Insulin-Like Growth Factor-IIAs A Tumour Marker In Patients With Hepatocellular Carcinoma

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Abstract: *Hepatocellular carcinoma (HCC) is one of the most common cancer worldwide that causes death and in Egypt the burden of HCC is increasing. Prognosis depends on tumor stage. Insulin-like growth factors (IGFs) play an important role in cancer risk. IGF-II is a polypeptide involved in the proliferation and differentiation of normal, transformed and malignant hepatocyte. It is anticipated that HCC may produce IGF-II, and serum IGF-II level may increase in patients with HCC so can be used as a serological test for the diagnosis of HCC. The aim was to assess the value of serum IGF-II in Egyptian patients with HCC. This study was included 40 patients with HCC, 30 patients with liver cirrhosis and 18 healthy controls. For all groups we studied clinical data, image findings, serum alpha fetoprotein(AFP) levels detected by enzyme immunoassay(EIA) kit and IGF-II serum levels were quantified using ELIZA technique. Tumor imaging characteristics of HCC including size, number and site were assessed. Tumor staging was done using Okuda ,Tokyo staging systems,CLIP and VISUM. The results showed that IGF-II values were higher in HCC cases than cirrhotics (P1=<0.001), higher in HCC than control (P2=0.003) but no significant difference between cirrhotics& control (P2= 0.869), with better diagnostic sensitivity and specificity for HCC group over non-HCC group (cirrhotic group and healthy control) with slightly better prognosis than AFP, these results suggest that IGF-II could be a potential diagnostic and prognostic marker for HCC investigations.*
Keywords:*Hepatocellular Carcinoma, IGF-II and Alpha fetoprotein.*

I. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer death
[**1**]**.**In Egypt, HCC is the second most common cancer in men and the sixth most common cancers in [**2**]**.** Hospital-based studies in Egypt have reported an increase in the relative frequency of all liver – related cancers, from 4% in 1993 to 7.3% in 2003 (Lehman and Wilson, 2009) and the increased survival rate among patients with cirrhosis allowing time for some of them to develop HCC
[**2**]**.**Prognosis for patients with HCC depends on tumor stage, with curative therapies only available for patients detected at an early stage, patients detected at an early stage can achieve 5-year survival rates of 70% with transplant or resection, whereas those with advanced HCC are only eligible for palliative treatments and have a median survival of less than one year
[**3**]**.**Up to 20% of HCC do not produce alphafetoprotein (AFP) even when very large (Ryder, 2003) and slight increase are usual in acute hepatitis, chronic hepatitis and cirrhosis and overlaps can cause diagnostic difficulties
[**4**]**.**Insulin-like growth factors (IGFs) play an important role in cancer risk[**5**]. Insulin-like growth factor II (IGF-II) is a polypeptide involved in the proliferation and differentiation of normal, transformed and malignant hepatocyte
[**6**]**.**Incresed expression of IGF-II at both mRNA and protein levels has been demonstrated in HCC tissues
[**7**]**.**Circulating IGF-II levels may decrease after transcatheter arterial chemoembolisation in patients with HCC
[**8**]**.**It is anticipated that HCC may produce IGF-II, and serum IGF-II level may increase in patients with HCC

II. Patients And Methods

This prospective observational study was carried out on 88 cases, classified into: Group(I) included 40 patients with hepatocellular carcinoma diagnosed by ultrasound (U/S) and/or confirmed by Triphasic Computed Tomography (CT) and Group (II) included 30 patients with cirrhosis diagnosed by clinical, laboratory, and U/S assessment. Admitted to gastroenterology, hepatology and infectious diseases & internal medicine departments of Benha University Hospital during the period from November 2014 to May 2015 . Third group 18 apparently healthy subjects (without HCC or cirrhosis and with normal imaging) served as controls.

The clinical/pathological data of the patients were recorded, including age, sex, viral infections {Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV)}, alcohol intakes, biochemical liver function test results, and AFP levels. Severity of liver cirrhosis by using Modified Child score &MELD score &Updated MELD score (1.27× log (1+creatinine mg/dl) + 0.94× log (1+bilirubin mg/dl) + 1.66× log (1+INR), And Veinna survival model for HCC (VISUM-HCC) (Sharma et al.,2008). HCC was diagnosed by abdominal US and serum AFP, with or without triphasicCT scan and/or liver histopathology. AFP was assayed by an enzyme immunoassay (EIA) Kit (Roche Mannheim, Germany). Tumor characteristics were detected by abdominal US with or without CT scan (including tumor size, number, site, halo sign and neovascularization). Tumor staging was done using Okuda **[10],** Tokyo [**11**]staging systems} and Cancer of the Liver Italian Program (CLIP) scoring system.

2.1 Blood sampling and biochemical assays: Fasting venous blood samples (5 ml) were collected by trained laboratory technicians. A portion of blood was allowed to clot and then centrifuged at 3500g for 5 min to separate the serum used for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and g-glutamyltranspeptidase (GGT), total bilirubin, direct bilirubin, albumin, creatnine and glucose concentrations were assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. &Trade-Giza, Egypt). Viral infection status (HCVAb and HBS Ag) were measured using Abbott, Axyam (USA, Supplied by al kamalcompany). Serum AFP level was determined using an enzymelinked binding protein assay kit. All assays were performed induplicate according to the manufacturer's instructions. Serum aliquots were stored at _80_C until assayed and thawedimmediately before the measurements levels. Another portion of blood was collected in vacutainer tubes containing EDTA to measure ILG-II.

2.2 Quantification of Human Insulin-like Growth Factor II (IGF-II):The test is quantitative level of IGF-II in the sample by ELISA, adopt purified human IGF-II to coat microtitre plate, make solid-phase antibody, then add samples or standards to wells with a labeled antibody specific to IGF-II, then add labeled HRP to the well. After washing completely, add TMB substrate solution, TMB substrate becomes blue color in wells that contain antibody-antigen-enzyme-antibody complex, reaction is terminated by the addition of a stop solution and the color change is measured at a wavelength of 450 nm. The concentration of IGF-II in the samples is then determined by comparing the O.D. of the samples to the standard curve. (Glory science Co., Ltd,USA).

2.3 statistical analysis: The collected data were summarized in terms of mean ± Standard Deviation (SD) and range for quantitative data and frequency and precentage for qualitative data. Comparisons between the different study groups were carried out using the Chi-square test (χ^2) and Fisher Exact test (FET) to compare proportions as appropriate. The Student t-test (t) was used to detect difference in the mean between two parametric data, while the Mann-Whitney test (z) was used to compare two non-parametric data.

Receiver Operating Curve (ROC) analysis was carried out to evaluate the diagnostic performance of AFP and IGF-II levels for HCC screening among cirrhotic patients. The best cutoff point and the corresponding sensitivity and specificity, Postive Predictive Value (PPV), Negayive Predictive Value (NPV) and Area Under the Curve (AUC) were estimated.Spearman Correlation coefficient (rho;ρ) was used to test for the correlation between AFP and IGF-II and estimated parameters.After the calculation of each of the test statistics, the corresponding distribution tables were consulted to get the "P" (probability value). Statistical significance was accepted at P value <0.05 (S). A P value <0.001 was considered highly significant (HS) while a P value >0.05 was considered non- significant.The statistical analysis was conducted using STATA/SE version 11.2 for Windows (STATA corporation, College Station, Texas).

III. Results

This study included 70 consecutive patients with HCC and 18 apparently healthy subjects. The epidemiologic characteristics of the two patients' groups were summarized in table 1. The mean age of HCC patients was 54.6± 6.55 years with a range between 43-73 years. In liver cirrhosis patients the mean age was 45.27± 5.77 years with a range between 28-55 years.Therewas a significant difference in the mean ages of HCC patientgroup, liver cirrhosis (p=<0.001). There was male predominance among the patients with HCC, 33 men (82.5) versus 7 women (17.5) with a male-to-female ratio of 4.7:1, in the liver cirrhosis patients there was 12 (40%) males versus 18 (60%) females, with a male-to-female ratio of 0.6:1 and there was significant difference in the sex ratios of HCC patients and liver cirrhosis patients (p=<0.001). As regards residence, occupation and smoking there was no significant difference (p = 0.49, 0.32, 0.36 respectively). The severity of liver cirrhosis assessed by child – Pugh classification table 2, most cases of HCC was Child B rather than Child A&C, but the frequency of Child A was predominant in cirrhotic patients than in HCC with statistical significance(p=<0.04). Also The severity of liver cirrhosis assessed by MELD, uMELD scores among the studied patients table 3 showed that both scores were significantly higher in HCC patients (p=0.001) (Table 1). Tumor imaging characteristics of HCC patients were illustrated intable4.

Table 1. Demographic features of the studied patients.

characteristics	Group(I) HCC N = 40		Group (II) cirrhosis N = 30		P-value
Age					<0.001*
Range	43-73		28-55		
Mean ± SD	54.6± 6.55		45.27± 5.77		
gender	N	%	N	%	P-value
Male	33	82.5	12	40.0	<0.001*
Female	7	17.5	18	60.0	
Male: female	4.7: 1		0.6: 1		
Residence		45	16	53.67	0.49
Urban	18				
Rural	22	55	14	46.67	0.32
Occupation					
Farmer	18	45	10	33.33	0.36
Non farmer	22	55	20	66.67	
Smoking	19	47.5	11	36.67	

*=Significant,SD=standard deviation.

Table 2.The severity of liver cirrhosis assessed by child – Pugh classification among the studied patients.

Child grade	Group (I) HCC N = 40		Group (II) cirrhosis N = 30		P-value
	N	%	N	%	
Child A	13	32.5	17	56.67	**0.04***
Child B	20	50	12	40	0.40
Child C	7	17.5	1	3.33	0.06

Table 3 . The severity of liver cirrhosis assessed by MELD &uMELD scores

	Group (I) HCC N = 40		Group (II) cirrhosis N = 30		P-value
	Range	Mean± SD	Range	Mean± SD	
MELD	7-23	12.57±3.97	6-24	8.77± 3.63	**<0.001***
uMELD	1.08-2.17	1.43±0.26	0.9-1.9	1.11±0.23	**<0.001***

MELD=model of end stage liver disease,uMELD=updated MELD.

Table 4 .C T finding and tumor characteristics in HCC patients.

	Group (I) HCC N = 40	
	N	%
Cirrhosis	40	100
Splenomegaly	38	95
Ascites	17	42.5
PVT	7	17.5
Focal lesion number		
Single	25	62.5
Multiple	15	37.5
Site		
Right Lobe	25	62.5
Left lobe	6	15
Both lobes	9	22.5
Size		
< 3 cm	16	40
3 – 5 cm	9	22.5
> 5 cm	15	37.5

PVT=portal vien thrombosis

Table 5.Tumor staging.

Stage	Group (I) HCC N = 40	
	N	%
Okuda staging system		
I	14	35
II	19	47.5
III	7	17.5
	CLIP staging system	

I	8	20
II	26	65
III	6	15
	VISUM staging system	
I	28	70
II	11	27.5
III	1	2.5
	Tokyo staging system	
Early < 5	21	52.5
Advanced □ 5	19	47.5

Table 6 .AFP and IGF-II values in the studied groups.

| Marker	Group (I) HCC N =40		Group (II) cirrhosis N =30		Group (III) control N = 18		P value
	Range	Mean± SD	Range	Mean ± SD	Range	Mean± SD	
IGF-II (ng/ml)	158.6-9691	1784 ± 2430	86.5-904.2	436.91 ± 264.1	91.3-838	360.2 ± 258.89	**<0.001***
AFP (ng/ml)	1.5- 3000	317.5 ± 617	0.99-77.8	10.64 ± 18.33	1.2-15.5	3.64 ± 3.21	**<0.003***

AFP=Alpha-fetoprotein,IGF-II= insulin-like growth factor-II

Abdominal CT showed Cirrhosis was present in 100% of cases with HCC, focal lesions by CT tend to be single (62.5), more in right Lobe (62.5), <3 cm in diameter (40) and PVT was present in 17.5% 0f cases. Regarding Okuda, and CLIP staging systems, most of HCC patients were relatively at advanced stage of the disease but as regard Tokyo staging system most HCC cases were early Tokyo stage table 5. As regards AFP levels, HCC patients had the highest mean value (317.5 ± 617ng/mL), with a significantly higher values (p<0.003), than the mean of AFP values in both liver cirrhosis patients (10.64 ± 18.33 ng/mL) and healthy control (3.64 ± 3.21ng/mL) groups. As regards IGF-II values were higher in HCC cases than cirrhotics (P1=<0.001), higher in HCC than control (P2=0.003), but no significant difference between cirrhotics& control (P2= 0.869)table&fig. 1.
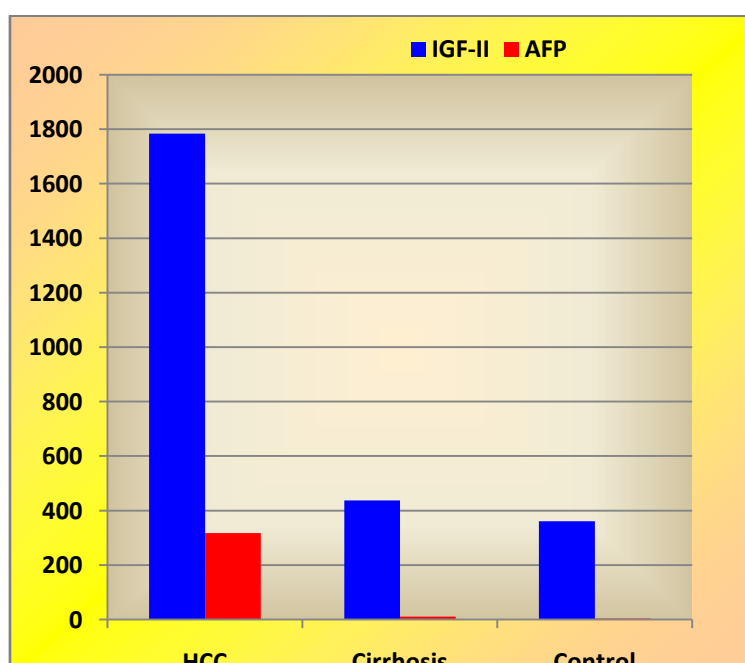

Fig. 1. IGF-II&AFP values among studied groups

In the receiver operating curve (ROC), the area under curve (AUC) for AFP was 80.7% when we use 6.15 ng/mL as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 75% and a specificity of 82%. with PPV 96.9% and NPV 94.7% . For IGF-II, the area under curve (AUC) was 84% when we use 530 cycle threshold as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 87.5% and a specificity of 62.5% with PPV86.5% and NPV 78.3% . (Fig. 2)& Table 3.
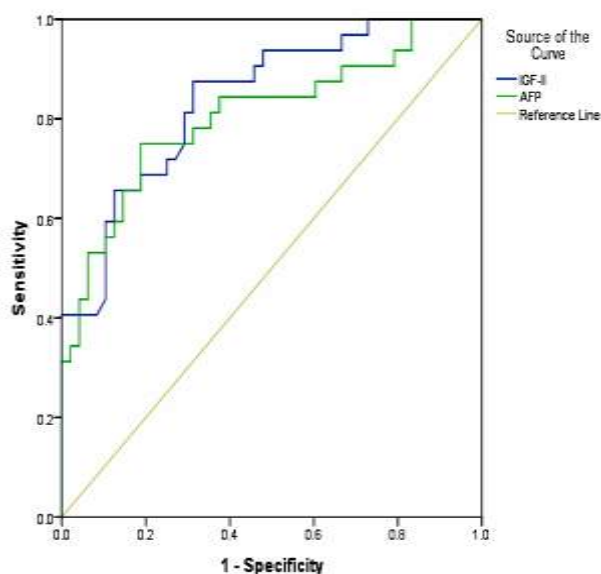
Fig. 2. In the receiver operating curve (ROC), the area under curve (AUC) for AFP was 80.7% when we use 6.15 ng/mL as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 75% and a specificity of 82%. with PPV 96.9% and NPV 94.7% . For IGF-II, the area under curve (AUC) was 84% when we use 530 cycle three shold as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 87.5% and a specificity of 62.5% with PPV86.5% and NPV 78.3% . (Fig. 2)& Table 3.

As regard prognosis, There was significant positive correlation between AFP and tumor number, IGF-II, Tokyo and VISUM staging system among HCC(P =0.008, 0.003, 0.023, 0.04 respectively). There was significant positive correlation between IGF-II and Child score, tumor number, AFP and Tokyo staging system among HCC cases(p=0.031, 0.042, 0.003, 0.02 respectively) figure 3,4.
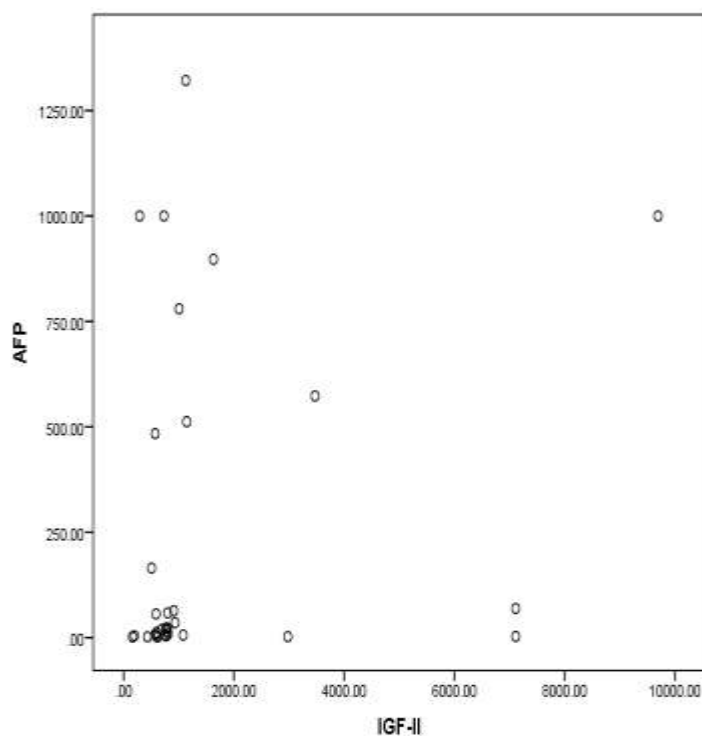
Fig. 3. Correlations between IGF-II&AFP values among HCC cases.
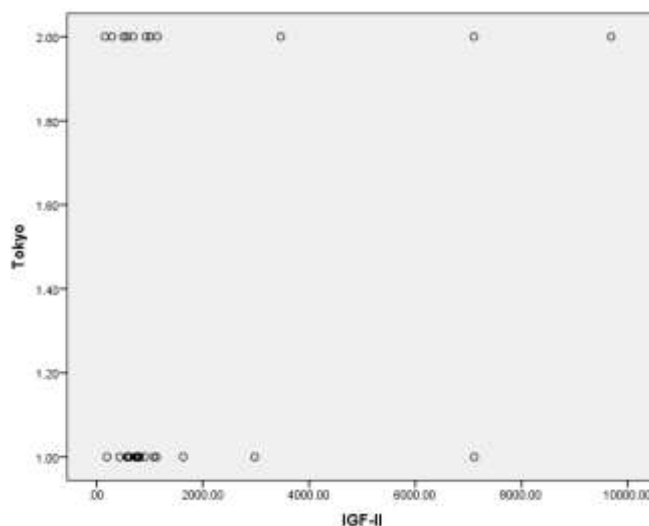
Fig. 4. Correlations between IGF-II values&Tokyo stage among HCC cases.

IV. Discussion

Worldwide, HCC is the fifth most common cancer in men and the seventh in women, with more than 748,000 new cases being diagnosed each year, accounting for 9.2% of all new global cancer cases [**12**]and[**13**]

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer death [**1**]

Prognosis for patients with HCC depends on tumor stage, with curative therapies only available for patients detected at an early stage. In this study we aimed at evaluating the diagnostic and prognostic value of IGF-II in patients with HCC.

In the current study, HCC commonly presented in males more than females with a male to female ratio 4.7:1, this was in agreement with[**14**] and[**15**]who reported that males predominated with male to female ratio of 4.7:1 and 5:1 among HCC patients respectively. The higher incidence of HCC in males might be due to the stimulatory effects of androgen and the protective effect of oestrogen. The biological activity of natural progesterones on HCC is contraverse. Concerning the age, patients with HCC had a mean age of 54.6 years with a range between 43-73 years old, while cirrhotic patients are younger with a mean age of 45.2 years and ranging between 28-55 years old.

These results were in agreement with[**16**]who reported that the age of the HCC patients ranged between 42 and 70 years and mean is 58.7 years, and also with[**17**]who reported that the peak age group for HCC was from 50-70 years with a mean age of 58.7 years.

In Egyptian series by [**18**]and[**19**] the peak age was 56 years and 55.74 years respectively.

In this study, 55% of HCC cases were from rural areas, this result was in agreement with [**20**] who stated that the incidence of HCC is more common in rural areas than urban.

This may be explained by increasing the incidence of exposure to many risk factors such as HCV and HBV infection, pesticides and diets contaminated by aflatoxin in rural areas [**21**].

In contrast,[**2**]stated that most of HCC cases resided in urban areas due to better access to medical facilities.

In this study, only 45% of HCC cases were farmers while 55% of HCC cases were non farmers, these results are in agreement with [**22**] who reoprted that only 41% of HCC cases were farmers.

These results were supported by [**23**]who stated that the relation between occupation and HCC has not been extensively studied, but prolonged exposure to toxic chemical substances may increase HCC incidence.

In contrast, [**24**] reported that HCC is more common in farmers (59.3%) compared with non farmers (40.7%). This may be due to increased exposure to carcinogenic factors especially HCV infection and lack of medical facilities.

The current study showed that 47.5% of HCC cases had a history of smoking, this result are in agreement with (Hara et al., 2008) who found that 46.8% of HCC cases are smokers especially in males, additionally [**25**]who found that 50% of HCC cases are smokers.

However, [**26**]reported that smoking was found in 64% of Egyptian patients with HCC compared to 38% in patients with liver cirrhosis.

Although alcoholism is a well identified risk factor for HCC as increasing level of alcohol consumption were associated with increased yearly incidence rates for HCC [**27**], however this role was not clear in this study as there is no history of alcohol intake between HCC cases.

This may be due to different socioeconomic and religious status that forbidden alcohol intake.

However, [18] in Egyptian series reported that 4.9% of HCC cases had history of alcohol intake. In contrast Alves et al. (2011) in their study in Brazil found that 22% of HCC patients were alcoholic's.

The clinical picture of HCC is very variable [4], in this study, the presenting symptoms in HCC group mostly were abdominal pain, yellowish discoloration of sclera, weight loss and abdominal enlargement, these results were constantly with that reported by [18], [22] and[28].

In this study, history of blood transfusion was 32.5% among HCC group, this result goes in agreement with [29] who stated that 28% of HCC cases had a history of blood transfusion.

The prominent physical signs in HCC patients were jaundice and hard liver, this goes in agreement with [30] who stated that jaundice and hard liver are common signs of HCC.

Physical examination usually shows other signs of cirrhosis with portal hypertension, including spider angiomas, palmar erythema, ascites, lower limb oedema, and splenomegaly, this goes in agreement with [31]

Concerning blood picture in this study hemoglobin, WBCs and platelets were lower in HCC patients than in cirrhotics. This results go in agreement with [32] who reported the same results exactly.

According to the liver biochemical profile in this study AST and serum bilirubin were higher in HCC patients. This results goes in agreement with [33] who reported that AST and serum bilirubin are higher in HCC patients than cirrhotics.

Other liver biochemical profile in this study as serum albumin was lower in HCC patients than cirrhotics, also prothrombin time was prolonged in HCC patients than cirrhotics. This results in agreement with [34]and[16] who reported the same results.

Concerning seroprevalence of HBsAg positivity among HCC cases none of cases were positive. This decrease of HbsAg positivity may be partially attributed to successful control measures of blood transfusion and vaccination and partially to the development of mutant or occult HBV infection, which requires costly assays for diagnosis [35]

However, in Egyptian studies by [18]and[20] reported that lower prevalence of 2.4% and 2.51% of HCC cases were HBsAg positive respectively

In this study, regarding the seroprevalence of HCVAb positivity among HCC cases, 90% of cases were positive for HCV Ab, this was in agreement with other egyptian series by [24]and[20] who reported that 89.19% and 91.32% of HCC cases were HCVAb positive respectively, also similar results by [36] and[17]who documented that 89.2% and 93% of HCC cases were HCVAb positive respectively.

These results are supported by [37] who stated that in areas with an intermediate rate of liver tumours such as southern Europe, Egypt and Japan, HCV is the predominant cause of HCC.

In this study, most of the patients with HCC (50%) were Child B, followed by Child A (32.5%) then Child C (17.5%), these results are in agreement with [38]who reported that (53.1%) of HCC cases were Child B followed by Child A (31.3%) then Child C (12.5%),and [39] who reported that (40%) 0f advanced HCC cases were Child B, followed by Child A (32.7%) then Child C (27.3%)

In contrast, [33] reported that (61.06%) of HCC cases were Child C, followed by Child A (20.3%) then Child B (18.5%).

This difference may be attributed partially to the type of patients and partially to the development of screening program for early detection of HCC in the other countries.

According to MELD and uMELD scores, in this study the mean scores for patients with HCC were 12.57 and 1.43 respectively, while the mean scores for patients with cirrhosis were 8.77 and 1.11 respectively, these results are nearly similar to that of [40]who reported that the mean MELD and uMELD scores for patients with HCC were 15.3 and 3.63 respectively, this may be explained by the fact that HCC comes on top of severe liver disease.

Abdominal ultrasonography was done to evaluate the liver status in the studied patients and all of the patients (100%) with HCC had sonographic evidence of liver cirrhosis, this goes in agreement with [40]and [28] who documented that cirrhosis of the liver is present in 100% of patients with HCC.

Also, [41]stated that 90-95% of people who develop HCC have underlying cirrhosis.

This was supported by [42]who documented that cirrhosis is the most important risk factor for HCC as more than (80%) of the HCC cases occur in the setting of cirrhosis.

Seven patients (17.5%) in this study were found to had portal vein thrombosis, this finding goes in agreement with

[35]and[28]who reported that 18% and 17.2% of HCC cases had portal vein thrombosis respectively.

A higher finding was reported by [43]and[19] who reported that 28% and 28.2% of HCC cases had portal vein thrombosis respectively as a considerable proportion of HCC cases in these studies had advanced disease.

Twenty five, representing 62.5% of HCC cases had single focal hepatic lesion, while multiple hepatic focal lesions were present in 37.5% of the cases, these results were in agreement with [44]and[22] who mentioned that single lesions found in 62.3% and 61% respectively.

Also, [40]documented that 66.7% patients had single lesion and 33.3% had multiple lesions.

In the present study 40% HCC cases had their tumor size less than 3 cm while 22.5% had their tumor size between 3cm and 5cm, and 37.5% had tumor size of more than 5 cm, meaning that 60% of patients had tumor size of more than 3cm.

This goes in agreement with [45]who reported that 49% with tumor size less than 3 cm, 23% with tumor size between 3 and 5 cm, and 28% with tumor size more than 5 cm, and [46]who documented that 35% with tumor size less than 3cm and 65% with tumor size more than 3cm.

In this study, the right lobe was predominantly more affected by tumor (62.5%) than left lobe (15%) and both lobes (22.5%), these results were in agreement with [47] who mentioned that 58.3% of his cases had their focal lesions within the right lobe, 18.3% within the left lobe, and 23.4% in both lobes, also [28] who reported that 65.5% of his cases had their focal lesions within the right lobe, 12% within the left lobe, and 22.5% in both lobes.

One patient (2.5%) of HCC cases in this study was found to had lymph node enlargement, this goes in agreement with [47]who mentioned that lymph node invasion or distant metastases was detected in (5.5%) patients at diagnosis.

Concerning Okuda staging, most of HCC cases were stage II (47.5%) followed by stage I (35%) then stage III (17.5%), these results were in agreement with [33]who reported that stage I was 37.2%, stage II was 42.5% and stage III was 20.3% and [47] who documented that stage I was 34.8%, stage II was 45.8% and stage III was 19.4%.Concerning CLIP staging, most of HCC cases were presented at the intermediate stage (65%) comparing to the patients presented at the early stage (20%) and the advanced stage (15%) respectively.

This goes in agreement with [48] who reported that (53.5%) of HCC cases were at intermediate stage, followed by the early stage (29%) then the advanced stage (17.5%).

Also, [49] who mentioned that (72.5%) of HCC cases were at intermediate stage, followed by the early stage (18%) then the advanced stage (8.5%), similarly Shao et al. (2012) who documented that most of HCC cases (66%) were at intermediate stage followed by the advanced stage (24%) then the early stage (10%).

According VISUM staging, most of HCC cases were stage I, this result was in agreement with [50] who reported that most of cases were in stage I.

According to Tokyo staging, most of HCC patients were presented at early stage (52.5%) comparing to the patients presented at advanced stage (47.5%).

Higher results were reported by [51] who mentioned that most of HCC cases (85.9%) were presented at early stage.

AFP is the most established tumor marker in HCC and the gold standard by which other markers for the disease are judged [52].

In the current study, AFP in HCC cases had a mean value of 317.5ng/ml which was statistically higher than that of patients with cirrhosis (10.6 ng/ml) and control (3.6 ng/ml) subjects, these results are in agreement with [53] who reported that AFP in HCC cases had a mean value of 384.6 ng/ml which was statistically higher than that of patients with cirrhosis (6.04 ng/ml) and control (2.9 ng/ml) subjects.

Furthermore, similar results by [54] who reported that AFP in HCC cases had a mean value of 295.9 ng/ml which was higher than that of patients with cirrhosis (19.5 ng/ml) and control (5.9 ng/ml).

ROC analysis of AFP used as a diagnostic test suggests that a value of about 6.15ng/ml provides the optimal balance between sensitivity and specificity, however at this level the sensitivity is only 75% and the specificity is 82%.

This goes in agreement with [53]who reported that sensitivity of AFP is about 79.7% and specificity is about 80.3% in HCC cases.

However, this value is considered to be within the normal level of serum AFP in adults which is less than 10ng/ml [55]

Accordingly, the role of AFP in the diagnosis of HCC is limited, these results were supported by [56]who mentioned that AFP is not elevated in all patients with HCC, its sensitivity for detecting HCC ranges between 25%-60%, and its specificity is also low because serum AFP can also be detected in patients with cirrhosis and chronic hepatitis.

This was supported by [57] who stated that AFP level are frequently normal in patients with small HCC and are not elevated in a significant portion in patients with early stage, potentially curable HCC. Also [58]concluded that the serum AFP level was a weak diagnostic predictor in HCC patients.

There is a debate in defining the AFP cut-off level for the diagnosis of HCC, an AFP value above 400-500 ng/ml has been considered to be diagnostic for HCC in patients with cirrhosis, however, such a cut-off value is problematic in absolute diagnostic terms, since high levels of this magnitude are not as common in the

presence of smaller tumors (< 5 cm) and furthermore, only 30% of HCC patients have levels higher than 100 ng/ml in this context [**59**]

In this study, there was non-significant correlation between AFP and the severity of the liver disease (Child, MELD and uMELD scores), tumor size, Okuda and CLIP stages, this finding is supported by [**58**]and [**18**] who reported that no correlation between AFP and severity of the liver disease.

But there was significant positive correlation between AFP and tumor number, IGF-II, Tokyo and VISUM staging system among HCC cases, these were in agreement with [**47**]and [**60**]who reported that there was a significant positive correlation between AFP and tumor number.

Regarding AFP as a marker for HCC, our results supported by [**58**]ﻝwho concluded that serum AFP is a weak prognostic predictor in HCC patients and [**61**]stated that the value of AFP is limited in the diagnosis and prognosis of HCC.

There is a growing body evidence to indicate that IGF-II play an important role in cancer risk. The addition of an assay for IGF-II may bring a significant improvement in the detection of small HCC [9]

Concerning Insulin-like Growth Factor II (IGF-II) mean value, HCC cases had a mean value of (1784 ng/ml) which was higher than that of patients with cirrhosis (436.9 ng/ml) and control (360.2 ng/ml) subjects, these results were in agreement with Yao et al. (2013) who reported that IGF-II was significantly higher in HCC patients (mean 450.3 ng/ml) than in cirrhosis (mean 174.2ng/ml) and healthy control (mean 149.7 ng/ml).

Similar results by [**62**] who stated that mean value of IGF-II was higher (888 ng/ml) in HCC patients than in cirrhotics (432 ng/ml).

Also [**63**] reported that mean value of IGF-II was significantly higher in HCC group than in cirrhotics or in the normal control group.

ROC analysis of IGF-II used as a diagnostic test suggests that a value of 530 ng/ml provides the optimal balance between sensitivity and specificity, at this level the sensitivity is 87.5% and specificity is 62.5%.

This goes in agreement with Aleem et al. (2012) who reported that at a cut-off value of 414 ng/ml, the sensitivity and specificity of IGF-II in HCC patients were 75% and 60% respectively.

Also, [62] stated that the sensitivity and specificity of IGF-II in HCC patients was 73% and 64% respectively.

Also [**64**] stated that expression of IGF-II may be useful markers for diagnosis of HCC and its extrahepatic metastasis, and monitoring recurrence.

There was significant positive correlation between IGF-II and severity of liver disease (Child-Pugh grade), tumor number, AFP and Tokyo stage, this goes in agreement with [**65**] who reported that there was positive correlation between IGF-II and AFP, Child-Pugh grade and number of lesions in HCC patients.

Also, [**66**] stated that there was significant positive correlation between IGF-II and AFP.

IGF-II has a significant role in assessment of severity of liver disease, this was supported by [**67**]who reported that determination of serum IGF-II levels is of help in judgment of severity of liver diseases as well as outcome prediction.

There was no significant correlation between IGF-II and the size of the tumor, this goes in agreement with [**63**]and [**68**]who reported that there was no significant correlation between IGF-II and tumor size.

Regarding IGF-II as diagnostic and prognostic marker of HCC, these results suggest that IGF-II level could be a potential diagnostic and prognostic marker for HCC, this suggestion was supported by [**9**]and[69] who documented that IGF-II can be used as prognostic and diagnostic indicators for HCC as its level increases especially in patients with small HCC.

According to this study, IGF-II level is elevated in HCC patients than cirrhotics and apparently healthy subjects, with better diagnostic sensitivity and specificity for HCC group over non-HCC group (cirrhotic group and healthy control) with slightly better prognosis than AFP, these results suggest that IGF-II could be a potential diagnostic and prognostic marker for HCC investigations [**70**] .

V. Conclusion

IGF – II couldbeaccurate a sensitive and specific serum marker for diagnosing HCC.

Consent
All authors declared that written informed consent was obtained from the patients for publication of this paper.
Ethical Approval
Ethical clearance was obtained from BenhaUniversityHospital's ethics committee.
Competing Interests
Authors have declared that no competing interests exist.

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