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Hypoxia Inducible Factor 1α as a Diagnostic and Predictor Test of Liver Fibrosis

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Authors' contributions

This work was carried out in collaboration between all authors. Author ESST designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MAET managed the literature searches, wrote the final draft of the manuscript and analyses of the study. Author OMH managed the experimental process and all investigations. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: Regulation of the the metabolic activities of the liver is done by oxygen, which is considered as a critical signaling molecule in that issue. Oxygen delivery dysregulation provoke hepatic steatosis and inflammation. Hypoxia inducible factors (HIF1α) control the expression of gene essential for adaptation to low level of oxygen. In the state of cell hypoxia, (HIF 1α) protein level increase.
Aim of the Work: Study the role of hypoxia inducible factor (HIF1α) as non-invasive marker and predictor of cirrhosis.
Patients and Methods: The study was conducted on 80 patients selected from chronic hepatitis c patients at National Liver Institute, Menofia university. Subjects were randomized into 2 main groups according to the degree of liver disease: Non-Cirrhotic group; included 40 cases with early fibrosis; while Cirrhotic group included 40 cases with liver cirrhosis; and finally, Control group: included 20 apparently healthy with no definite fibrosis by liver biopsy(historical control). Excusion

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criteria included, Hepatic tumors, or hepatic focal lesion, Vascular liver diseases or, portal, superior mesenteric, thrombosis, and unfit for US guided liver biopsy, all subjects had liver function tests, virology screen, complete blood counts, serum creatinine, fasting and two hours postprandial blood sugar, abdominal ultrasound, recent liver biopsy and hypoxia inducible factor (HIF1α) assay.

Results: Non-Cirrhotic group showed moderate significant positive correlation between grade of fibrosis and HIF1α level, while there was inverse, moderate, significant correlation between albumin and HIF1α. Cirrhotic group, showed significant positive correlation between HIF1α, and total bilirubin, direct bilirubin, PT and INR while there was significant negative correlation with albumin. HIF1α was significantly increased with increased fibrosis grade. HIF1α level significant increased in Cirrhotic group compared to Non-Cirrhotic (2.16±0.65 vs 1.02±0.41 respectively), and in decompensated cases compared to compensated cases (2.50±0.46 vs 1.72±0.60 respectively). Finally, HIF1α had a predictor values for diagnosis of early fibrosis as area under the curve (AUC) was 0.97. The best cut off value was 0.68, with 92.5% sensitivity and 90.0% specificity.

Conclusion: HIF1α can be a useful sensitive, diagnostic, and predictor marker of hepatic fibrosis.

Keywords: Hypoxia; liver disease; fibrosis.

1. INTRODUCTION

Liver fibrosis process initiated by widespread deposition of collagen and extracellular matrix inside the liver in the context of chronic injury. The spark of this process happens when liver insult encourages cells to form and secrete certain growth factor as platelet derived growth factor (PDGF) along with other activator of hepatic stellate cells with the end result formation of collagen from per biliary fibroblast [1]. Other mediators as matrix metalloproteinase that modulate matrix turnover are essential for the initiation and progression of hepatic fibrosis [2]. Regulation of the the metabolic activities of the liver.is done by oxygen, which is considered as a critical signaling molecule in that issue. Dysregulation of oxygen delivery inside the hepatic cells can provoke hepatic steatosis and inflammation [3]. Impairment of cellular oxygenation affects gene expression through the transcription factor. HIF1α control the expression of gene critical for adaptation to low oxygen levels. Hypoxia inducible factors has 2 subunits, alpha subunit (HIF-1α, HIF-2α) and beta subunit (HIF-1β) [4]. Normally, HIF1α is produced and targeted for proteolytic degradation. In the state of cell hypoxia, the mechanism that targets HIFα subunit for degradation is inhibited resulting in HIF1α protein level to increase [5].

1.1 Aim of the Work

The aim of this study: Study the role of hypoxia inducible factor (HIF1α) as non-invasive marker and predictor of cirrhosis.

2. PATIENTS AND METHODS

This study was done on 80 patients having chronic hepatitis C (HCV) and attending National Liver Institute, hepatology clinic. Written consent and National Liver Institute IRB (ethical committee) approval were obtained. Patients were randomized into 2 main groups according to liver pathology. NON-Cirrhotic Group: Included 40 non-cirrhotic patients, and Cirrhotic Group: included 40 cases with HCV related- liver cirrhosis (compensated, and decompensated). Both groups were compared to 20 apparently healthy with recent report of normal liver pathology recruited as a historical control group.

2.1 Exclusion Criteria

Includes; Hepatic tumors, or hepatic focal lesion, Vascular liver diseases, portal, superior mesenteric, thrombosis, and patient unfit for US guided liver biopsy, patient who had hepatic steatosis, or other liver insult than CHCV, diabetes mellitus, hypertension; and ischemic heart diseases.

All patients had, complete history taking, Clinical examination, liver function tests, virology screeining (HBsAg, HBcAb, HCV Ab by using third generation ELISA and quantitative HCV RNA), Complete Blood Counts, Serum creatinine, Fasting and two hours' postprandial blood sugar, Hypoxia inducible factor -1 alpha (HIF1α) assay and Abdominal Ultrasound.

Except for patient with clinical evidents of liver cirrhosis, all patient had Ultrasound guided liver biops, or recent report of liver biopsy within 6 months' don in the same or other facility.

Liver biopsy slides were reviewed by 2 experienced histopathology experts, Metavir score was done and revised for every case.

The metavir scoring includes grading (from 0-4), and a staging (A1, and A2) system. The staging reflect the activity or amount of inflammation while the grading represents fibrosis or scarring [6].

Hypoxia inducible factor alpha-1 (HIF1α) was assessed using ELISA Kit Catalog No: E0798h (produced by EIAab Company, Wuhan EIAab Science Co., Ltd. 3rd Floor, Building A2, Biopark, Optics Valley, Wuhan, CHINA).

2.2 Statistical Analysis of Data

The collected data were organized, tabulated and statistically analyzed using statistical package for social science (SPSS) version 16, running on IBM compatible computer using Microsoft windows ® 7 as an operating system. For quantitative data, mean, standard deviation (SD), minimum and maximum were calculated, and for comparison between 2 means, the student's sample (t) test was used. The one-way analysis of variance (ANOVA; F) test was used for comparison between more than 2 means. For qualitative data, the frequency and percent distribution were calculated and for comparison between groups, the Chi square (X^2) test was used. Correlation between two parameters was done calculation of Pearson's correlation coefficient (r); it was mild if less than 0.3, moderate if more than 0.3 and less than or equal to 0.7 and powerful if more than 0.7; it was proportional if the sign is positive and inverse if the sign is negative. For interpretation of results, the p value less than or equal to 0.05 was considered significant.

3. RESULTS

Both study groups were matched as regards gender distribution (Males represented 87.5% and 72.5% from Non-Cirrhotic group and Cirrhotic group respectively). The mean age was 42.50±9.70 years, and 53.07±10.05 years in Non-Cirrhotic group and Cirrhotic group respectively, while control group was slightly younger 35.05±8.51 years. All cases in both groups had proved Chronic Hepatitis C, and all cases in Cirrhotic group considered to have grade 4 fibrosis (F4); while Non-Cirrhotic group had 5% fibrosis grade 0; 27.5% were fibrosis grade 1; 37.5% were fibrosis grade 2 and 30% were fibrosis grade 3. No case had fibrosis grade 4 in group A according to Metavir scoring (Table 1).

Table 2 showed Comparison between studied groups as regards descriptive data.

Table 3 Correlation between HIF1α and other studied parameters in different study groups.

It revealed that, in Non-Cirrhotic group, there was moderate ($r \geq 0.3$ and ≤ 0.7), significant and positive correlation between grade of fibrosis and HIF1α ($r = 0.67$, p value < 0.001). This correlation means that, when fibrosis grade increased, the HIF1α was increased and vice versa. On the other hand, when albumin decreased, HIF1α increased and vice versa, denoting, significant inverse, correlation ($r=-0.53$, $p < 0.001$).

Regarding Cirrhotic group, there was considerable positive correlation between HIF1α and total bilirubin ($r=0.51$, $p <0.001$), direct bilirubin ($r =0.48$, $p =0.002$) and INR ($r =0.52$, $p = 0.001$). Similarly, a powerful significant negative correlation between HIF1α and serum albumin ($r = -0.93$, $p < 0.001$). Both correlations denoted that, HIF1α is increased with advancement of liver disease (marked by increased fibrosis grade and decreased albumin levels). On the other hand, there no significant correlation between HIF from one side and each of ALT, AST, total& direct bilirubin, INR, creatinine, HB, platelets and TLC (Table 3).

In Cirrhotic group, there was significant positive correlation between HIF1α and each of total bilirubin ($r=0.51$, $p <0.001$), direct bilirubin ($r =0.48$, $p =0.002$), PT ($r=0.63$, $p <0.001$) and INR ($r =0.52$, $p =0.001$). On the other hand, there was powerful significant negative correlation between HIF1α and albumin ($r=-0.93$, $p < 0.001$). These correlations revealed the relation between HIF1α level and liver fibrosis, and deterioration of its synthetic functions. Actually, other liver function tests showed significant correlation with HIF1α (total and direct bilirubin, PT and INR). Contrarily, we did not find significant correlation between HIF1α and each of ALT, AST, creatinine, Hb, platelets or TLC)

Finally, in healthy control group, there was no significant correlation between HIF and any other studied parameters (the r value was 0.03, -0.25. 0.03, 0.250, 0.18, 0.13, -0.28, 0.22, 0.27, 0.19 and -0.22 for ALT, AST, albumin, total bilirubin, direct bilirubin, PT, INR, creatinine, Hb, platelets and TLC respectively) (Table 3).

In the present work, in all patients, HIF1α significantly increased with increased fibrosis grade. HIF1α was 0.62±0.14 in fibrosis grade 0, 0.79±0.18 in fibrosis grade 1, 0.86±0.13 in fibrosis grade 2; 1.4±0.45 in fibrosis grade 3 and 2.16±0.65 in fibrosis grade 4 (Table 4).

Our study, showed, a significant increase of HIF1α in Cirrhotic group compared to Non-Cirrhotic group (2.16±0.65 vs 1.02±0.41 respectively, $p < 0.001$). Moreover, there was significant increase of HIF1α in decompensated cases compared to compensated cases in Cirrhotic group (2.50±0.46 vs 1.72±0.60 respectively) (Table 5).

In the present study, HIF1α had a predictor values for diagnosis of early fibrosis as area under the curve (AUC) was 0.97. The best cut off value for HIF1α was 0.68, with 92.5% sensitivity and 90.0% specificity (Table 6, Fig. 1).

Table 1. Comparison between different groups as regard to categorical data

		Non-cirrhotic group (40)	Cirrhotic group (40)	Control group (20)
Sex	Male	35(87.5%)	29(72.5%)	15(75.0%)
	Female	5(12.5%)	11(27.5%)	5(25.0%)
Age		42.50±9.70	53.07±10.05	35.05±8.51
HCV antibody		40(100.0%)	40 (100.0%)	0(0.0%)
Fibrosis grade	F0	2 (5.0%)	0(0.0%)	20(100.0%)
	F1	11 (27.5%)	0(0.0%)	0(0.0%)
	F2	15 (37.5%)	0(0.0%)	0(0.0%)
	F3	12(30.0%)	0(0.0%)	0(0.0%)
	F4	0(0.0%)	40 (100.0%)	0(0.0%)

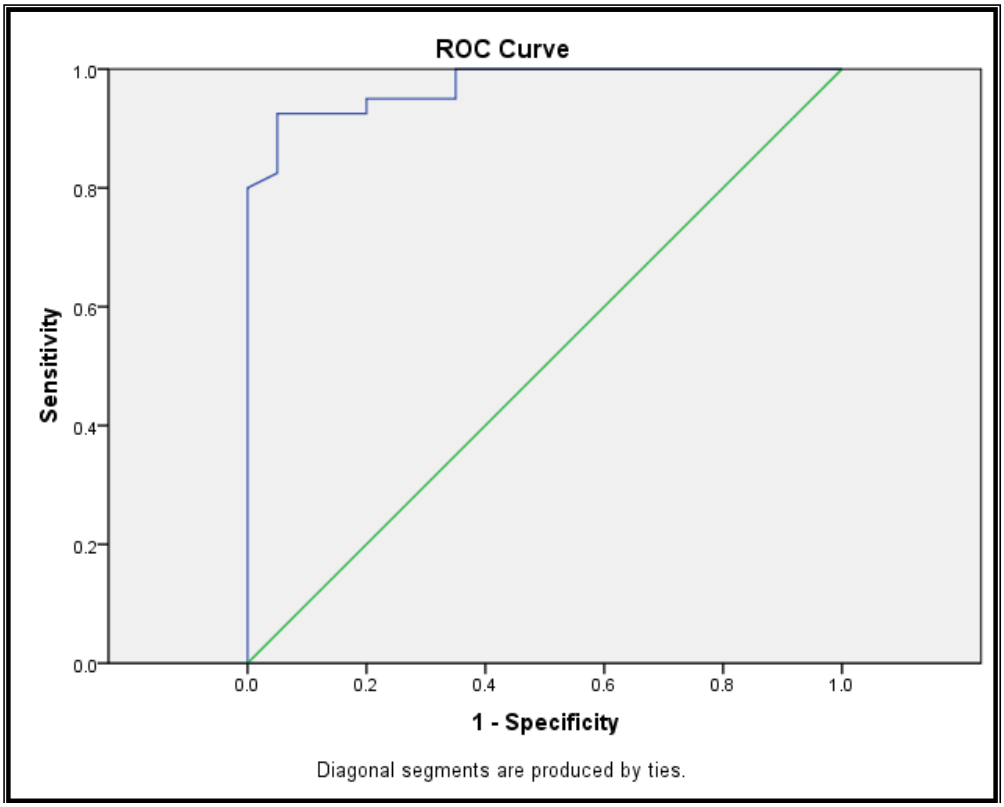

Fig. 1. ROC for HIF1α in diagnosis of fibrosis

Table 2. Comparison between studied groups as regard to descriptive data

	Non-cirrhotic group (40)	Cirrhotic group (40)	Control group (20)	P1	P2	P3
	Mean±S. D	Mean±S. D	Mean±S. D			
ALT	38.09±27.79	37.79±20.94	34.35±5.84	0.418	0.33	0.95
AST	38.86±32.75	**53.15±31.45**	31.00±6.31	0.150	**<0.001***	**0.05***
Albumin	**4.33±0.39**	**3.00±0.55**	**4.59±0.32**	**0.009***	**<0.001***	**<0.001***
Total bilirubin	0.85±0.36	**1.80±0.73**	0.85±0.18	0.997	**<0.001***	**<0.001***
Direct bilirubin	0.39±0.27	**1.30±0.65**	0.41±0.23	0.700	**<0.001***	**<0.001***
PT	12.65±2.02	**15.77±2.03**	12.20±0.36	0.176	**<0.001***	**<0.001***
INR	1.10±0.14	**1.36±0.24**	1.10±0.07	0.896	**<0.001***	**<0.001***
Creatinine	0.83±0.14	0.84±0.15	0.77±0.12	0.092	0.08	0.91
HB	13.03±2.18	**11.15±1.57**	13.65±1.29	0.175	**<0.001***	**<0.001***
Platelets	**178.25±62.92**	**107.20±38.51**	**233.50±44.67**	**<0.001***	**<0.001***	**<0.001***
TLC	5.40±2.55	5.32±2.32	5.52±2.03	0.848	0.72	0.87

P1 = comparison between Non-cirrhotic group and control group: P2 = comparison between cirrhotic group and control group; and P3= Comparison between Non-cirrhotic group, and cirrhotic groups

Table 3. Correlation between HIF1α and other studied parameters in different study groups

	Non-cirrhotic group (40)		Cirrhotic group (40)		Control group(20)	
	r	p	r	p	r	p
ALT	-0.04	0.77	-0.12	0.45	0.03	0.87
AST	0.08	0.60	-0.12	0.44	-0.25	0.28
Albumin	**-0.53**	**<0.001***	**-0.93**	**<0.001***	0.03	0.88
Total bilirubin	0.24	0.12	**0.51**	**0.001***	0.20	0.39
Direct bilirubin	0.29	0.06	**0.48**	**0.002***	0.18	0.42
PT	-0.28	0.07	**0.63**	**<0.001***	0.13	0.56
INR	0.20	0.21	**0.52**	**0.001***	-0.28	0.22
Creatinine	-0.13	0.41	0.05	0.78	0.22	0.34
HB	-0.24	0.12	-0.18	0.25	0.27	0.23
Platelets	-0.17	0.29	-0.23	0.14	0.19	0.41
TLC	0.15	0.35	0.29	0.06	-0.22	0.33
Grade of fibrosis	**0.67**	**<0.001***				

Table 4. HIF1α level in different grades of fibrosis, in non-cirrhotic, and all patients

		Non-cirrhotic group		All cases (Non-cirrhotic and cirrhotic)	
		Mean	SD	Mean	SD
Fibrosis	F0	0.62	0.14	0.62	0.14
	F1	0.79	0.18	0.79	0.18
	F2	0.86	0.13	0.86	0.13
	F3	1.4	0.45	1.4	0.45
	F4	-	-	2.16	0.65
Statistics	F	15.59		28.82	
	P	<0.001*		<0.001*	

Table 5. HIF1α in non-cirrhotic group, and cirrhotic group (compensated, and decompensated)

	Group	Mean	S. D	t	p
HIF-1α	Non cirrhotic	1.023	0.41		
	Cirrhotic	2.168	0.65	9.34	<0.001*
	Comp. cirrhosis	17	1.72	4.61	<0.001*
	Decomp. cirrhosis	23	2.50		

Table 6. Sensitivity and specificity of HIF in diagnosis of fibrosis

	AUC
AUC	0.972
P value	<0.001*
Best cutoff	0.68
Sensitivity	92.5%
Specificity	90.0%

4. DISCUSSION

Liver fibrosis is a common pathology following liver injury, it is characterized by excessive accumulation of extracellular matrix (ECM), mainly collagen materials in the hepatic cells, hepatic stellate cell (HSC) is responsible for ECM formation [7]. The hepatic inflammation and damage are leading to hypoxia in local micro-environment. In hypoxic state, the oxygen dependent degradation pathway is suppressed and HIF1α dimerizes with HIF1beta and bind with hypoxia-responsive elements (HRE) of target genes, thus, allowing cells survive in hypoxia [8,9].

The role of HIF1α in the pathogenesis of chronic liver disease is still a matter of debate; to our knowledge the present study is a unique in measuring HIF1α in liver fibrosis cases. Thus, results of the present study may be considered a preliminary result and the first to examine the role of HIF1α in diagnosis and prediction of liver fibrosis.

This study was designed to evaluate the role of HIF1α in the development of liver fibrosis, through mediators produced by injured liver cells and to use the serum HIF as a non-invasive marker to monitor the grade of liver fibrosis and response to therapy. The study was conducted on 80 subjects selected from outpatient and inpatients departments from Hepatology Department of the National Liver Institute as well as 20 healthy subjects was enrolled in the study as a control group. According to laboratory, abdominal US and liver biopsy, the patients were divided into 2 groups: *Non-cirrhotic group* included 40 cases with early fibrosis; while *Cirrhotic group* included 40 cases (17 had compensated liver cirrhosis and 23 had decompensated liver cirrhosis). All participates were subjected to complete history taking, clinical examination, routine liver and kidney function tests, complete blood count, fasting and two hours postprandial blood sugar, and serum HIF1α assay.

Our results showed no significant difference was detected regarding age and gender within the groups, the patient selection was matched regarding fibrosis grade. Serum albumin and platelets were significantly decreased in cirrhotic compared to non-cirrhotic patients or control. This is explained by the hypo-albuminaemia and decrease of PT concentration was more common among individuals with chronic liver disease reflecting both severe liver damage and decreased albumin synthesis [10]. 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 [11].

Regarding HIF1α, it is significantly increased in cases with a high fibrotic grade compared to cases with early fibrotic grades, and higher levels were detected in cases with liver cirrhosis. Moreover, the negative relationship was detected between HIF1α and serum albumin levels in cirrhotic and non-cirrhotic groups. Additionally, HIF1α was positively correlated with bilirubin, PT and INR in Cirrhotic group. All of these findings are denoting the role of HIF1α in the pathogenesis of disease progression, however further larger studies are needed to confirm that. No correlation between HIF1α and different parameters in control group, suggesting the connection between HIF1α and development of liver fibrosis or cirrhosis.

Furthermore, in the present study, HIF1α had a predictor values for diagnosis of early fibrosis as area under the curve (AUC) was 0.97. The best cut off value for HIF1α was 0.68, with 92.5% sensitivity and 90.0% specificity.

Stellate cell activation has been described as an initiating factor in liver fibrosis, and stellate cells cultured under hypoxia had increased collagen mRNA transcripts. Exposure of the hepatic stellate cell line to hypoxia stimulated HIF1α and VEGF mRNA accumulation by 8 hours, and was associated with evidence of increased signaling through the transforming growth factor-beta (TGF-β)-SMAD dependent pathway. Also, hypoxia revealed several targets, including fibroblast growth factor-4, that have been implicated in fibrogenesis or inflammation [12]. Isolated stellate cells from HIF1α-deficient mice also demonstrated that genes involved in fibrosis, including angiogenic and collagen deposition factors, were at least partially dependent on functional HIF1α [13,14].

However, increased levels of HIF-1α in cirrhotic cases when compared to non-cirrhotic cases adds an evidence that, fibrosis itself has a profound effect on HIF-1α production and hepatitis C-infection alone cannot explain these findings.

Taking into account that, all cases in the present study were hepatitis C-positive can give an interpretation for increased HIF-1α in studied cases. It was reported that; hepatitis C infection may interact with the HIF1α pathway by way of multiple mechanisms. Huh7 cells expressing the HCV core protein were reported to have increased VEGF expression and increased HIF-1α DNA binding and stabilization and the HIF-1α stability was accompanied by production of functional VEGF. Additionally, this stabilization again appeared to be dependent on multiple kinase and transcriptional pathways, [15]. Furthermore, HIF-1α stabilization by HCV was demonstrated to be insensitive to antioxidant treatment and dependent on derangement of mitochondrial respiration in HCV-infected cells [16].

5. CONCLUSION

The overall results of our study highlight a beneficial role of HIF1α level in diagnosis and potential role in monitoring progress of liver fibrosis. This test is less-invasive, easily obtained and can be repeated on regular intervals. There is potential role need more studies to prove if HIF1α level assay, could be used as predictor of response to ant fibrotic therapy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastro-enterology. 2008;134: 1655–1669.
2. Han HK, Han CY, Cheon EP, Lee J, Kang KW. Role of hypoxia-inducible factor-alpha in hepatitis-B-virus X protein-mediated MDR1 activation. Biochem Biophys Res Commun. 2007;357:567–573.
3. Jungermann K, Kietzmann T. Oxygen: Modulator of metabolic zonation and disease of the liver. Hepatology (Baltimore, Md.). 2000;31:255–260.
4. Coleman ML, Ratcliffe PJ. Oxygen sensing and hypoxia-induced responses. Essays Biochem. 2007;43:1-15.
5. Cash TP, Pan Y, Simon MC. Reactive oxygen species and cellular oxygen sensing. Free Radic Biol Med. 2007; 43(9):1219-25.
6. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 1996;24:289–293
7. Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. Best Pract Res Clin Gastroenterol. 2011;25:195-206.
8. Troeger JS, Schwabe RF. Hypoxia and hypoxia-inducible factor 1α: Potential links between angiogenesis and fibrogenesis in hepatic stellate cells. Liver Int. 2011;31: 143-145.
9. Nath B, Szabo G. Hypoxia and hypoxia inducible factors: Diverse roles in liver diseases. Hepatology. 2012;55:622-633.
10. Selim FO, Ahmed AM. The possible role of serum soluble Endoglin level in the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis: Bio Med Research International. 2014;2(9):229-240.
11. Zekri AR, Alam El-Din HM, Bahnassy AA, Zayed NA, Mohamed WS, El-Masry SH, Gouda SK, et el. Serum levels of soluble Fas, soluble tumor necrosis factor-receptor II, interleukin-2 receptor and interleukin-8 as early predictors of hepatocellular carcinoma in Egyptian

patients with hepatitis C virus genotype-4. Comp Hepatol. 2010;9(1):1.

12. Novo E, Cannito S, Zamara E, Caligiuri A. Proangiogenic cytokines as hypoxia-dependent factors stimulating migration of human hepatic stellate cells. Am. J. Pathol. 2007;170:1942–53.
13. Copple BL, Bai S, Burgoon LD, Moon JO. Hypoxia-inducible factor- 1alpha regulates the expression of genes in hypoxic hepatic stellate cells important for collagen deposition and angiogenesis. Liver Int 2010a;31:230-244.
14. Copple BL, Bai S, Moon JO. Hypoxia-inducible factor-dependent production of profibrotic mediators by hypoxic Kupffer cells. Hepatol Res. 2010b;40:530-539.
15. Nasimuzzaman M, Waris G, Mikolon D, Stupack D, Siddiqui A. Hepatitis C virus stabilizes hypoxia-inducible factor 1alpha and stimulates the synthesis of vascular endothelial growth factor. J Virol. 2007;81:10249-10257.
16. Ripoli M, Quarato G, Gouttenoire J and Scrima R. Hepatitis C virus-linked mitochondrial dysfunction promotes hypoxia-inducible factor 1 alpha-mediated glycolytic adaptation. J Virol. 2010;84:647-660.

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