2

1) Cirrhosis group *versus* control:

When using the ROC curve, to improve the specificity and sensitivity of Ang-2, the cutoff value of 1700 pg/ml yielded a sensitivity and specificity of 90% and 97%, respectively (best cutoff).

2) HCC group *versus* control:

When using the ROC curve, to improve the specificity and sensitivity of Ang-2, the cutoff value of 3900 pg/ml yielded a sensitivity and specificity of 93% and 100%, respectively (best cutoff).

3) HCC group *versus* cirrhosis:

When using the ROC curve, to improve the specificity and sensitivity of Ang-2, the cutoff value of 8100 pg/ml yielded a sensitivity and specificity of 70% and 80%, respectively (best cutoff) (Figure 3).

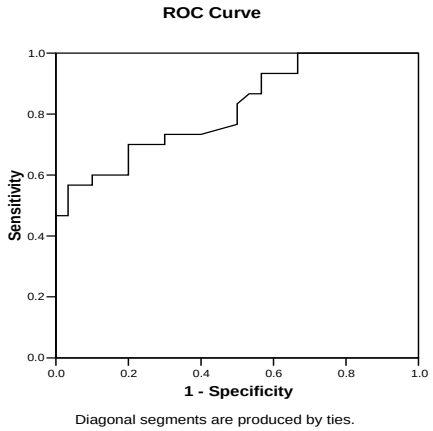


Figure (3): ROC curve for Ang-2 (cirrhosis versus HCC)

Table (3) showed the correlation between the level of Ang-2 and other parameters in the studied groups.

Figure (4) showed the inverse correlation between serum Ang-2 and serum albumin in patients with cirrhosis.

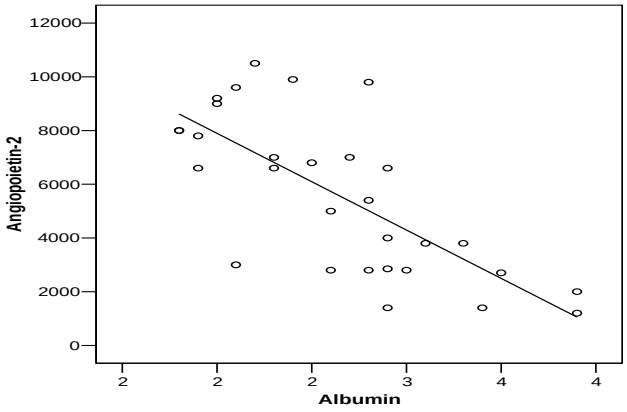


Figure (4): *Correlation between Ang-2 and albumin in cirrhosis patients.*

Table (3): ***Correlation between Ang-2 level and other parameters in the studied groups***

Variable / Correlated	Cirrhosis (n= 30)		HCC (n= 30)	
	Correlation coefficient (r)	p value	Correlation coefficient (r)	p value
Age	0.224	0.233 NS	0.117	0.537 NS
TLC	0.101	0.596 NS	0.045	0.815 NS
Haemoglobin	-0.144	0.448 NS	0.005	0.979 NS
Platelets	-0.273	0.144 NS	-0.306	0.100 NS
ESR	0.201	0.288 NS	0.176	0.352 NS
PC	-0.520	0.003 HS	-0.122	0.522 NS
T. bilirubin	0.359	0.052 NS	0.313	0.092 NS
D. bilirubin	0.415	0.023 S	0.291	0.118 NS
AST	-0.029	0.880 NS	0.030	0.877 NS
ALT	-0.204	0.279 NS	-0.163	0.389 NS
ALP	0.218	0.248 NS	0.184	0.330 NS
T. Protein	0.003	0.986 NS	-0.433	0.056 NS
Albumin	-0.737	0.001 HS	-0.327	0.077 NS
Urea	0.237	0.207 NS	-0.313	0.092 NS
Creatinine	0.214	0.256 NS	0.131	0.490 NS
FBS	0.300	0.107 NS	-0.094	0.622 NS
AFP	-0.226	0.229 NS	0.172	0.363 NS

NS= non significant P> 0.05 S= significant P< 0.05 HS= highly significant P< 0.01

Table (4) showed the correlation between serum Ang-2, serum AFP and Child score in both cirrhosis and HCC groups.

Table (4): ***Correlation between serum Ang-2, serum AFP and Child score in cirrhosis and HCC patients:***

Variable / Correlated	Cirrhosis (n=30)		HCC (n= 30)	
	Correlation coefficient (r)	**p value**	**Correlation coefficient (r)**	**p value**
Ang-2 vs. Child score	0.807	0.001 HS	0.339	0.067 NS
AFP vs. Child score	-0.222	0.238 NS	0.643	0.001 HS

NS= non significant P> 0.05 HS= highly significant P< 0.01

There was a statistically significant positive correlation between the serum Ang-2 level in HCC patients with the number of focal lesions (r= 0.95; p= 0.031) and no significant correlation between serum Ang-2 and the size of hepatic focal lesions (Table 5).

Table (5): ***Correlation between Ang-2 with the number and size of focal lesions in HCC patients***

Variable / Correlated	HCC (n= 30)	
	Correlation coefficient (r)	p value
Ang-2 vs No. of focal lesions	0.395	0.031 S
Ang-2 vs size of focal lesions	0.197	0.298 NS

NS= non significant P> 0.05 S= significant P< 0.05

There was no significant correlation between serum level of AFP and the size or number of hepatic focal lesions.

There was a significant difference ($p < 0.05$) between the mean value of Ang-2 in HCC patients with portal vein thrombosis (5 patients) (19500.00 ± 4320.88) and those without (25 patients) (*9092.5 ± 3726)*.

4. Discussion:

At present, AFP is the most commonly used tumor marker in early HCC screening in populations at high risk, however serum AFP is associated with two main problems; first, the transient high rise in the serum level of AFP during exacerbation of hepatitis on top of CLD (serum level >100 ng/ml) and slight rise in the serum AFP in chronic hepatitis and cirrhosis (serum level >20 ng/dl) causing diagnostic difficulties (low specificity) (Kuntz & Kuntz, 2006). The second is that among all patients diagnosed with HCC, AFP levels may be normal in up to 40% of patients, particularly during the early stages (low sensitivity) (Sherman., 2001).

Serum angiopoietin-2 levels were elevated in patients with cirrhosis, implicating a possible role of the angiopoietin-Tie-2 system for neoangiogenesis in cirrhosis, and were further elevated in patients with HCC, suggesting the potential use of angiopoietin-2 as a marker for the detection of cirrhosis and HCC (Scholz et al., 2007).

This study was thus done to further investigate the potential role of Ang-2 as a diagnostic serum marker for HCC in patients with liver cirrhosis and to assess its sensitivity and specificity compared to AFP and whether -in association with AFP- Ang-2 improves the diagnostic power of HCC.

Chronic HCV infection, as a cause of cirrhosis, accounted for 90% of our HCC patients reflecting the close relationship between HCV and HCC. Our results are in agreement with El-Zayadi et al. (2005) who reported that HCV accounted for 86.9% of HCC cases during a single center study for HCC in Egypt over a decade. Darwish et al. (1997) reported also that viral hepatitis is strongly associated with the development of HCC in Egyptian patients and HCV seems to play a predominant role compared with HBV.

In our study HBV carriers were 10% in HCC group which was in agreement with El-Zayadi et al. (2005) who noticed a significant decline of HBV infection in HCC patients from 38.6% to 20.5% and attributed that partially to successful control measures of blood transfusion introduced in the mid-seventies and partially to the presence of undiagnosed cases of mutant or occult HBV infection, which requires costly assays for diagnosis. Yates et al. (1999) concluded that infection with HCV and HCV-HBV double infection, but not HBV alone, is strongly correlated with HCC in Egypt.

On using the ROC curve to reach the value of the best sensitivity and specificity of AFP; it has been shown that the sensitivity and specificity of AFP varied with the different cutoff values used. At a value of 75.5 ng/ml (the best cutoff), the sensitivity was 53% and the specificity was 87%. Bruix and Sherman (2005) reported the diagnostic cutoff of HCC at 200 ng/ml. In our study, when using this cutoff, the sensitivity declined to 24% while the specificity was 100%. This finding was comparable to that of Oka et al. (1994), who reported low sensitivity (13%) and a specificity of 97% at AFP values over 200 ng/ml. When we further increased the cutoff of serum AFP> 400 ng/ml, the specificity increased and the sensitivity decreased to 10% which was close to the results obtained by Rapaccini et al. (2004), who reported a sensitivity of 7.2% at a cutoff > 400 ng/ml.

In this study, AFP was elevated (>200 ng/ml) in only 23.3% of HCC patients and this was in agreement with Huo et al. (2004), who concluded that serum AFP level was a weak diagnostic predictor in HCC patients.

In this study, no significant correlation was found between AFP levels and the number and size of focal hepatic lesions. This is in accordance with Sato et al. (1994), who concluded that the rise in the serum AFP level did not usually correlate with the tumor size. This could be explained by the fact that tumor differentiation and its ability to secrete AFP are more important than the tumor size in determining the level of AFP produced by HCC (Okaet al., 1994).

In the current study, there was a significant correlation between AFP and the Child classification in the HCC group and this may be attributed to the presence of underlying CLD with subsequent cirrhosis and the progressive deterioration of the liver condition.

Our results revealed that there was a statistically highly significant elevation ($p< 0.001$) in the mean serum Ang-2 in cirrhosis group (5578.33 ± 2928.21 pg/ml) when compared to control group (480.67 ± 202.3 pg/ml).

These results are consistent with Scholz et al. (2007) who reported a statistically highly significant elevation of Ang-2 serum levels in cirrhosis patients when compared to control subjects. Although there are few data available regarding the expression of angiopoietins in cirrhosis in humans, many theories could be speculated in explanation:

Ang-2 is released in inflammatory conditions such as chronic HCV infection (Scholz et al., 2007). Salcedo

et al. (2005) reported that chronic HCV patients showed elevated serum baseline VEGF and Ang-2 levels. After treatment by interferon alpha 2b plus ribavirin, both factors were decreased, whereas antiangiogenic sTie-2 was increased, indicating a shift toward an "anti-angiogenic" profile of serum markers in chronic HCV patients.

In situ hybridization data had shown that Ang-2 mRNA expression was absent from hepatocytes of cirrhotic livers. Apart from endothelial cells of blood vessels, Ang-2 positive cells were found also within the connective tissue strands spanning between the portal tracts. Ang-2 expressing cells may include endothelial cells as suggested by their typical cellular morphology, as well as inflammatory and mesenchymal cells (Scholz et al., 2007). These data may argue against inflammation as the sole reason for elevated Ang-2 levels in serum of cirrhotic patients and emerge the fibrosis process as an extra explanation (Scholz et al., 2007).

Angiopoietins are overexpressed in the rat liver after partial hepatectomy but their role is less fully understood (Sato et al., 2001).

Other similar findings include generation of neovessels in livers of primary biliary cirrhosis patients accompanied by the increased expression of VEGF, Ang-2, and Tie-2 (Medina et al., 2005). Taken together, these published data suggest a causative role of angiogenic factors such as Ang-2 in the remodeling of the cirrhotic liver.

Furthermore, our results revealed that there was a statistically highly significant elevation ($p< 0.001$) in the mean serum Ang-2 in HCC group (10855.0 ± 5321.92 pg/ml) when compared to cirrhosis group (5578.33 ± 2928.21 pg/ml). These results are consistent with Scholz et al. (2007) who reported a statistically highly significant elevation of Ang-2 serum levels in HCC patients when compared to cirrhotic patients and also reported that Ang-2 mRNA was expressed in most of HCC cryopreserved biopsies (using in situ hybridization) in addition to positive staining of the intratumoral vessels.

In our study, when using ROC curve to improve the specificity and sensitivity of Ang-2, the cutoff value of 8100 pg/ml yielded a sensitivity and specificity of 70% and 80%, respectively (best cutoff). These results are consistent with Scholz et al. (2007) who reported a sensitivity of 70.56% and specificity of 73.28% when the high cutoff value of Ang-2 was used (12350 pg/ml), the sensitivity and specificity were 40% and 100% respectively. These results demonstrate that the high cutoff value of serum Ang-2 may show better results of specificity than the best cutoff value of (8100 pg/ml) at the expense of much decrease of sensitivity value.

Concerning the demographic features, hematological tests, ESR and liver function tests, our study revealed that there was no significant correlation between all of the previous parameters and serum Ang-2 levels, among both cirrhosis and HCC groups, with the exception of a significant positive correlation between direct bilirubin and serum Ang-2 level in cirrhosis group, and a significant inverse correlation between serum albumin, PC and serum Ang-2 in the cirrhosis group; consequently, there was a significant positive correlation between serum Ang-2 levels and Child classification in the cirrhosis group. Although Scholz et al. (2007) had reported no significant correlation between serum Ang-2 levels and Child classification, they found that discrimination between cirrhotic and control individuals by Ang-2 serum levels improved somewhat with the progression of cirrhosis. These interesting data are in need to be further studied to assess the significance of Ang-2 as a serum marker in liver cirrhosis and illustrate its role in liver remodeling process.

On the other hand, there was no significant positive correlation between serum Ang-2 levels and Child classification in the HCC group in our study, as was the case in Scholz et al. (2007). This may be explained by the fact that Ang-2 levels increase or decrease according to degree of differentiation of HCC, tumor density and portal vein invasion and not according to the degree of deterioration in liver functions.

In addition, serum Ang-2 did not exhibit a significant correlation with the tumor pathology or the size of focal hepatic lesions which was also in agreement with Scholz et al. (2007). However, there was a significant correlation between the number of hepatic focal lesions and serum Ang-2 level, but this may be attributed to the small number of patients having more than 2 hepatic focal lesions (16.6%) as compared to those with 1 or 2 lesions (83.3%), so, further studies are needed using larger number of patients with groups of comparable sizes to verify such correlation.

In our results, there was a significant difference ($p < 0.05$) between the mean value of Ang-2 in HCC patients with portal vein thrombosis (19500.00 ± 4320.88) and those without (9092.5 ± 3726). Li et al. (2006) reported a high expression of Ang-2 mRNA (using immunohistochemistry) in HCC cases with portal vein tumor thrombosis in comparison with those without; concluding that Ang-2 can promote tumor thrombus formation by modulating angiogenesis. Also, Kuboki et al. (2008) reported a significant relationship between high Ang-2 levels in hepatic vein and portal vein invasion. However, further studies are needed to verify such

correlation using larger number of patients and imaging modalities that can differentiate between malignant and non-malignant portal vein thrombosis.
There was no correlation between serum Ang-2 and serum AFP in patients of both the cirrhosis and HCC groups in our study. This was in agreement with Scholz et al. (2007) who reported no correlation between both markers in patients with HCC and in patients with cirrhosis.

In our results, the combined use of the two markers (AFP and Ang-2) led to an increase in the sensitivity of AFP from 53.3% to 83.3%. These results were in agreement with Scholz et al. (2007) who concluded that detection rates could increase from 71.0% when using AFP alone to 89.2% when using both markers.

5. Conclusion:

• Serum Ang-2 levels are elevated in patients with liver cirrhosis secondary to HCV infection, implicating a possible role of the Ang-2 in chronic HCV infection and remodeling in cirrhotic patients.
• The use of Ang-2 as an independent tumor marker in the diagnosis of HCC is to be considered as serum Ang-2 levels were more elevated in HCC patients when compared to cirrhotic patients.
• Ang-2 may be a helpful tool in the diagnosis of vascular invasion in patients with HCC, as it was significantly elevated in HCC patients with portal vein thrombosis when compared to those without.
• AFP was found to be a weak diagnostic predictor with low sensitivity. Decreasing the cutoff value was associated with improvement of sensitivity at the expense of specificity.
• Ang-2 could be combined with AFP to increase its sensitivity in HCC detection, as combined use of both markers gave the highest index of detection of HCC.

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