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Glypican-3 as a tumor marker for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma(HCC) is one of the most frequent cancers in the world.The burden of HCC has been increasing in Egypt with a doubling in the incidence in the past ten years. The prognosis of most patients is unsatisfactory due to rapid clinical deterioration after the initial diagnosis. Therefore, it is very important to detect HCC and the recurrence at its earlier period. Glypican-3(GPC3) is a cell-surface protein, which is a member of the heparan sulfate proteoglycan family. GPC3 was indentified in the serum of patients with HCC and can be used as a serological test for the diagnosis of HCC. The aim was to assess the value of serum GPC3 in Egyptian patients with HCC. This study was included 30 patients with HCC, 30 patients with liver cirrhosis and 20 healthy controls. For all groups we studied clinical data, image findings, serum alphafetoprotein(AFP) levels detected by enzyme immunoassay(EIA) kit and GPC3 gene expression was detected by real time polymerase chain reaction(PCR).Tumour characteristics were assessed including size, number and site. Tumour staging was done using Okuda &Tokyo staging systems.The data showed that HCC patients had a significantly higher mean GPC3 values (p=0.000)than both cirrhotics and healthy control groups. GPC3 has a positive significant correlation with tumour size (p=0.015)and Tokyo staging system(p=0.047).GPC3 could be a useful diagnostic & prognostic marker for detection of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the seventh most common malignant tumor over the world (Yao *et al.,* 2011), representing more than 5% of all cancers (Michiellsen *et al.,* 2005), and the third most common cause of cancer related deaths (Liovet *et al.,* 2004; Grieco *et al.,* 2005). The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years (El-Shenawy *et al.,* 2012).

In Egypt HCC is the second most common malignant tumour (Soliman *et al.,* 2010), accounting for about 7.2% of chronic liver disease patients (El-Zayadi *et al.,* 2005). The prognosis of HCC is generally grave (Lopez, 2005; Liv *et al.,* 2010), approximately 75% of patients with hepatocellular carcinoma present with advanced ,unresectable disease and some element of hepatic dysfunction (Vauthey *et al.,* 2002). HCC can. only be cured if diagnosed at an early stage (Okochi *et al.,* 2002), so there is a pressing need either to prevent the tumor or to diagnose it at a presymptomatic stage ,when surgical interventions still possible (Kew, 2002).

Up to 20% of HCC do not produce alpha-fetoprotein (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 (Sherlock and Dooley , 2002). Glypican-3 (GPC3), a heparin sulphate proteoglycan anchored to the plasma membrane and it is an oncofetal protein that is over expressed in HCC .Serum GPC3 has been shown to be significantly higher in HCC than in liver cirrhosis or in healthy controls thus a novel serological marker for the early detection of HCC (Lopez, 2005). GPC3 has been reported to be expressed in the majority (>70%) of hepatocellular carcinoma (HCC) as a diagnostic marker (Jia *et al.,* 2007). This study was aimed to evaluating the diagnostic and prognostic value of Glypican-3 in comparison to the old established biomarker AFP levels in patients with HCC.

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PATIENTS AND METHODS

Patients

This study was approved by the Ethics and Research Committee of the Benha faculty of Medicine, Benha University,Egypt. Serum samples were obtained from sixty patients with chronic liver disease, divided into two groups: Group (I) included thirty patients with HCC, Patients with cancers other than HCC or metastatic liver cancer were excluded. Group (II) included thirty patients with liver cirrhosis and without any evidence of HCC. Twenty healthy adults were recruited as controls (Group III). All patients included in this study had the procedure thoroughly explained to them. HCC was diagnosed by abdominal US and serum AFP, with or without triphasic CTscan and/or liver histopathology. AFP was assayed by an enzyme immunoassay (EIA) Kit (Roche Mannheim, Germany). 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 disease was assessed by and MELD (model for end stage liver disease) score (Kamath *et al.*, 2001) and the updated MELD (uMELD) score (Sharma *et al.*, 2008). . 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 (Okuda *et al.*, 1985), and Tokyo (Tateishi *et al.*, 2005) staging systems}.

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-glutamyl transpeptidase (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 (HCV Ab and HBS Ag) were measured using Abbott, Axyam (USA, Supplied by al kamal company). Serum AFP level was determined using an enzyme-linked binding protein assay kit. All assays were performed in duplicate according to the manufacturer's instructions. Serum aliquots were stored at _80_C until assayed and thawed immediately before the measurements levels. Another portion of blood was collected in vacutainer tubes containing EDTA to separate lymphocyte cells.

Quantification of GPC3 expression by real-time PCR

GPC3 gene expression was assyed for all samples and controls. Preparation of PBMCs obtained from peripheral blood of all patients and controls were isolated by Ficoll density centrifugation and sedimentation. RNA was extracted from PBMCs cells using QIAamp_viral RNA extraction kit. Quantification of was performed using TaqMan® Gene Expression assay (Applied Biosystems Inc, Foster City, CA, USA). β -actine was used as housekeeping gene (endogenous reference cDNA). Fractional threshold cycles (CT) were expressing the initial concentration of target sequence. Relative mRNA quantification was calculated using the arithmetic formula 2-Δct, where ΔCT is the difference between the CT of a given target cDNA and an endogenous reference cDNA. Thus, this value yields the amount of the target normalized to an endogenous reference.

Statistical Analysis

Statistical package (SPSS, version 10.0) was used for data management. Descriptive statistics was presented as mean±standard deviations for continuous variables, number and percentage for categorical variables (frequency distribution). Unpaired Student t-test (two sided) was used to test the significance of difference between the mean value of studied groups and chisquare test was used for comparison of categorical variables. The diagnostic value for each marker was assessed using Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values. Receiver operating characteristic curves (ROC) were constructed to assess the validity of the markers in predicting HCC by calculating the area under the curve (AUC). Pearson correlation test was used to identify the correlation between Glypican-3 and different clinicopathological variables. The significance level was set at $p<0.05$.

RESULTS

The demographic features and characteristics of the two patients' groups were summarized in Table 1. A total of 60 adults, which comprised 30 patients with HCC, 30 patients with liver cirrhosis and 20 apparently-normal control subjects were studied. The mean age of HCC patients was 57.6±9.7 years with a range between 40 and 73 years. In liver cirrhosis patients the mean age was 52.2±9.8 years with a range between 34and 70 years.There was a significant difference in the mean ages of HCC patient group, liver cirrhosis (p=0.037). There was male predominance among the patients with HCC, 21 men (70.0%) versus 9 women (30.0%), with a male-to-female ratio of 2.3:1, in the liver cirrhosis patients there was 16 (53.3%) males versus 14 (46.7) females, with a male-to-female ratio of 1.1:1but there was no significant difference in the sex ratios of HCC patients, liver cirrhosis patients (p=0.14) Regarding HCC etiology, the results showed that the frequency of HBV positivity had no statistically significant difference between the studied patients. It was detected only in10.0% in HCC cases and in 13.3% in cirrhotic cases. HCV was present in 100.0% of HCC cases and 100.0%of cirrhotic cases with no statistically significant difference between the two groups. The severity of liver cirrhosis assessed by MELD, uMELD scores among the studied patients showed that both scores were significantly higher in HCC patients (p=0.000) (Table 1). Tumor imaging characteristics of HCC patients were illustrated in Table 2.

Table. 1: Demographic features and characteristics of the studied patient groups.

Characteristics	HCC patients (n=30)	Liver cirrhosis (n=30)	P-value
Age(years)			
Range	40-73	34-70	0.037*
Mean±SD	57.6±9.7	52.2±9.8	
Gender			
Male	21(70%)	16(53.3%)	0.14
Female	9(30%)	14(46.7%)	
Etiology			
Smoking	9(30%)	13(43.3%)	0.21
Alcohol	5(16.7%)	4(13.3%)	0.5
HCV	30(100%)	30(100%)	-
HBV	3(10%)	4(13.3%)	0.5
Severity of liver disease			
MELD score			
Range	10-25	6-21	0.000*
Mean±SD	15.3±4.1	10±4.1	
uMELD score			
Range	3.1-4.6	2.3-4.1	0.000*
Mean±SD	3.63±0.42	2.91±0.48	

*=Significant,SD=standard deviation,HCV=hepatitis C virus,HBV=hepatitis B virus, MELD=model of end stage liver disease,uMELD=updated MELD.

Table. 2: Tumour-related findings and characteristics of the HCC patient groups.

Characteristics	HCC patients (n=30)	Percentage(%)
Computed tomographic features		
Tumor size		
<3cm/3-5cm/>5cm	8/7/15	26.7/23.3/50
No. of nodules		
Single/2 or more	20/10	66.7/33.3
Site of tumor		
Right lobe/left lobe	11/19	36.7/63.3
Shape		
Rounded/oval	22/8	73.3/26.3
Portal vein invasion	3	10
Metastasis	1	3.3
Tumor staging		
Okuda stage		
I/II/III	4/20/6	13.3/66.7/20
Tokyo stage		
Early(0-4)	9	30
Advanced(5or more)	21	70
AFP(ng/ml)level		
Range	3-1060	
Mean	296.7	
Glypican-3(cycle threshold)		
Range	256-262144	
Mean	37725.87	

AFP=Alpha-fetoprotein.

Abdominal CT showed the dominant occurrence of HCC on top of cirrhosis (100%) and a higher percentage of portal vein thrombosis (PVT) (10%) and the higher incidence of the focal lesion to be single (66.7%), large (50%) and affecting the left lobe (63.3%). Metastasis was present in 3.3% of HCC cases.

Regarding Okuda, and Tokyo staging systems, most of HCC patients were relatively at advanced stage of the disease. As regards AFP levels, the data revealed that HCC patients had the highest mean value (296.7±213 ng/mL), with a significantly higher values (p=0.0001), than the mean of AFP values in both liver cirrhosis patients (39.6±33.4 ng/mL) and healthy control (5.8±2 ng/mL) groups. As regards GPC3 the data showed that HCC patients had the highest mean values (37725.87±54470.4cycle threshold), with a significantly higher values (p=0.0001), than the mean of GPC3 values in both liver cirrhosis patients (8194.1±14893.86cycle threshold) and healthy control (114±123.4 cycle threshold) groups. Figure 1.

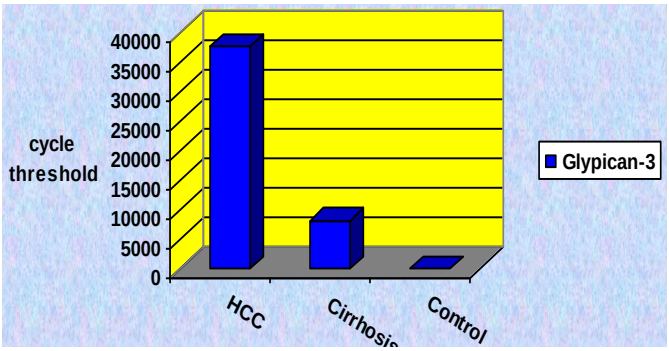

Fig. 1: Glypican-3 values among the studied groups.
As regards GPC3 the data showed that HCC patients had the highest mean values (37725.87±54470.4cycle threshold),with a significantly higher values (p=0.0001), than the mean of GPC3 values in both liver cirrhosis patients (8194.1±14893.86cycle threshold) and healthy control (114±123.4 cycle threshold) groups.

In the receiver operating curve (ROC), the area under curve (AUC) for AFP was 74.1% when we use 17 ng/mL as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 63.3% and a specificity of 60%. with PPV 100% and NPV 64.52% .

For GPC3, the area under curve (AUC) was 88.3% when we use 3072 cycle threshold as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 86.7% and a specificity of 80% with PPV100% and NPV 83.3% . (Fig. 2)& Table 3.

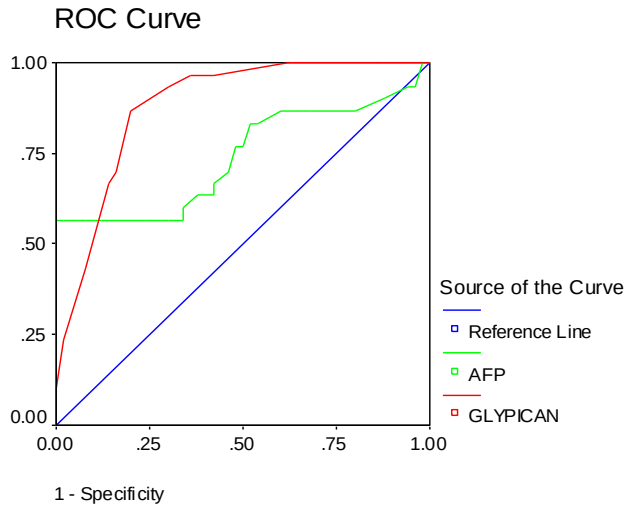

Fig. 2: In the receiver operating curve (ROC), the area under curve (AUC) for AFP was 74.1% when we use 17 ng/mL as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 63.3% and a specificity of 60%. with PPV 100% and NPV 64.52% . For GPC3, the area under curve (AUC) was 88.3% when we use 3072 cycle threshold as a cutoff point which gives the optimum balance between sensitivity, specificity the sensitivity was 86.7% and a specificity of 80%. with PPV100% and NPV 83.3%.

Table. 3: ROC curve analysis of AFP and Glypican-3 as markers for HCC.

Test	Cut off	Sensitivity %	Specificity %	PPV %	NPV %	AUC %
AFP (ng / ml)	17	63.3	60	100	64.52	74.1
Glypican – 3 (cycle threshold)	3072	86.7	80	100	83.3	88.3

PPV=positive predictive value, NPV=negative predictive value,AUC=area under curve.

In relation to prognosis, there was no significant correlation between AFP and the tumor number & size and Okuda& Tokyo staging among patients with HCC. There was significant positive correlation between GPC3 and the size (p=0.0015) and Okuda& Tokyo staging (p=0.047) among patients with HCC (Fig. 3&4).

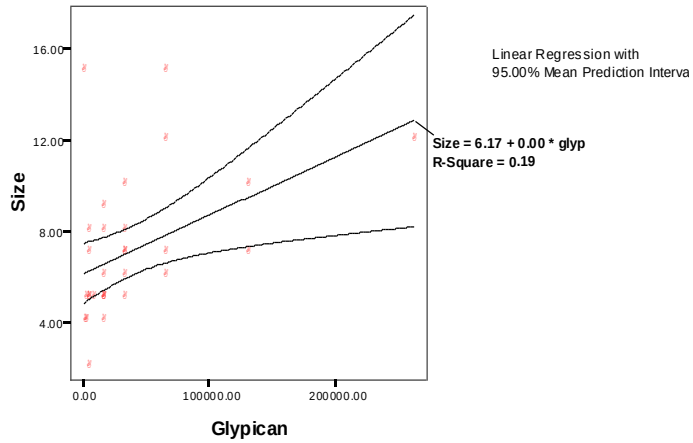

Fig. 3: Correlations of Glypican-3 values and tumour size among HCC cases. There was significant positive correlation between glypican and tumour size among patients with HCC. (P = 0.0015).

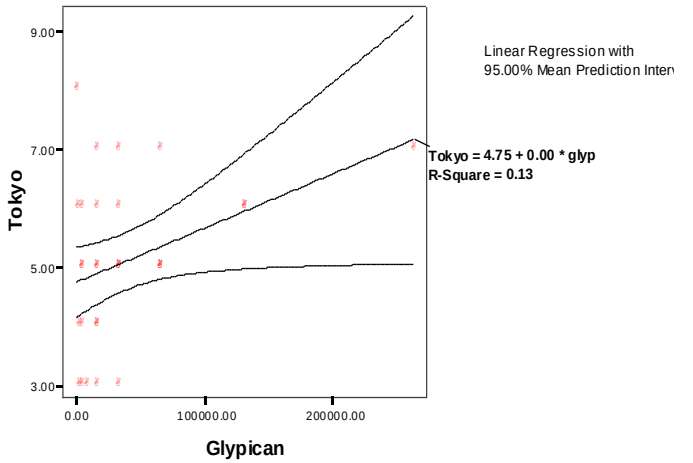

Fig. 4: Correlations of Glypican-3 values and Tokyo stage among HCC cases. There was significant positive correlation between glypican and Tokyo staging among patients with HCC.(P = 0.047).

DISCUSSION

AFP is the most established tumour marker in HCC and the gold standard by which other markers for the disaease are judged (Lopez, 2005).Concerning AFP mean value,HCC cases had a mean value of (297.6 ng/ml) which was higher than that of patients with cirrhosis (39.6 ng/ml) and control (5.9 ng/dl) subjects this results agree with Atta *et al.,* 2008 who reported a higher mean values for HCC cases. ROC analysis of AFP used as a diagnostic test suggests that a value of about 17 ng/ml provides the optimal balance between sensitivity and specificity.

However at this level the sensitivity is only 63.3% and the specificity is 60% Bruix and Sherman (2005), Atta *et al.,* (2008) reported exactly the same results. Concerning AFP,it was obvious from this study that there was no significant correlation between AFP and parameters of severity of liver disease as documented by Hou *et al.,* (2004) and Atta *et al.,* (2008). Also no significant correlation between AFP and tumour size ,number or Okuda and Tokyo staging and this goes in agreement with Ryder, 2003 and Qin and Tang, 2002 respectively.

Regarding AFP as a marker for HCC ,our results supported by Huo *et al.,* (2004) who concluded that serum AFP is a weak prognostic predictor in HCC patients and Guan *et al.,* (2006) stated that the value of AFP is limited in the diagnosis and prognosis of HCC. Concerning Glypican-3 mean value,HCC cases had a mean value of (37725.87 ct) which was higher than that of patients with cirrhosis (8194.1 ct) and control (114 ct) subjects this results agree with El-Shenawy *et al.,* 2012 who reported a higher mean values for HCC cases. ROC analysis of Glypican-3 used as a diagnostic test suggests that a value of 3072 ct provides the optimal balance between sensitivity and specificity. At this level the sensitivity is 86.7% and specificity is 80% ,a lower values were reported by El-Shenawy *et al.,* 2012 who reported a sensitivity of only 63.5% and a specificity of 70%.

There was no significant correlation between Glypican-3 and severity of liver disease, this was in agreement with El-Shenawy *et al.,* 2012.

There was significant correlation between Glypican-3 and tumour size and Tokyo staging and this goes in agreement with El-Shenawy *et al.,*2012 who reported a significant correlation between Glypican-3 and tumour size and CLIP staging. Regarding Glypican-3 as a marker for HCC, our results supported by Liu *et al.,* 2010 who concluded that Glypican-3 is a sensitive and specific marker for HCC diagnosis, also Yao *et al.,* 2011 reported that Glypican-3 may be promising marker for diagnosis of HCC. Zou *et al.,* 2010 concluded that Glypican-3 is a diagnostic and prognostic marker and expected to be an ideal target for the therapy of HCC.

CONCLUSION

Glypican-3 could be a sensitive, specific and accurate serum marker for diagnosing HCC.

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CONFLICT OF INTEREST

The author declare that she have no competing interests or conflict of interest.

REFERENCES

Atta M.M., El- Masry S.A., Abdel -Hameed M., Baiomy H.A., and Ramadan N.E. Value of serum anti-p53 antibodies as a prognostic factor in Egyptian patients with hepatocellular carcinoma. Clinical Biochemsitry. 2008; 41: 1131-1139.

Bruix J., and Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42(5): 1208-36.

El-Shenawy S.Z., El. Sabawi M.M., Sheble N., Abd El-Raof M., Allam M.M., and Fath Allah S.K. Diagnostic Role of Serum Glypican -3 as a tumor Marker for Hepatocellular Carcinoma. Nature and Science. 2012; 10(4): 32-38.

El-Zayadi A.R., Badran H.M, Barakat E.M., Attia M.E., Shawky S., Mohamed M.K., Selim O., and Saeid A . Hepatocellular carcinoma in Egypt: a single center study over a decade. World J. Gastroenterol. 2005; 11(33): 5193-5198.

Grieco A., Pompili M., Caminiti G., Miele L ., Covino M ., Alfei B., Rapaccini, G.L., and Gasbarrini G. Prognostic factors for survival in patients with early intermediate hepatocellular carcinoma undergoing non- surgical therapy: comparison of Okuda, CLIP, and BCLC staging systems in a single Italian center. Gut. 2005; 54:411-418.

Guan Y-S., He Q., and La Z. Roles of p53 in carcinogenesis, Diagnosis and hepatocellular carcinoma: an analysis of 403 patients. Gut. 2005; 54: 419-425.

Huo T-L., Huang Y-H., Lui W-Y., Wu J-C., Lee P-C., Chang F-Y., and Lee S-D. Selective prognostic impact of serum alpha-fetoprotein level in Patients with hepatocellular carcinoma: analysis of 543 patients in single center .Oncology Reports. 2004;11: 543-550.

Jia H.L , Ye Q.H., Qin L.X., Budhu A., Forgues M., Chen Y., Liu Y.K., Sun H.C., Wang L., Lu H.Z., Shen F., Tang Z.Y., and Wang X.W. Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma. Clin .Cancer Res. 2007; 13(4): 1133-9.

Kamath P.S., Wiesner R.H., Malinchoc M., Kremers W., Therneau T.M., Kosberg C.L., D'Amico G., Dickson E.R., and Kim W.R. A model to predict survival in patients with end-stage liver disease. Hepatology. 2001; 33: 464-470.

Kew M.C. 2002. Hepatic tumours and cysts. in: Gatrointestinal and liver Disease: phathophysiology, diagnosis, management. Feldman M., Friedman L.S.,and Sleisenger M.H. (eds), 7th Editon, Saunders, Philadelphia, London, New York, St. Louis, Sydney. 1577-1602.

Liovet J.M., Fuster J., and Bruix J.The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. Liver Transplantation. 2004; 10(2): supp11: S115-S120.

Liu H., Li P., Zhai Y., Qu C-F., Zhang L-J., Tan Y-F., Li N., and Ding H-G. Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. World Gastroenterol.2010; 16(35): 4410-4415.

Lopez J.B. Recent development in the first detection of hepatocellular carcinoma.Clin. Biochem. Rev. 2005; 26: 65-79.

Michielsen P. P., Francque, S. M., and Dongen, J.L. Viral hepatitis and hepatocellular carcinoma.World Journal of Surgical Oncology. 2005; 3: 27.

Okochi O., Hibi K., Uemura T., Inoue S., Takeda S., Kaneko T., and Nakao A.Detection of mitochondrial DNA alterations in the serum of hepatocellular carcinoma patients. Clinical Cancer Research.2002; 8: 2875 – 2878.

Okuda K., Ohtsuki T., Obata H., Tomimatsu M., Okazaki N., Hasegawa H., Nakajima Y., and Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment study of 850 patients. Cancer. 1985;56(4): 918-28.

Qin L-X., and Tang Z-Y. The prognostic significance of clinical and pathological Features of hepatocellular carcinoma. World J. Gastroenerol. 2002;8(2): 193-199.

Ryder S.D. Guidelins for the diagnosis and treatment of hepatocellular carcinoma. (HCC) in adults. Gut. 2003; 52 (suppl III): 1-8.

Sharma P., Shaubel D.E., Sima C.S., Merino R.M., and Lok A.S. Re-weighting the model for end-stage liver disease score components. Gastroenterology. 2008; 135: 1575-1581.

Sherlock S., and Dooley J. 2002. Diseases of the liver and biliary system. 11th ed., Blackwell S.C., Oxford London, Eninburgh. 537 – 561.

Soliman A.S., Hung C-W., Tsodikov A., Seifeldin I.A., Ramadan M., Al-Gamal D., Schiefelbein E.L., Thummalapally P., Dey S., and Ismail K. Epidemiologic risk factors of hepatocellular carcinoma in a rural region of Egypt. Hepatol. Int .2010; 4:681–690.

Tateishi R., Yoshida H., Shiina S., Imamura H., Hasegawa K., Teratani T., Obi S., Sato S., KoikeY., Fujishima T., Makuuchi M ., and Omata M. Proposal of a new prognostic model for treatment of hepatocellular carcinoma. Journal of Cancer Molecules, 2006; 2(5): 191-197.

Vauthey B.J., Lauwers G.Y., Esnaola N.F., Do K-A., Belghiti J., Mirza N., Curley S.A., Ellis L.M., Regimbeau J-M., Rashid A., Cleary K.R., and Nagorney D.M. Simplified staging for hepatocellular carcinoma. Journal of Clinical Oncology. 2002; 20(b): 1527-1536.

Yao M., Yao D-F., Bian Y-Z., Zhang C-G., Qui L-W., Wu W., Sai W-L., Yang J-L., and Zhang H-J. Oncofetal antigen glypican -3 as apromising early diagnostic marker for hepatocellular carcinoma. Hepatobiliary pancreat .Dis. Int .2011; 10: 289-294.

Zou Z-Q., Ding Y-P., Long B., Yu J-G., Xu A-L., Lang Z-W., Zou S-Y., Liu Y-D., Ding K., and Li Y-Y. Gpc-3 is a Notable diagnostic, prognostic and a latent targeted therapy marker in hepatocellular carcinoma. Hepato-Gastroenterology. 2010 ; 57: 1285-1290.

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