Gene Cell Tissue. 2015 October; 2(4): e33012. doi: 10.17795/gct-33012

Published online 2015 October 28. **Editorial**

Application of Tumor Biomarkers as Screening Tools in Early Detection of Hepatocellular Carcinoma

Zahra Heidari[1,2] and Bita Moudi[1,2,*]

[1]Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran
[2]Department of Histology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, IR Iran

*Corresponding author: Bita Moudi, Department of Histology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, IR Iran. Tel: +98-5433295794, Fax: +98-5433295794, E-mail: bita.moudi@yahoo.com

Received 2015 September 12; **Accepted** 2015 September 23.

Keywords: Hepatocellular Carcinoma, Diagnostic Markers, Liver

Hepatocellular carcinoma (HCC) is an aggressive tumor that arises in subjects with chronic hepatitis B and C infection. Frequently, HCC is a primary solid tumor of the liver. It is a disseminative cancer that leads to poor prognosis and causes approximately 5% of all malignant lesions (1). The prevalence of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection is rising throughout the world, especially in Asia and Africa, and it's consequence is an increase in the incidence of HCC (2, 3). Therefore, the number of patients that are at risk of developing HCC is constantly rising.

Incidence rates of HCC are higher in males than females (2-4:1), and also there is a higher incidence in African-Americans and Asians than Caucasians. It usually occurs between 30 to 50 years of age.

Liver cirrhosis is the main risk factor of HCC. In South Asia, HBV is the main cause of HCC and may develop among young patients. Some of these patients have cirrhosis and a long period of infection is the main determinant of HCC in these people (4).

Hepatocellular Carcinoma prevalence is high in the Far East and Africa (> 20 cases/100,000 inhabitants/year). In Iran, its prevalence is considered to be in the medium range (5 to 20 cases/100,000 inhabitants/year). The actual incidence of HCC in Iran is unknown. Since HBV infection is known as a probable cause of HCC in at least 80% of cases worldwide and about 5% of the world's population (350 million people) is chronically infected with HBV, it seems that death rate from HCC in HBV patients has increased in the recent years in Iran. It has been estimated that about 1.5 million people in Iran are infected with hepatitis B, of which more than 40% are at risk of cirrhosis and HCC (5, 6).

Up to now, prognosis of HCC has remained poor, because of late detection in patients who have tumors with poor prognostic features. Large size, multiplicity and vascular invasion are good signs for early detection of HCC yet these modes do not always occur. Therefore, sometimes it is difficult to identify patients with HCC at early stages (4).

Nowadays, because of increased cancer mortality rate, lack of screening programs, lack of early detection, increased incidence of HCC and the low survival rate of patients with the diagnosis of HCC at advanced levels in Iran and thought the world, introduction of new methods for early detection and diagnosis are essential (4).

In the recent years, screening of patients with HCC is done by different methods. Assessment of Alpha-Fetoprotein (AFP), imaging with ultrasonography, spiral computerized tomography (CT) of the liver and magnetic resonance imaging (MRI) with contrast enhancement and biopsy as the most powerful detection method, are the best prognosis methods for HCC (4, 7).

There is no agreement on application of these diagnostic procedures for patients with HCC worldwide, and several proposals have been published. These are three major methods, which compete as first-line detective options for large multiple nodules in patients with no preserved liver function. In this regard, research on tumor tissues can provide biological information about tumors; thus, the search for tumor biomarkers is crucial. Hepatocellular carcinoma tumor cell-derived biomarkers have been identified in the recent decades. On the other hand, genomic, proteomic and metabolomics as scientific studies of chemical processes involving metabolites, have enabled the identification of novel biomarkers for HCC. Furthermore, HCC biomarkers are mainly used as screening tools in surveillance programs. The most important goal of these programs is reduction of HCC mortality. It should be noted that multiple marker-based approaches are more useful than a single marker-based approach because the biology of HCC is very complex. In addition, the association between host, viral and environmental factors could affect the natural history of HCC. Biomark-

ers are sensitive and specific tools at mRNA and protein levels that are being used for the detection and diagnosis of diseases.

Hepatocellular carcinoma is a multistage process of tumor progression. Histopathological studies have indicated the multistep development of HCC (8-10). Atypical adenomatous hyperplasia (AAH) and adenomatous hyperplasia (AH) are precancerous lesions that appear in chronically diseased livers. Clinical findings have revealed that AH and AAH modes can develop into early HCC. This mode is associated with microinvasive carcinoma that develops into progressive HCC (7, 11).

Early HCC is a hypercellular nodular lesion with structural atypia including the remodeling of the cord structure, formation of acini and thin trabeculae. A lot of portal tracts are present within the nodules and diffuse fatty change is seen in the tumor. Tumor cells can attack portal tracts. There is no capsule formation between tumor and nontumoral cells. These features correspond to microinvasive carcinoma of the liver that changes to early HCC (12).

Distinction of normal tissue from malignant lesions is still difficult yet immunohistochemical markers and molecular techniques can better address the diagnosis of early HCC. In this article, we focused on some candidate molecular markers that could potentially be appropriate for early diagnosis of HCC.

1. Embryonic Antigen

1.1. Alpha-Fetoprotein (AFP)

AFP, the most available tumor biomarker, is currently used for early detection of HCC. Serum AFP had a sensitivity of 41% - 65% and specificity of 80% - 94% (13). Alpha-Fetoprotein has a positive rate ranging from 60% to 80%. Furthermore, AFP is positive during pregnancy, embryonic tumors and some gastrointestinal tumors. It is a major biomarker in benign liver diseases and exists in the serum and liver of patients with HCC. When total AFP is 10 - 200 ng/mL, the diagnostic specificity for HCC reaches 100%. Moreover, AFP does not correlate with other biomarkers, thus it can be used as an independent factor for the early diagnosis of HCC (14-16).

2. Proteantigen

2.1. Glypican-3 (GPC3)

GPC3 is a potential marker for HCC. It links to the cell membrane by a glycosylphosphatidylinositol anchor. It is a heparan sulfate proteoglycans that is involved in regulating cell growth. Furthermore, GPC3 can remove tumorigenic growth factors (such as hepatocyte growth factor and vascular endothelial growth factor) from the cell surface and inhibit the growth of HCC (17, 18). There is no correlation between GPC3 expression and AFP level, tumor size and stage (19).

2.2. Heat Shock Protein 70 (HSP70)

HSP70 is a potential marker for HCC. It is expressed when someone is exposed to carcinogens. It is a conserved stress response protein and can promote cells to repair damages. Immunohistochemical staining, showed that the positive rate of HSP70 was 56.3 in HCC (20). Its stain intensity was associated with tumor size and stage. The sensitivity and specificity of HSP70 in detecting HCC was 57.5 and 85%, respectively (21, 22).

3. Cytokines

3.1. Transforming Growth Factor- β 1 (TGF- β 1)

TGF- β 1 is a growth factor involved in the regulation of cell proliferation and immune function. It is expressed in tumor cells. It can inhibit the proliferation of Cytotoxic T Lymphocytes (CTL) and promote the growth of tumor cells. Furthermore, TGF- β 1 may be used as an indicator to diagnose HCC related to HBV with sensitivity and specificity of 89.5 and 94.0%, respectively (23-25).

3.2. Vascular endothelial growth factor (VEGF)

VEGF has a vital role in tumor angiogenesis. It can induce new vessel formation and promote tumor metastasis. The level of VEGF is higher in HCC patients than healthy individuals. It has been revealed that the expression of VEGF is correlated with tumor prognosis and recurrence. It seems that overexpression of VEGF is a useful biological marker of tumors (26, 27).

4. Genetic Biomarkers

4.1. Alpha-Fetoprotein mRNA

This is a marker for spreading of HCC in the blood in active HCC cells. It is a predictor for HCC recurrence and has a positive rate of 82.4% in recrudescent patients (14).

4.2. MicroRNAs

These are non-coding RNAs that block translation by inducing the degradation of target mRNAs. MiR-500 is a new biomarker for HCC. It could downregulate liver development and then upregulate cirrhosis (28). Thus, MiR-500 is a promising biomarker of HCC.

5. Enzymes and Isozymes

5.1. Des-γ-Carboxyprothrombin (DCP)

DCP is induced by the absence of vitamin K. Vitamin K-dependent carboxylation system fails and causes the production of DCP in malignant liver cells. Its level is associated with a larger tumor. It is an accurate tumor marker compared with AFP (29, 30).

5.2. Gamma-Glutamyl Transferase (GGT)

This enzyme is secreted by endothelial cells of the bile duct and hepatic Kupffer cells. Its activity increases in HCC tumors. In addition, cholestasis and inflammation can improve the level of GGT. Gamma-Glutamyl Transferase mRNA is widely distributed in liver tissues of HCC patients. Therefore, GGT can be a biomarker for diagnosis of HCC.

5.3. Glutamine Synthetase (GS)

GS induces the synthesis of glutamine. Glutamine is an important energy source for tumor cells. The level of GS increases in patients with precancerous lesions, which can change to advanced-HCC. It has been reported that GS is a new target in development of HCC (specificity 89%; sensitivity 100%) (31, 32).

6. New Discoveries

6.1. Hepatocyte Paraffin 1 (HepPar 1)

This antigen can differentiate between normal and malignant hepatocytes. It is expressed in normal human liver cells. Decreasing expression of HepPar 1 is seen in HCC. It seems that reduction of HepPar 1-positive cells is associated with HCC (33). Therefore, HepPar 1 can be considered as a new valuable marker for the diagnosis of HCC.

7. Multi-Marker Panel

The use of panels with multiple biomarkers increases the accuracy of the diagnosis. Findings have indicated that concomitant use of GS, GPC3 and HSP70 has sensitivity and specificity of 70% and 100%, respectively in HCC diagnosis. This panel has an accuracy of 57% and specificity of 100% in grade 1 HCC patients (34, 35). In addition, a four-marker panel has been introduced, which has a diagnostic accuracy of 84.3% in small HCC tumors (36). It seems that multi-marker panels need to be further investigated to achieve valuable detective methods.

As mentioned above, there are some biomarkers for HCC detection. Hepatocellular Carcinoma can be diagnosed by analysis of the expression of specific genes by quantitative methods such as Reverse transcription polymerase chain reaction (qRT-PCR). In this regard, different expression levels of genes have been associated with protein levels, which are related to HCC development. In addition, immunohistochemical techniques are useful in HCC diagnosis. Hepatocellular Carcinoma is able to synthesize tumor-related proteins. Therefore, biomarker-related researches are needed.

Regarding the relationship between biomarkers and HCC, selecting useful biomarkers in HCC detection is essential. Despite limitations, it seems that AFP is the most important tumor marker for HCC. The results indicated that AFP and DCP were better than AFP alone in detecting early HCC. In addition, some biomarkers including GPC3, GS, HepPar 1 and HSP70 could be supplementary to AFP in HCC detection. In addition, in some HCC patients, DCP or AFP may be negative while other biomarkers may be positive; therefore, multi-marker panels can modify the precision of the diagnosis.

Considering the importance of this lethal cancer in the world and considering the mortality rate of HCC, it is essential to introduce better methods for diagnosis and prognosis of HCC patients. Medical researches have primarily affected public health and have an important role in all human activities. One of the most important aspects of medicine is proper interpretation of data. However, medical decision-making is difficult because processing a lot of data is a difficult task. In this regard, diagnosis is very important. Error at this level can have considerable consequences. One of the main problems related to HCC patients, is the lack of proper diagnosis of disease. As a result of wrong diagnosis or no diagnosis at an early stage, a person may even develop complications that lead to death. Rapid and correct diagnosis of HCC should be determined based on a predetermined pattern. In this regard, molecular biology-related researches in regards to HCC could reveal valuable information about the natural history of HCC including metastasis and recurrence. In the recent years, specific biomarkers have been reported that have diagnostic values. However, some of them can't diagnose the disease at early stages. Thus, studies should be focused on prognostic values of such biomarkers. These biomarkers not only lead to the prediction of HCC in patients but also provide useful information for selection of appropriate treatment, leading to increased survival of patients.

Acknowledgments

The authors of this article appreciate the vice chancellor of research and technology of Zahedan university of medical sciences for assistance and facilities provided for this project.

Footnote

Authors' Contributions: Both authors conducted the entire research.

References

1. Bosch FX, Ribes J, Borras J. Epidemiology of primary liver cancer. *Semin Liver Dis.* 1999;**19**(3):271–85. doi: 10.1055/s-2007-1007117. [PubMed: 10518307]
2. Njei B, Rotman Y, Ditah I, Lim JK. Emerging trends in hepatocellular carcinoma incidence and mortality. *Hepatology.* 2015;**61**(1):191–9. doi: 10.1002/hep.27388. [PubMed: 25142309]
3. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? *Hepatology.* 2014;**60**(5):1767–75. doi: 10.1002/hep.27222. [PubMed: 24839253]
4. Franca AV, Elias Junior J, Lima BL, Martinelli AL, Carrilho FJ. Diagnosis, staging and treatment of hepatocellular carcinoma. *Braz J Med Biol Res.* 2004;**37**(11):1689–705. [PubMed: 15517086]

5. Merat S, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. *Arch Iran Med.* 2000;**3**(4):192–201.
6. Shamszad M, Farzadegan H. Hepatitis B related cirrhosis and hepatocellular carcinoma in Iran. *J Irn Med Council.* 1982;**8**:238.
7. Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, et al. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. *Hepatology.* 1998;**28**(5):1241–6. doi: 10.1002/hep.510280511. [PubMed: 9794907]
8. Takayama T, Kosuge T, Yamazaki S, Hasegawa H, Okazaki N, Takayasu K, et al. Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma. *Lancet.* 1990;**336**(8724):1150–3. doi: 10.1016/0140-6736(90)92768-d. [PubMed: 1978027]
9. Tsuda H, Hirohashi S, Shimosato Y, Terada M, Hasegawa H. Clonal origin of atypical adenomatous hyperplasia of the liver and clonal identity with hepatocellular carcinoma. *Gastroenterology.* 1988;**95**(6):1664–6. [PubMed: 2846405]
10. Arakawa M, Kage M, Sugihara S, Nakashima T, Suenaga M, Okuda K. Emergence of malignant lesions within an adenomatous hyperplastic nodule in a cirrhotic liver. Observations in five cases. *Gastroenterology.* 1986;**91**(1):198–208. [PubMed: 3710069]
11. Sakamoto M, Hirohashi S. Natural history and prognosis of adenomatous hyperplasia and early hepatocellular carcinoma: multi-institutional analysis of 53 nodules followed up for more than 6 months and 141 patients with single early hepatocellular carcinoma treated by surgical resection or percutaneous ethanol injection. *Jpn J Clin Oncol.* 1998;**28**(10):604–8. [PubMed: 9839500]
12. Hamilton SR, Aaltonen LA, International Agency for Research on Cancer, World Health Organization. *Pathology and genetics of tumours of the digestive system.*Lyon: IARC press; 2000.
13. Debruyne EN, Delanghe JR. Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. *Clin Chim Acta.* 2008;**395**(1-2):19–26. doi: 10.1016/j.cca.2008.05.010. [PubMed: 18538135]
14. Singhal A, Jayaraman M, Dhanasekaran DN, Kohli V. Molecular and serum markers in hepatocellular carcinoma: predictive tools for prognosis and recurrence. *Crit Rev Oncol Hematol.* 2012;**82**(2):116–40. doi: 10.1016/j.critrevonc.2011.05.005. [PubMed: 21680198]
15. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, et al. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol Hepatol.* 2007;**5**(3):394–402. doi: 10.1016/j.cgh.2006.12.005. [PubMed: 17368240]
16. Kobayashi M, Hosaka T, Ikeda K, Seko Y, Kawamura Y, Sezaki H, et al. Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre- and postoperatively. *Hepatol Res.* 2011;**41**(11):1036–45. doi: 10.1111/j.1872-034X.2011.00858.x. [PubMed: 21883741]
17. Zittermann SI, Capurro MI, Shi W, Filmus J. Soluble glypican 3 inhibits the growth of hepatocellular carcinoma in vitro and in vivo. *Int J Cancer.* 2010;**126**(6):1291–301. doi: 10.1002/ijc.24941. [PubMed: 19816934]
18. Capurro MI, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res.* 2005;**65**(14):6245–54. doi: 10.1158/0008-5472.CAN-04-4244. [PubMed: 16024626]
19. Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. *Int J Oncol.* 2009;**34**(3):649–56. [PubMed: 19212669]
20. Joo M, Chi JG, Lee H. Expressions of HSP70 and HSP27 in hepatocellular carcinoma. *J Korean Med Sci.* 2005;**20**(5):829–34. [PubMed: 16224158]
21. Tremosini S, Forner A, Boix L, Vilana R, Bianchi L, Reig M, et al. Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. *Gut.* 2012;**61**(10):1481–7. doi: 10.1136/gutjnl-2011-301862. [PubMed: 22287594]
22. Luk JM, Lam CT, Siu AF, Lam BY, Ng IO, Hu MY, et al. Proteomic profiling of hepatocellular carcinoma in Chinese cohort reveals heat-shock proteins (Hsp27, Hsp70, GRP78) up-regulation and their associated prognostic values. *Proteomics.* 2006;**6**(3):1049–57. doi: 10.1002/pmic.200500306. [PubMed: 16400691]
23. Zhou L, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. *World J Gastroenterol.* 2006;**12**(8):1175–81. [PubMed: 16534867]
24. Balzarini P, Benetti A, Invernici G, Cristini S, Zicari S, Caruso A, et al. Transforming growth factor-beta1 induces microvascular abnormalities through a down-modulation of neural cell adhesion molecule in human hepatocellular carcinoma. *Lab Invest.* 2012;**92**(9):1297–309. doi: 10.1038/labinvest.2012.94. [PubMed: 22732936]
25. Dong ZZ, Yao DF, Yao M, Qiu LW, Zong L, Wu W, et al. Clinical impact of plasma TGF-beta1 and circulating TGF-beta1 mRNA in diagnosis of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int.* 2008;**7**(3):288–95. [PubMed: 18522884]
26. Xiang ZL, Zeng ZC, Fan J, Tang ZY, Zeng HY, Gao DM. Gene expression profiling of fixed tissues identified hypoxia-inducible factor-1alpha, VEGF, and matrix metalloproteinase-2 as biomarkers of lymph node metastasis in hepatocellular carcinoma. *Clin Cancer Res.* 2011;**17**(16):5463–72. doi: 10.1158/1078-0432.CCR-10-3096. [PubMed: 21712445]
27. Zhang L, Wang JN, Tang JM, Kong X, Yang JY, Zheng F, et al. VEGF is essential for the growth and migration of human hepatocellular carcinoma cells. *Mol Biol Rep.* 2012;**39**(5):5085–93. doi: 10.1007/s11033-011-1304-2. [PubMed: 22161247]
28. Yamamoto Y, Kosaka N, Tanaka M, Koizumi F, Kanai Y, Mizutani T, et al. MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. *Biomarkers.* 2009;**14**(7):529–38. doi: 10.3109/13547500903150771. [PubMed: 19863192]
29. Yamamoto K, Imamura H, Matsuyama Y, Kume Y, Ikeda H, Norman GL, et al. AFP, AFP-L3, DCP, and GP73 as markers for monitoring treatment response and recurrence and as surrogate markers of clinicopathological variables of HCC. *J Gastroenterol.* 2010;**45**(12):1272–82. doi: 10.1007/s00535-010-0278-5. [PubMed: 20625772]
30. Naraki T, Kohno N, Saito H, Fujimoto Y, Ohhira M, Morita T, et al. γ-Carboxyglutamic acid content of hepatocellular carcinoma-associated des-γ-carboxy prothrombin. *Biochimica et Biophysica Acta .* 2002;**1586**(3):287–98. doi: 10.1016/s0925-4439(01)00107-7. [PubMed: 11997080]
31. Osada T, Sakamoto M, Nagawa H, Yamamoto J, Matsuno Y, Iwamatsu A, et al. Acquisition of glutamine synthetase expression in human hepatocarcinogenesis. *Cancer.* 1999;**85**(4):819–31. doi: 10.1002/(sici)1097-0142(19990215)85:4<819::aid-cncr9>3.0.co;2-e. [PubMed: 10091759]
32. Cadoret A, Ovejero C, Terris B, Souil E, Levy L, Lamers WH, et al. New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. *Oncogene.* 2002;**21**(54):8293–301. doi: 10.1038/sj.onc.1206118. [PubMed: 12447692]
33. Minervini MI, Demetris AJ, Lee RG, Carr BI, Madariaga J, Nalesnik MA. Utilization of hepatocyte-specific antibody in the immunocytochemical evaluation of liver tumors. *Mod Pathol.* 1997;**10**(7):686–92. [PubMed: 9237179]
34. Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E, et al. Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology.* 2007;**45**(3):725–34. doi: 10.1002/hep.21531. [PubMed: 17326147]
35. Di Tommaso L, Destro A, Seok JY, Balladore E, Terracciano L, Sangiovanni A, et al. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. *J Hepatol.* 2009;**50**(4):746–54. doi: 10.1016/j.jhep.2008.11.014. [PubMed: 19231003]
36. Di Tommaso L, Destro A, Fabbris V, Spagnuolo G, Laura Fracanzani A, Fargion S, et al. Diagnostic accuracy of clathrin heavy chain staining in a marker panel for the diagnosis of small hepatocellular carcinoma. *Hepatology.* 2011;**53**(5):1549–57. doi: 10.1002/hep.24218. [PubMed: 21520170]