Characterization of Hypoxia-Inducible Factor-1α and Its Impact on Diagnosis and Prognosis of Hepatocellular Carcinoma in Hepatitis C Patients

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Abstract: Hypoxia is a common feature of many solid tumors, including hepatocellular carcinoma (HCC). It can promote tumor progression and induce radiation and chemotherapy resistance. Hypoxia inducible factor-1 (HIF-1) is one of the major mediators of hypoxic response, it has been demonstrated that a high level of HIF-1 in the HCC microenvironment leads to enhanced proliferation and survival of HCC cells. Accordingly, overexpression of HIF-1 is associated with poor prognosis in HCC. The study included 20 healthy subjects that served as control group, 33 patients with liver cirrhosis and 30 patients with HCC. All subjects were subjected to thorough history and clinical examination, abdominal ultrasound and laboratory investigations including; complete blood picture, liver function tests, in addition to measurement of alpha fetoprotein (AFP) and HIF-1 serum levels. The serum levels of HIF-1α were significantly ($P < 0.001$) increased in HCC patients as compared to those with HCV and controls subjects. The circulating HIF-1α has a high sensitivity (100%) and specificity (90.7%) for HCC prediction than AFP (87.5% and 67.4%, respectively) and the associated high level of HIF-1α with metastatic HCC cases suggesting its role in the prognosis of HCC and it could be a useful molecular marker in HCC diagnosis, and monitoring prognosis. In conclusion, serum HIF-1α is highly sensitive and specific for detecting HCC alone or if combined with AFP. Therefore, it may play an important role in early diagnosis of HCC.
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Key Words: hepatocellular carcinoma; hepatitis C virus; early diagnosis; prognosis; hypoxia; hypoxia inducible factor-1

1. Introduction

Hepatocellular carcinoma (HCC), is one of the most common malignant tumors in the world, accounts for > 5% of all human cancers and for 80-90% of primary liver cancer **(Altekruse *et al.*, 2009)**. HCC is the sixth most common neoplasm and the third most frequent cause of cancer death. HCC is the leading cause of death among patients with cirrhosis **(Alazawi *et al.*, 2010)**. Yearly 550,000 people worldwide die from HCC. Its incidence is increasing dramatically **(Jemal *et al.*, 2007)**.

According to the data of regional oncology centers in Egypt, liver tumors represent the fourth most common cancer in Egypt. They are the second most common cancer in males (after bladder cancer) and the sixth in females. Mortality from liver cancer represented 14.8% of all cancer deaths **(El-Atar, 2005).** Multiple risk factors are associated with HCC disease etiology, with the highest incidence in patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), although other factors such as genetic makeup and environmental exposure are involved **(Raza *et al.*, 2007, Feo *et al.*, 2009, Hui, 2009)**.

Currently, surgical resection, liver transplantation, and local ablation are considered curative therapeutic practices for HCC. The diagnosis of HCC without pathologic confirmation is achieved by analyzing serum alpha-fetoprotein (AFP) levels combined with imaging techniques, including ultrasonography, magnetic resonance imaging, and computerized tomography **(Zhu *et al.*, 2013)**. Although progress has been made in the diagnosis and management of HCC, its prognosis remains dismal. Various new technologies have identified numerous novel biomarkers with potential diagnostic as well as prognostic value. These biomarkers not only help in the early diagnosis and prediction of prognosis, but also assist in identifying potential targets for therapeutic interventions.

Hypoxia inducible factor-1 alpha (HIF-1α) regulates the expression of gene critical for adaptation to low oxygen levels **(Haase *et al.*, 2001).** In normoxic cells, HIF-1α is produced and targeted for

proteolytic degradation. When cells become hypoxic, the mechanism that targets HIF-1α subunit for degradation is inhibited allowing HIF-1α protein level to increase **(Cash *et al*, 2007)**.

Significant evidence indicates that the HIFs play an important role in the pathogenesis and pathophysiology of HCC **(Tajima *et al*., 2009).** HIF-1α and vascular endothelial growth factor (VEGF) were found to be expressed at higher levels in dysplastic nodules and implicated in malignant transformation **(Nakamura *et al*., 2007).**

This study evaluated to investigate the levels of HIF-1α expression in a range of patients with liver disease, HCV and HCC at various stages, in order to determine how it can be used in the diagnosis of HCC and in establishing prognosis which could shed light on new therapeutic approaches for the treatment of HCC.

2. Materials and Methods

Subjects

Patients with post HCV and different grades of HCC were recruited from the Outpatient Clinics and Inpatient Department, National Liver Institute, Menoufiya University (Menoufiya, Egypt). HCV liver cirrhosis cases (33) were diagnosed by enzyme-linked immunosorbent assay (ELISA) for antibodies to HCV and confirmed by qualitative reverse transcriptase polymerase chain reaction (RT-PCR) for HCV RNA in serum (Cobas Amplicor HCV Test, Roche Diagnostics, Branchburg, NJ). HCC cases (30) were diagnosed by AFP and confirmed by triphasic CT. Cases with chronic inflammatory hepatitis other than HCV, autoimmune hepatitis and chronic inflammatory diseases other than liver are excluded by clinical and laboratory investigations. In addition, 20 healthy volunteers with matched age and sex were included.

*The study was approved by the National Liver Institute (Menoufiya University), Ethics Committee, and all patients and controls gave written informed consent.

Clinical Evaluation

A- Thorough history taking: Stress was laid on history of HCV infection, Bilharziasis, autoimmune hepatitis, hematemesis, melina, abdominal enlargement, jaundice, lower limb swelling, and encephalopathy.

B- Complete clinical examination: Stress was made on jaundice, lower limb edema, hepatomegaly, splenomegaly, and ascites.

C- Triphasic CT: To assess the grade of HCC, size and echo pattern of the liver and presence of periportal fibrosis (PPF), the size of the spleen, the presence of ascites or any other abnormalities in the abdomen and Doppler studies of hepatic veins, inferior vena cava and portal vein were taken into consideration.

D- Liver biopsy to patients of chronic hepatitis C: Hepatic injury was assessed using the histological activity index (HAI) as modified by **Ishak (1994).** This consisted of a necro-inflammatory grading score (range 0 to 18; 0 = no activity, 18 = severe activity) and a fibrosis staging score (range 0 to 6; 0 = no activity, 6 = cirrhosis).

Laboratory Investigation

Sampling

Ten ml of venous blood samples were drawn from each subject without frothing and after minimal venous stasis. Serum samples were separated and stored at -80ºC until the time of assay. Blood and serum samples were subjected to the following laboratory investigations:

Complete Blood Count (CBC)

Complete blood picture was done on Sysmex XT 1800 ***(Germany)***.

Liver Function Tests (LFTs)

- LFTs were done on Integra 800 Auto analyzer ***(Roche-Germany Catalogue number; M, 87432).***
- ALT, AST and albumin were measured according to the International Federation of Clinical Chemistry **(Bergmeyer *et al.*, 1986).**
- Serum total and direct bilirubin was performed according to Diazo method **(Malloy and Evelyn, 1973).**

Determination of Serum AFP by Sandwich ELISA

AFP concentration was measured in serum using a solid phase ELISA kit from Sorin Biomedica (USA). Briefly, 25 µl of each calibrator and samples were pipetted into appropriate wells of microtiter plate coated with anti-AFP mAb. One hundred µl of enzyme conjugate was added to each well and incubated for 30 minutes (min) at room temperature. After washing, 100 µL/well of substrate solution was added and incubated for 10 min at room temperature. The enzymatic reaction was stopped by adding 50 µL of stopping solution to each well. The absorbance was measured by reading (within 10 min) the plate in dual wavelength mode, at 450 and 630 nm filter that was used to measure the reference absorbance. Biotek Elx 800-UV microtiter plate reader (Murex Biotech S.A. (Pty) Ltd, Republic of South Africa, Ref. No., 9F80-01/9F80-05 was used for measurement).

Determination of Anti-HCV and Anti-HBsAg Antibodies by Indirect ELISA

Anti-hepatitis C virus (anti-HCV) and anti-hepatitis B surface antigen (anti-HBsAg) antibodies were qualitatively determined in serum using an indirect ELISA kit from Abbott Murex (Murex Biotech). Briefly, 180 µl of sample diluent was pipetted into each well, 20 µl of samples and controls were added to the appropriate wells for 1 hr at 37°C. After washing, 100 µl/well of conjugate was added and incubated for 30 min at 37°C. Hundred µl of

substrate solution was added to each well, after washing 30 min at 37°C avoiding direct sunlight. The absorbance was measured as previously mentioned. Biotek Elx 800-UV microtiter plate reader was used for measurement and blanked against air (Murex Biotech).

Determination of Hypoxia Inducible Factor-1α Concentration

It was determined by human HIF-1α ELISA Kit Catalog No: E0798h (EIAab company, Wuhan EIAab Science Co., Ltd. 3rd Floor, Building A2, Biopark, Optics Valley, Wuhan, China).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for HCV-RNA

The procedure follows the general principle of RT-PCR kits (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions. The lower detection limit is < 10 IU/mL. Reverse transcription quantitative real-time PCR (RT-qPCR) is the most commonly used method for the quantification of mRNA expression levels **Wong and Medrano (2005)**.

Statistics

The data collected were tabulated and analyzed by SPSS statistical package version 20 on IBM compatible computer. Quantitative data were expressed as mean ± standard deviation (M±SD) and analyzed by applying a student *t*-test for normally distributed variables and Mann Whitely U test for non normally distributed variables. ANOVA test (f-test) was used for comparison of more than two groups of normally distributed variables.

Qualitative data were expressed as number and percentage (No and %) and analyzed by applying chi-square test (X^2). Pearson correlation (r) was used to detect association between quantitative variables. Receiver Operating Characteristic (ROC) was used to test the diagnostic performance of HIF for comparison with that of AFP by calculating the sensitivity and specificities at several cut off points. All these tests were used as tests of significance at $P < 0.05$.

3. Results

For the present study, 33 patients suffered from post HCV cirrhosis and 30 patients suffered from HCC, who attend at the National Liver Institute Hospital, Menoufiya University at the period from October 2013-October 2014 were included. Blood was taken from healthy control, cirrhotic HCV and HCC patients for different analysis.

Clinical Data

The clinical investigation results showed that cirrhotic patients consisted of 22 (67.4%) males and 11 (32.6%) females, their ages ranged between 28 to 79 years old. Patients with HCC consisted of 19 (62.5%) males and 11 (37.5%) females, their ages ranged between 28 to 77 years old compared to healthy control of approximately the same ages (27-65 years old), and comprised 15 (75%) males and 5 (25%) females **(Table1)**.

Triphasic CT showed that post HCV cirrhotic patients included 13 (39.5%) compensated and 20 (60.5%) decompensted while HCC patients included 9 (29.2%) metastatic and 21 (70.8%) non metastatic compared to control **(Table 2).**

Table 1. The comparison between three studied groups regarding age and sex.

	The studied groups						Test of sign.	*P* value
	Cirrhotic N = 33		HCC N = 30		Control N = 20			
Age (year)								
M ± SD	53±9		53±11		47±12		F ration	
Range	28 – 79		28 – 77		27 – 65		2.21	0.12
	N	%	N	%	N	%	X^2	
Sex								
Male	22	67.4	19	62.5	15	75.0	0.68	0.71
Female	11	32.6	11	37.5	5	25.0		

M: Mean, SD: Standard deviation, F test = F test of ANOVA, N: Number,
X^2 = Chi square, HCV: Hepatitis C Virus, HCC: Hepatocellular carcinoma.

TABLE 2. Frequency of cirrhotic and hepatocellular cases.

	N	**%**
Cirrhotic cases (N = 33):		
Compensated	13	39.5
Decompensated	20	60.5
HCC cases (N = 30):		
Metastasis	9	29.2
Non metastasis	21	70.8

N: Number, Hepatocellular carcinoma (HCC).

Laboratory Data

Complete Blood Count (CBC)

Cirrhotic and HCC patients showed a significant decrease ($P < 0.001$) in Hb concentration and platelets count compared to healthy control, Hb concentration was significantly lower in cirrhotic cases than HCC patients ($P= 0.01$) **(Table 3)**. While no significant differences was recorded in platelets count between the two patient groups ($P= 0.41$) **(Table 3)**.

As regard total leucocytic count (TLC), there was a significant decrease in cirrhotic patients compared to control ($P= 0.02$), while there was no statistically significant difference compared HCC with control group or cirrhotic patients **(Table 3)**.

Table 3. Comparison between the studied groups as regard complete blood count.

	The studied groups			*t*- test	*P* value
	Cirrhotic group N = 33	**HCC group N = 30**	**Control N = 20**		
Hb (g/dl)				2.54	0.01[1]
M ± SD	11.23±2	12.33±2	13.86±1	.91	<0.001[2]
Range	8.7 – 14.8	8.8 – 15.8	11.5 – 16	2.77	0.009[3]
Platelets($x10^3$)/m^3				0.84	0.41[1]
M ± SD	107.44±32	114.65±36	241.38±46	10.61	<0.001[2]
Range	53 – 160	53 – 197	180 – 320	9.56	<0.001[3]
TLC($x10^3$)/m^3				≠ 1.41	0.16[1]
M ± SD	5.478±2.384	6.278±2.166	6.650±1.670	2.35	0.02[2]
Range	2200 –12800	3200 –13000	4200 – 10300	1.0	0.32[3]

≠ = **Mann Whitney U.**

M: Mean, SD: Standard deviation, N: Number, HCC: Hepatocellular carcinoma, Hb: Haemoglobin, TLC (Total leucocyte count).

1 = comparing cirrhotic cases and HCC cases. 2 = comparing cirrhotic cases and control.

3 = comparing HCC cases and control.

Liver Function Parameters

The data obtained showed a significant increase (P <0.001) in AST activity and bilirubin serum levels in cirrhotic and HCC patients as compared to control, while no significant difference was detected between both groups as regard ALT, albumin or PT concentration **(Table 4)**.

Comparing cirrhotic with control group, a significant increase was detected in the levels of AST and serum bilirubin and a significant decrease was detected in serum albumin and PT concentration in cirrhotic patients **(Table 4)**.

As the comparison between HCC and control group revealed that, a significant increase in the levels of ALT, AST and serum bilirubin in HCC cases, while albumin and PT concentration were significantly decreased **(Table 4).**

On the other hand, although there was not any significant difference was detected in ALT activity between cirrhotic patients and healthy control (P= 0.54), there was a significant increase in ALT activity in HCC patients compared to both healthy control and cirrhotic patients (P= 0.007). Moreover, a statistical significant decrease ($P < 0.001$) was observed in albumin and PT concentration serum levels in cirrhotic and HCC patients compared to control **(Table 4)**.

Immunological Parameters

ELISA was used to investigate anti-AFP, anti-HCV, anti-HBsAg and anti-HIF-1α antibodies in sera of patients as well as healthy control. The results ensured that patients with cirrhosis did not have any antibodies against HBsAg.

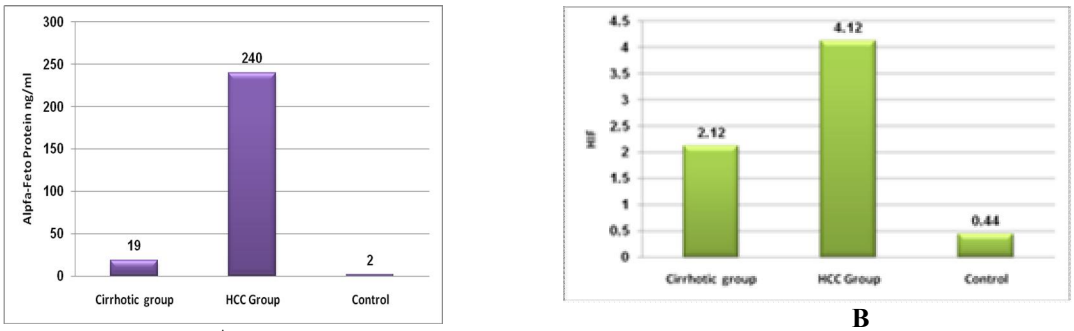
Sandwich ELISA was used to determine anti-AFP Abs and anti-HIF-1α Abs in sera of cirrhotic and HCC patients as well as healthy control. **Fig. (1a) s**howed a significant ($P < 0.001$) increase in AFP serum levels of cirrhotic and HCC cases compared to healthy control, also a significant ($P < 0.001$) increase was observed in AFP levels in HCC patients' serum compared with cirrhotic ones.

As regard HIF-1α**, Fig. (1b)** showed a significant ($P < 0.001$) increase in HIF-1α serum levels of patients with HCC compared to cirrhotic cases and control group and compared cirrhotic to control group.

Table 4: Comparison between three studied groups as regard liver function.

	The studied groups			Mann Whitney U	*P* value
	Cirrhotic group N = 33	**HCC group N = 30**	**Control N = 20**		
ALT(IU/L)				2.68	0.007[1]
M ± SD	38.4±21	60.21±45	34.75±6	0.61	0.54[2]
Range	13 – 115	20 – 236	24 – 44	2.69	0.007[3]
AST(IU/L)				1.45	0.15[1]
M ± SD	52.12±30	66.58±49	30.81±7	3.61	<0.001[2]
Range	14 – 185	10 – 234	20 – 46	3.33	0.001[3]
Total Bilirubin (mg/dl)				*	0.02[1]
M ± SD	3.57±2.31	1.63±1.44	0.85±0.21	2.38	<0.001[2]
Range	0.8 – 24.7	0.4 – 7.4	0.4 – 0.21	5.55	0.009[3]
				2.62	
Direct Bilirubin (mg/dl)				*	<0.001[1]
M ± SD	2.19±1.61	0.80±0.66	0.46±0.25	5.02	<0.001[2]
Range	0.32 – 15	0.1 – 4.2	0.1 – 0.9	5.18	0.40[3]
				0.85	
Albumin(g/dl)				1.69	0.10[1]
M ± SD	3.10±0.52	3.45±0.71	4.88±1.14	8.26	<0.001[2]
Range	2.2 – 4.5	1.3 – 4.3	4 – 9	5.21	<0.001[3]
PT (%)				*	0.78[1]
M ± SD	72.48±14	71.38±18	95.63±4	0.28	<0.001[2]
Range	45 – 75	29 – 85	87 – 100	9.36	<0.001[3]
				6.21	

* :*t*–test, M: Mean, SD: Standard deviation, N: Number, ALT: Alanine Transaminase, AST: Aspartate Transaminase, PT: Prothrombine, HCV: Hepatitis C Virus, HCC: Hepatocellular carcinoma.
1 = comparing cirrhotic cases and HCC cases.
2 = comparing cirrhotic cases and control.
3 = comparing HCC cases and control.



A B

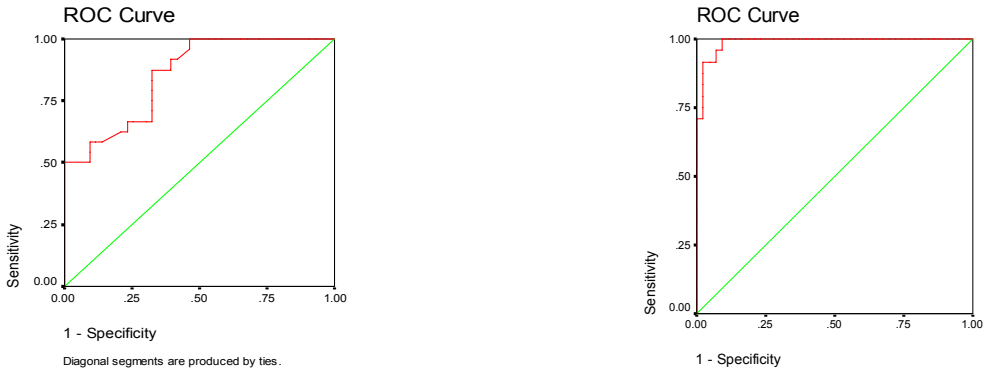
Fig. 1. Comparison between the three studied groups as regards alpha-fetoprotein (a) and hypoxia inducible factor-1 alpha (b).

At the cut off 13.2 ng/ml, AFP curve can discriminate between cirrhotic and HCC patients with a sensitivity of 87.5%, specificity of 67.4 %, positive predictive value (PPV) 60%, negative PV (NPV) 90.6% and area under the curve (AUC) 0.85 with a diagnostic accuracy of 74.6% **(Table 5 & Fig. 2)**. When HIF-1α used to discriminate between cirrhotic and HCC patients, a sensitivity of 100%, specificity of 90.7%, PPV 85.7%, NPV100% and AUC 0.99 with a diagnostic accuracy of 94% were recorded at a cut off 2.906 ng/ml **(Table 5 & Fig.2)**, suggesting higher sensitivity, specificity, PPV and NPV of HIF-1α than AFP level. By using a combined two markers the sensitivity and NPV were improved to 100% **(Table 5)**.

TABLE 5. The value of AFP and HIF-1 α for the prediction of cirrhosis and HCC.

Comparison	AFP(ng/ml)	HIF-1α (ng/ml)	Combined AFP&HIF-1α
Cut off point	13.2	2.906	-
P value	<0.001	<0.001	-
AUC	0.85	0.99	-
Sensitivity	87.5%	100%	100%
Specificity	67.4%	90.7%	65.1%
PPV	60%	85.7%	61.5%
NPV	90.6	100%	100%
Accuracy	74.6	94%	77.6%

AFP: Alpha-fetoprotein. HIF-1α: Hypoxia inducible factor-1 alpha. AUC: Area under the curve.
PPV: Positive predictive value. NPV: Negative predictive value.



A B

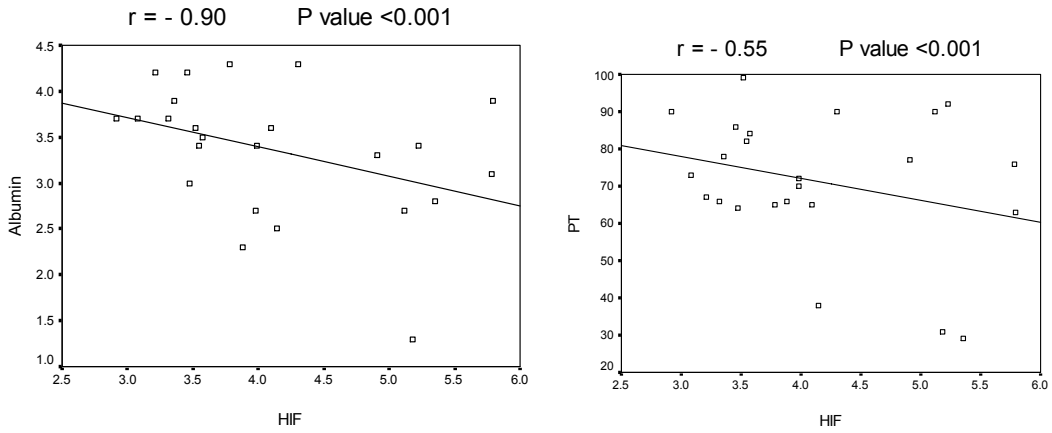
Fig. 2. Receiver operator characteristic (ROC) curve for alpha fetoprotein (a) and hypoxia inducible factor-1 alpha (b) as discriminant of hepatocellular carcinoma vs cirrhosis patients.

Spearman's correlation coefficient did not find any correlation between AFP of cirrhotic or HCC patients and different variables such as age and laboratory parameters (Hb, TLC, platelets and PT) and LFT (ALT, AST, albumin and bilirubin) **(Table 6).** However, there was a significant negative ($P < 0.001$) correlation between HIF-1α and both of albumin **(Fig. 3a)** and PT **(Fig. 3b)** in cirrhotic group.

Table 6. Correlation between alpha fetoprotein and other parameters among cirrhotic and hepatocellular carcinoma groups.

Parameters	AFP(ng/ml)			
	Cirrhotic group		HCC group	
	R	*P* value	r	*P* value
Age (/Year)	- 0.01	0.97	0.07	0.75
ALT(IU/L)	0.03	0.84	0.17	0.42
AST(IU/L)	0.06	0.69	0.27	0.19
Albumin (g/dl)	0.07	0.64	0.25	0.25
Total bilirubin (mg/dl)	- 0.09	0.52	- 0.09	0.68
Direct bilirubin (mg/dl)	- 0.09	0.57	- 0.08	0.71
PT (%)	0.04	0.82	0.05	0.82
Hb (g /dl)	- 0.03	0.86	- 0.05	0.81
Platelets(x10^3)/m^3	0.04	0.81	- 0.04	0.87
TLC (x10^3)/m^3	0.14	0.37	- 0.06	0.80
HIF-1α (ng/ml)	- 0.003	0.99	0.26	0.23

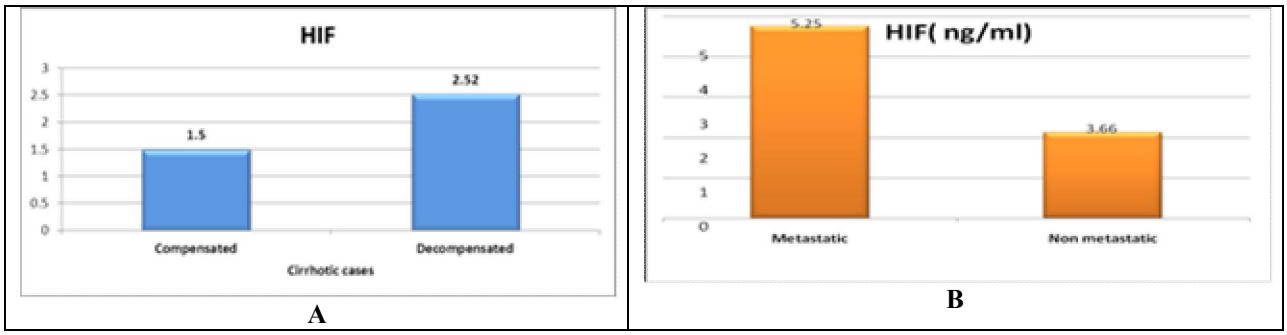
AFP: Alpha-fetoprotein, HCV: Hepatitis C Virus, HCC: Hepatocellular Carcinoma, r: Correlation Coefficient, ALT: Alanine Transaminase, AST: Aspartate Transaminase, PT: Prothrombine Time, Hb: Hemoglobin, TLC: Total lymphocyte counts, HIF-1α: Hypoxia inducible factor-1 alpha.



A **B**

Fig. 3. Correlation between hypoxia inducible factor-1 alpha and either albumin (a) or prothrombin (b) among cirrhotic hepatitis C virus and hepatocellular carcinoma patients.

By comparing AFP and HIF-1α in cirrhotic and HCC subgroup cases using *t*-test and Mann Whitney U, there was no statistically significant differences as regard AFP between both sub groups in contrast significant ($P < 0.001$) increase of HIF-1α level in decompensated cirrhotic and metastatic HCC patients was detected as compared to compensated **(Fig. 4a)** and non-metastatic **(Fig. 4b)** ones, respectively.



A **B**

Fig. 4. Comparison between cirrhotic (a) and hepatocellular carcinoma (b) subgroups cases as regards hypoxia inducible factor-1 alpha.

4. Discussion

The infection with HCV is almost always acquired in adulthood, mainly as a result of the illicit use of injectable drugs and sexual transmission. Eighty percent or more of individuals acutely infected with HCV become chronic carriers of the virus and about 60% develop chronic hepatitis. Of the latter approximately 20% progress to cirrhosis over a period of 20 to 50 years, and a portion of these develop HCC **(Kiyosawa *et al.*, 2004).** Although progress has been made in the diagnosis and management of HCC, its prognosis remains dismal.

Several studies have suggested that HIF-1α is a prognostic factor for tumor in human and murine HCC **(Dai *et al.*, 2009).** For the present study, 33 patients recurrence with post-HCV liver cirrhosis (20 de-compensated and 13 compensated), 30 patients with proved HCC (9 metastatic and 21 non metastatic) and 20 healthy volunteers were included.

Cirrhotic and HCC patients showed a significant decrease ($P < 0.001$) in Hb concentration and platelets count compared to healthy control, Hb concentration was significantly lower in cirrhotic cases than HCC patients ($P= 0.01$), while no significant differences

was recorded in platelets count between the two patient groups (P= 0.41).

As regard TLCs, there was a significant decrease in cirrhotic patients compared to control (P=0.02), while there was no statistically significant difference when comparing HCC with control group or cirrhotic patients studied groups**,** and this agreed with **Zakaria *et al.* (2004)** who stated that the complete blood picture showed a significant anaemia, thrombocytopenia and leucopenia in the cirrhosis and HCC groups compared to control.

Comparing cirrhotic and HCC with control group, a significant increase was detected in the levels of AST, serum bilirubin and a significant decrease was detected in serum albumin and PT concentration in cirrhotic patients. While a significant increase in ALT activity was recorded only in HCC patients compared to both healthy control and cirrhotic patients (P= 0.007). This is because **Selim and Ahmed (2014)** found that any damage occurred in the liver, the liver cell (hepatocyte) membrane becomes more permeable and some of the enzymes leak out into the blood circulation. Also serum bilirubin concentration is a well established marker of cirrhosis, which is accompanied with hepatocellular destruction. Therefore, when fibrosis progresses, bilirubin increases because of reduced hepatic excretion and less enterohepatic circulation attributable to portal systemic shunt.

In agreement with **Selim and Ahmed (2014)** who detected that hypo-albuminaemia and decrease of PT concentration (P < 0.001) were more common among individuals with chronic liver disease reflecting both severe liver damage and decreased albumin synthesis. Our study detected a statistical significant decrease (P < 0.001) in Albumin and PT concentration serum levels in cirrhotic and HCC patients compared to control. The decrease of PT concentration in advancing liver fibrosis indicates a damage of liver parenchyma resulting in decreased production of coagulation proteins with increased risk of bleeding tendencies.

In the present study, there was a significant increase in HIF-1α serum levels was detected in patients with HCC compared to cirrhotic cases and control group. Liver cirrhosis is considered as a premalignant state, as about 80% of HCC cases are associated with liver cirrhosis. Our study also did not find any correlation between AFP or HIF-1α and the other studied parameters except a significant negative (P < 0.001) correlation was recorded between HIF-1α and both of albumin and PT in cirrhotic cases**.** This is in contradiction to **Selim and Ahmed (2014)** results which showed positive correlation between AFP and ALT.

Bertout *et al.* (2008) found that HCCs do not usually express HIF-1α; in meta-analysis, of 953 HCC patients, 475 (50%) had HIF-1α overexpression. However, once cancer cells acquire HIF-1α expression, they transform to more aggressive and metastatic behavior. In accordance, the present study showed that there was a significant increase of HIF-1α in metastatic patients than non metastatic one. Accordingly, **Li et al. (2011)** found high HIF-1α expression could be detected in all patients with HCC with extra-hepatic metastasis.

The low sensitivity associated with serum AFP levels, elevated false-negative rate, and poor discriminatory ability in the diagnosis of HCC have all significantly limited reliance on AFP as a diagnostic test **(Gonzalez and Keeffe, 2011).**

Whereas HIF-1α can discriminate between cirrhotic and HCC patients, at a cut off 2.906 ng/ml, with higher sensitivity (100%), specificity (90.7%), PPV (85.7%), NPV (100%) and AUC (0.99) than AFP with a diagnostic accuracy of 94%. Moreover a combined two markers improved the sensitivity and NPV to 100%. This agree with **Li *et al.* (2011)** who stated that the level of serum HIF-1α in HCC patients was significantly higher (P <0.001) and the incidence of HIF-1α abnormality was 100 % in HCC, 89.2% in liver cirrhosis using the area under ROC curves 0.909 in HIF-1α.

In conclusion, hepatic HIF-1α expression is associated with the development and prognosis of HCC, and circulating HIF-1α level is a useful marker for HCC diagnosis and prognosis. It may shed light on novel strategies for the follow-up and HIF-1α can be tested in the selected patients for the better chance of a longer survival.

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