Review Article

Diagnostic biomarkers in hepatocellular carcinoma

Shumaila Naz*, Safina Iqbal and Madiha Rashid

Department of Biosciences, University of Wah, Wah Cantt-Pakistan
Corresponding author's email: dr.shumyla.naz@outlook.com

Abstract

Being the second most lethal type of cancer, Hepatocellular carcinoma (HCC) annually kills 650,000 individuals worldwide, approximately, prompting communal malignant tumor that accounts for more than 90% of liver cancer. We aimed to clarify the functioning of different diagnostic markers of incidence and progression of hepatocellular carcinoma. Biomarkers are molecular indicators that are very imperative for the management of different diseases. Different biomarkers of HCC, such as serum and tumor biomarkers have been diagnosed and their presence in urine, tissues and serum indicates the development of the tumor. The diagnoses at early stages helps in the prevention of HCC. In the case of HCC, more than 70% of patients showed an increased level of alpha-fetoprotein (AFP) due to excretion of the tumor and thus the level of AFP in the body is one of the most important biomarkers of HCC. Another important biomarker is AFP-L3 is considered as more precise in the diagnosis of HCC than AFP. Percentages of AFP-L3 are measured with high sensitivity and hence known as the most important early predictor at low AFP levels. One of the significant biomarkers is DCP (Des-gamma-carboxy-prothrombin) that is relatively more accurate than the other two diagnostic pointers. While differentiating malignant HCC with non- malignant liver disease, it was clear that the diagnostic value of DCP is better than AFP. DCP is a great deal more effective than AFP in diagnosing HCC on the comparatively lower level. When compared these diagnostic carcinoma biomarkers, showed the potency in a descending order from DCP>AFP> AFP-L3.

Keywords: AFP; AFP-L3; Des-gamma-carboxy prothrombin; Hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) elucidates for more than 85-90% of primary cancer of liver and causes many deaths worldwide [1]. Among the type of cancers, it is at number six in the world [2]. It kills annually more than 650,000 people in the world [3]. HCC was known as the second most lethal cancer [4]. In early stages liver cancer shows no symptoms, after its adequate progression clinical symptoms are noticeable. Earlier studies predict that analysis of HCC at the initial stage and its efficient treatment enhance the lifetime of patients having liver cancer. 60-80% patients of HCC have a condition called cirrhosis [5]. Patients of HCC which have liver cirrhosis, lead to chronic liver disease. In the case of liver cirrhosis there is a decrease in proliferation of hepatocytes that reduce the liver regenerative capacity and leads to fibrous tissues and damage to liver cells which then causes cancerous nodules [6].

Myofibroblasts are the unique population of smooth muscle-like fibroblasts. These cells play a role in the secretion of growth factors, wound healing, matrix deposition and degradation. That's why myofibroblasts help in both human physiology and pathology. Fibroblasts of cancer tissues are similar to myofibroblasts [7]. In the wound healing process cancer-associated fibroblasts (CAFs) are active just like normal fibroblasts and thus promotes tumor growth by secreting cytokines and growth factors [8]. Chronic HBV and HCV cause hepatic fibrosis and results in the development of HCC. Worldwide, HCC is mainly caused by chronic hepatitis B while its primary cause is hepatitis C. Individuals that are suffering from hepatitis B can develop HCC without any cause of cirrhosis [9].

For HCC finding, various types of innovative techniques like imaging apparatuses are accessible for clinical staff to utilize. These advances involve Digital or computerized tomography and resonance imaging and use of tumor biomarkers, similar to alpha-fetoprotein (AFP), Golgi protein 73, and Vit-K absence or antagonistic-II. As of now in clinical practice, serum AFP levels are for the most part utilized by numerous specialists for diagnosing HCC. Even though that, serum AFP levels are additionally higher in generous liver diseases, similar to liver cirrhosis and hepatitis, and in up to 40% of patients with HCC levels of AFP can be typical. That is the reason, the poor explicitness of AFP is a worry for its application in the finding of disease. [10-12]. To cure HCC, surgical resection and liver transplantation is the most efficient way but, in most cases, surgery is not adapted because of intrahepatic and distant metastasis during diagnosis and these are suitable only for small tumors [13].

HCC-diagnostic biomarkers
Biomarkers in serum
Alpha-fetoprotein (AFP)

The fetal liver produces a specific glycoprotein known as alpha-fetoprotein. After birth concentration of serum falls rapidly and in adulthood its synthesis is repressed. More than 70% of patients of HCC have a higher concentration of AFP in the blood due to the excretion of the tumor. AFP in serum is an important tumor marker in diagnosing HCC patients. The Level of AFP in serum higher than 400ng/ml is measured as diagnostic however such higher percentages are observed in few patients of HCC [14, 15]. Patients of HCC that have higher serum concentrations of AFP (>400ng/ml) have larger tumor sizes, thrombosis in the portal vein, bi-lobar involvement and have a lower survival rate [16]. Ultrasonography and serum AFP level are two important determinants for HCC diagnosis at an early level [17]. Although the determination of serum AFP, impart a chief role in the diagnosis of HCC patients but some reports designated that AFP does not differentiate HCC from benign chronic liver diseases because of its more false-negative and false-positive counts [18]. AFP is secreted by almost 50% of HCC [19].

AFP level in serum, resulting in two main problems;

First, gradually increased level of AFP in serum in case of chronic liver disease like hepatitis (>100 ng/ml), chronic hepatitis, acute hepatitis and cirrhosis. Secondly, in up to 40% of patients showed normal levels of these biomarkers mostly during early stages. Thus, it results in false-negative and positive values [18].

AFP in tumor tissues is available in bound state, conjugated to different proteins and furthermore released in the circulatory framework. In vitro and in vivo frameworks created to see the take-up of AFP by tumor cells have demonstrated positive outcomes. Tumors of the muscle tissues show endocytosis of AFP corresponding to the fetal and neonatal muscle tissues. As tumor

cells like fetal endodermal, ectodermal and mesodermal cells are yet not separated, so it is presumed that AFP take-up happens just in undifferentiated cells and neoplastic cells. Followed by take-up, its essence has been pointed by radio-imaging in cell film invaginations and golgi bodies arrange that encompasses the core [19].

Lens Culinaris agglutinin-(LCA)

According to bind capacity to lens Culinaris agglutinin (LCA), there are three distinct sorts of markers that change just in sugar chains (AFP-L1, AFP-L2, AFP-L3). In serum of non-threatening incessant liver illness patients, the significant sort of AFP is AFPL1 (not bound to LCA part). Rather than that, the most important type of AFP in HCC patient's serum is Lens Culinaris-reactive AFP, also called AFP-L3. AFP-L3 is a fucosylated specie of AFP (an isoform of AFP) which is the product of GDP-fucose in the presence of alpha 1-6 fucosyltransferase. In HCC tissues, the activity of this enzyme is higher than normal tissues, so AFP-L3 is known as more specific in the diagnosis of HCC than AFP. An increase in AFP-L3 levels indicates HCC in patients along with the constant increase in serum AFP level [20]. With the help of a newly developed immunoassay system, AFP-L3 percentages are measured with high sensitivity. This high sensitivity of AFP-L3 is used as a predictor in the early stages of HCC observation. High level of AFP-L3 is considered as an early prognostic factor in HCC development even at low serum concentrations of AFP as well as in the absence of ultrasound findings [21]. Lens culinaris agglutinin-reactive AFP (AFP-L3) is a glycoform variation of AFP and is communicated as a level of the all-out AFP level. It tends to be identified in the serum of roughly 33% of patients with little HCCs (< 3 cm) where sliced off degrees of 10% to 15% are utilized. At higher cut-off degrees of $> 15\%$, AFP-L3 shows an affectability of 75% to 96.9% and

explicitness of 90% to 92% [60,61]. The utility of this marker is restricted as studies have just been led in East Asian populaces in whom AFP levels are as of now raised [22].

Des-gamma-carboxy-prothrombin (DCP) or (PIVKA-II)

DCP is a protein present in serum that is secreted by HCC cells thus commonly used in the prognosis of HCC. It is known as, abnormal prothrombin induced by the absenteeism of a fat-soluble vitamin that is K or antagonist II (PIVKA-II) that lack 10-glutamic acid carboxylation on its N-terminal. DCP thus shows a lack of coagulation activity. As it lacks carboxylation of carbon at its γ-position thus known as des-γ-carboxy prothrombin. DCP activates the proliferation of HCC cells [23]. The malignant potential of DCP has been described as an autologous growth factor that triggers HCC growth and thus during hepatocytes malignant transformation, it activates and increases angiogenic factors like endothelial growth factor receptor and vascular endothelial growth factor (VEGF). DCP activates HCC progression through stimulation of the DCP-Met-JAK1-STAT3 signaling pathway. Metastasis and HCC invasion are increased by activation of matrix metalloproteinase (MMPs) signaling pathway [24]. In patients of HCC, increased levels of DCP are found. DCP has high sensitivity and specificity in the detection of HCC [25].

Des-gamma-carboxy prothrombin (DCP), otherwise called protein actuated by nutrient K nonattendance II (PIVKA-II), has been researched solely in Asian nations as a helpful liver tumor marker over the most recent 2 decades. DCP contrasts from prothrombin by the arrangement of amino-corrosive deposits, coming up short on the capacity to associate with other coagulation factors. This "irregular prothrombin" was at first found in the blood of patients introducing nutrient K insufficiency or

accepting nutrient K enemies. In 1984, Liebman et al detailed just because that serum DCP was likewise expanded in HCC patients. The specific component by which DCP is created by the tumor isn't yet completely saw, yet the standardization of DCP levels after healing malignant growth treatment obviously shows that the tumor is the wellspring of its creation [26].

Gamma-glutamyl transferase (GGT)

Gamma-glutamyl transferase (GGT) is an enzyme that bounds to membrane and involved in nucleic acid metabolism, biotransformation and tumorigenesis. High level of γ-GGT levels in serum results in increased cancer risk [27]. It helps in the breakdown of glutathione (GSH) extracellularly. Many tissues produce this enzyme but in serum higher level of GGT is derived from the liver. GGT is carried out by albumin and lipoproteins in the serum [28]. Increased activity of γ-glutamyl transferase (GGT) results in liver injury and mortality in the general population. Although it is present in many tissues only the GGT of the liver is detectable in the serum [29].

Its level increments in the event of liver ailment. It is extremely fundamental for the endurance of people and assumes products jobs in elements of epithelial cells for example liver and kidney. The degree of GP73 in the serum of HCC patients is higher

and, in this way, it is otherwise called a serum marker for the location of HCC [30].

Golgi proteins 73 (GP73)

The epithelial cells of numerous human tissues regularly express a protein called Golgi protein-73 (GP73) that is a sort II Golgi-confined vital film protein [31]. Its level increments in the event of liver ailment. It is extremely fundamental for the endurance of people and assumes products jobs in elements of epithelial cells for example liver and kidney. The degree of GP73 in the serum of HCC patients are higher and, in this way, it is otherwise called a serum marker for the location of HCC [32].

The strategies for looking at the GP73 incorporate Western blotting, immunohistochemical staining and enzyme linked immunosorbent assay (ELISA). a few specialists utilized Western blotting to identify GP73 in the serum of sound individuals and liver malignant growth patients, and they found that the affectability was 78% and particularity was 93.5% when hatched with polyclonal antibodies. The affectability when brooded with monoclonal antibodies was 84.7%, and explicitness of 93.5%. The solidness and reproducibility of monoclonal antibodies are better than that of polyclonal antibodies. [33]. The diagnostic serum levels of biomarkers are shown in the Table 1.

Table 1. Diagnostic levels of different biomarkers in the serum of HCC patients

Biomarkers	Diagnostic levels in serum	References
DCP	More than 400 ng/mL	[32]
AFP	More than 400 ng/mL	[14]
GGT	5.5 IU/L	[28]
GP73	median 107.3 μg/L	[33]

Tumor biomarkers of HCC
Glypican-3 (GPC3)

Glypican-3 is a specific and precise histochemical and serological marker for HCC. Glypican-3 is a representative of the glypican family and belongs to the group of heparin sulfate proteoglycan that is bound to

the outer surface of the cell membrane with the help of glycosylphosphatidylinositol anchor [34]. In the case of mammals there are six followers of this family that include GPC1-GPC6. GPC3 has been perceived in fetal liver and placenta but not present in other adult organisms. In the case of hepatic

cancer, GPC3 level increases in HCC tissues and thus discharged into the serum. In many HCC cases, there is an increase in the expression of GPC3 that is determined through cDNA microarray analysis. In about 40-53% patients of HCC, GPC3 can be detected [35]. This demonstrated GPC3 is a particular tumor marker if there should be an occurrence of HCC. Be that as it may, as of recently the connection between overexpression of GPC3 and conclusion of HCC has not yet been explained [36].

In patients of HCC, GPC3 is overexpressed both at quality and protein levels, and its demeanor conjecture a poor prognosis. Earlier examinations indicated that GPC3 capacities in HCC intrusion by the official to atoms like Wnt flagging proteins and development factors. Moreover, GPC3 has been utilized as an objective for atomic imaging and remedial mediation in HCC. As of not long ago, GPC3-focused on attractive reverberation imaging, near infrared imaging and positron emission tomography have been explored for the discovery of HCC [37].

Until this point in time, GPC3-GPC3-targeted magnetic resonance imaging, HSPs are overexpressed in cancer cells that are necessary for their subsistence in lethal conditions. The role of HSPs-70 in HCC is poorly understood although higher intracellular concentration of HSPs results in HCC, thus considered as a useful marker for the progression of HCC. It has been exemplified that higher level of HSPs may act as diagnostic and prognostic markers in HCC [43, 44].

HSP47 is encoded by the SERPINH1 gene, which is situated on chromosome 11q13.5, one of the most every now and again intensified areas in human malignant growth. The adjusted articulation levels of the HSP47 have been related with a few kinds of malignancy, for example, liver, cervical, bosom, pancreatic and gastric tumors. Studies have indicated that the HSP47

positron emission tomography, and near-infrared imaging have been explored for early HCC location, and different immunotherapeutic protocols focusing on GPC3 have been created, including the utilization of acculturated anti-GPC3 cytotoxic antibodies, treatment with peptide/DNA immunizations, immunotoxin treatments, and hereditary treatments [38].

Heat shock proteins 70 (HSPs)

Heat shock proteins are found ubiquitously within the cells and they act an influential molecular chaperone in case of stress like carcinogenesis. These are known as extremely conserved proteins, which under normal conditions are expressed at low levels but mostly activated in response to cellular stresses. HSPs play a vital role in apoptosis, protein homeostasis, invasion and signals transduction at the cellular level. In heat shock response, stimulation of HSPs leads cancer cells to adapt to stress conditions and thus due to continuous stress environment in tumor cells, many human cancers show overexpression of HSPs [38].

advances tumor angiogenesis, development, movement and metastatic limit [45].

Tumor associated glycoproteins 72 (TAG-72)

The tumor-related glycoprotein (TAG-72) is a layer glycoprotein complex of roughly 220–400 kDa that is available on the outside of numerous malignancy cells [46]. It is indicated that TAG-72 is overexpressed in different kinds of human tumors, for example, colon disease, ductal bosom malignant growth, lung malignant growth, epithelial ovarian malignancy, hepatocellular carcinoma, and oral malignancy, however isn't communicated by most typical tissues [47].

Ki-67 antigen

In proliferating cells, a nuclear antigen is present that is known as Ki-67. In cancerous

cells, it is one of the most widely used proliferation-associated markers. Various studies suggest that Ki-67 had a close connection with metastasis. Researchers found Ki-67 score was an independent prognostic molecular marker to predict

distant metastasis in soft tissue sarcoma **[48]**. The higher the expression of Ki-67 indicates the faster proliferation of tumor cells **[49]**. Different tumor biomarkers along with their sensitivity, specificity, and serum level is given in the Table 2.

Table 2. Sensitivity and specificity of some HCC biomarkers

HCC Biomarkers	Sensitivity (%)	Specificity (%)	Cutoff Value	Reference
AFP	62	90.2	>20ng/mL	**[39]**
AFP-L3	33	100	>35%	**[40]**
DCP	51	91.2	>10ng/ mL	**(39)**
GGT	60	72.9	128U/L	**(25)**
GP73	82	80	85.5mg/L	**(31)**
GPC3	69	93	3.8ng/mL	**[41]**
HSPs	70	73	456.5 pg./mL	**[42]**

Role of microRNAs in HCC

MicroRNAs (miRNAs) are little non-coding RNA silencing particles that control/regulate quality articulation contrarily by an official to the 3′-untranslated locales of target flag-bearer RNAs (mRNAs). MicroRNAs are comprised of around 19 to 23 nucleotides. Albeit the human genome comprises an exceptionally little part of miRNAs yet they assume an indispensable job in numerous formative procedures as a result of post-transcriptional guidelines of numerous qualities. In this way, these administrative RNAs are known as significant players of carcinogenesis **[50]**. Incredible efforts have been made in the investigations of miRNAs in HCC. The key administrative instruments of miRNAs include expansion, apoptosis, intrusion, metastasis, epithelial-mesenchymal progress (EMT), angiogenesis, sedate opposition and autophagy in HCC. Exosomal miRNAs likewise assume significant action in expansion, attack, metastasis, and medication opposition in HCC by directing quality articulation in the objective cells. Also, some miRNAs, including exosomal miRNAs, can be as potential demonstrative and forecast markers in HCC **[51]**.

Hepatocellular carcinoma (HCC) is the most widely recognized essential liver neoplasia and is portrayed by a poor guess. MicroRNAs (miRNAs) are associated with the guideline of articulation of the significant tumor-related pathways in carcinogenesis and may go about as oncogenes or tumor silencer qualities. mTOR is the most spoken to miRNA-managed pathway in HCC. miRNAs saw as deregulated in HCC could be utilized in clinical practice as indicative, prognostic or restorative targets **[52, 53]**.

Many types of cancers have an abnormal expression of miRNAs. In the case of HCC miRNAs are usually deregulated and some specific miRNAs are involved in clinicopathological features of HCC. Many studies have publicized that micro RNAs play an imperative role in HCC progression by increasing cell proliferation **[54, 55]**. They are an endless class of RNA, every one of which can control several quality targets and direct assorted natural procedures, for example, organogenesis, hematopoiesis, apoptosis and cell expansion. Irregular articulation of miRNA adds to tumorigenesis and malignant growth movement. MicroRNAs are as of late saw as another class of malignant growth biomarkers in HCC. They can without much of a stretch be

disconnected from tissues, plasma, serum, urine, excrement and are utilized as prognostic devices to analyze and forestall HCC [56].

Conclusion

HCC results in cancerous death globally. People having diseases like hepatitis A or C, chronic liver diseases, obese or diabetic people and heavy drinkers are at risk. Chronic liver cirrhosis sponsors to HCC because in this case hepatocytes growth and proliferation are waned or decreased that results in tumor formation. The aim of this appraisal is to discover the biological tools for the prevention of HCC. In tissues and body fluid of the human body, there are biomolecules and in case of abnormal conditions like carcinogenesis the level of these biomolecules is altered. So, these molecules are called biomarkers and are known as prognostic tools in the early diagnosis of HCC. Many types of biomarkers have been detected in various stages of HCC that includes enzymes, glycoproteins, peptides, mRNAs and miRNAs that are either obtained from serum or liver tissues of HCC patients. Past assessments showed that DCP is clearly preferable tumor marker over AFP and AFP-L3. The outcome of AFP and AFP-L3 are comparative. Regardless, there is no need for AFP-L3 in the being of AFP. By relating estimations of DCP, AFP and AFP-L3 in the examination of HCC, the result was orchestrated by DCP from the start then AFP and AFP-L3 at third. AFP-L3 is basic in the early affirmation of tumors. DCP had a quick association with tumor size and its critical levels are not found in any patients without HCC. That is the reason DCP is increasingly sensitive and has positive prescient worth (PPV) in the determination of HCC. In this manner, for HCC identification, DCP is utilized as principal serum test. In the serum of HCC patients, both AFP and DCP are performing self-rulingly and contend with each other for the discovery of HCC. The mixes of AFP and DCP help in aggregate affectability and explicitness hence commonly utilized for HCC analysis. If there should be an occurrence of early acknowledgment of HCC, AFP is viewed as more predominant than DCP. Be that as it may, if there should be an occurrence of attacks, DCP is more trustful than AFP as pinpointing or demonstrative device for HCC.

Authors' contributions

Conceived and designed the experiments: S Naz, Performed the experiments: S Iqbal, Analysed the data: S Naz, S Iqbal & M Rashid, Wrote the paper: S Naz, S Iqbal & M Rashid.

References

1. Nordenstedt H, White DL & El-Serag HB (2010). The changing pattern of epidemiology in hepatocellular carcinoma. *Digest Liver Dis* 42(3): S206-S214.
2. Alves RCP, Alves D, Guz B, Matos C, Viana M, Harriz M & Polletti P (2016). Advanced hepatocellular carcinoma, review of targeted molecular drugs. *Ann Hepatol* 10(1): 21-27.
3. Yue M & Mingshou H (2017). Current epidemiology, prevention and treatment of hepatitis B in China. *Infect Dis Trop Med* 2017; 3 (3): E412
4. Mazzanti R, Arena U & Tassi R (2016). Hepatocellular carcinoma: Where are we? *Jpn J Exp Med 6*(1): 21-36.
5. Chedid MF, Kruel CR, Pinto MA, Grezzana-Filho TJ, Leipnitz I, Kruel CD & Chedid AD (2017). Hepatocellular carcinoma: diagnosis and operative management. *Arq Bras Cir* 30(4): 272-278.
6. Sanyal AJ, Yoon SK & Lencioni R (2010). The etiology of hepatocellular carcinoma and consequences for treatment. *Oncologist* 15(S 4): 14-22.
7. Bagalad BS, Kumar KM & Puneeth HK (2017). Myofibroblasts: Master of disguise. *J Oral Maxillofac Pathol 21*(3): 462-463.

8. Song J, Ge Z, Yang X, Luo Q, Wang C, You H & Chu W (2015). Hepatic stellate cells activated by acidic tumor microenvironment promote the metastasis of hepatocellular carcinoma via osteopontin. *Cancer Lett 356*(2): 713-720.

9. Zamor PJ, Delemos AS & Russo MW (2017). Viral hepatitis and hepatocellular carcinoma: etiology and management. *J Gastrointest Oncol 8*(2): 229-242.

10. Jiang K, Li W, Zhang Q, Yan G, Guo K, Zhang S & Liu Y (2016). GP73 N-glycosylation at Asn144 reduces hepatocellular carcinoma cell motility and invasiveness. *Oncotarget 7*(17): 23530-23541.

11. Zhao Y, Wang M, Cui C, Zhang L, Liao F, Li H & Wu X (2015). Significance of combined tests of serum golgi glycoprotein 73 and other biomarkers in diagnosis of small primary hepatocellular carcinoma. *Cancer Biomark* 15(5): 677-683.

12. Gao J & Song P (2017). Combination of triple biomarkers AFP, AFP-L3, and PIVAKII for early detection of hepatocellular carcinoma in China: Expectation. *Drug Discov Ther 11*(3): 168-169.

13. Mlynarsky L, Menachem Y & Shibolet O (2015). Treatment of hepatocellular carcinoma: Steps forward but still a long way to go. *J Hepatol* 7(3): 566-574.

14. Chan MY, She WH, Dai WC Tsang SHY, Chok KSH, Chan ACY, & Cheung TT (2019). Prognostic value of preoperative alpha-fetoprotein (AFP) level in patients receiving curative hepatectomy-an analysis of 1,182 patients in Hong Kong. *Trans Gastroenterol Hepatol 4*. doi.org/10.21037/tgh.2019.06.07.

15. Mehinovic L, Islamagic E, Husic-Selimovic A, Kurtovic-Kozaric A, Vukobrat-Bijedic Z & Suljevic D (2018). Evaluation of diagnostic efficiency of alpha-fetoprotein in patients with liver cirrhosis and hepatocellular carcinoma: single-center experience. *Open Access Macedonian J Med Sci* 6(9): 1668-1673.

16. Ma WJ, Wang HY & Teng LS (2013). Correlation analysis of preoperative serum alpha-fetoprotein (AFP) level and prognosis of hepatocellular carcinoma (HCC) after hepatectomy. *World J Surg Onc* doi.org/10.1186/1477-7819-11-212

17. Reichl P & Mikulits W (2016). Accuracy of novel diagnostic biomarkers for hepatocellular carcinoma: An update for clinicians. *Oncol Rep* 36(2): 613-625.

18. Malek NP, Schmidt S, Huber P, Manns MP & Greten TF (2014). The diagnosis and treatment of hepatocellular carcinoma. *Dtsch Arztebl Int* 111(7): 101-106.

19. Naz Z, Usman S, Saleem K, Ahmed S, Bashir H, Bilal M & Sumrin A (2018). Alpha-fetoprotein: A fabulous biomarker in hepatocellular, *Gastric Rectal Cancer* 29(12): 613-625.

20. Song PP, Xia JF, Inagaki Y, Hasegawa K, Sakamoto Y, Kokudo N & Tang W (2016). Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. *World J Gastroenterol* 22(1): 262-274.

21. AlSalloom AAM (2016). An update of biochemical markers of hepatocellular carcinoma. *Int J Health Sci 10*(1): 121-136.

22. Kim JU, Shariff MI, Crossey MM, Gomez-Romero M, Holmes E, Cox IJ& Taylor-Robinson SD (2016). Hepatocellular carcinoma: Review of disease and tumor biomarkers. *J Hepatol* 8(10): 471-484.

23. Kumada T, Toyoda H, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Satomura S (2014). High-sensitivity Lens culinaris agglutinin-reactive alpha-fetoprotein assay predicts early detection of hepatocellular carcinoma. *J Gastroenterol* 49(3): 555-563.

24. Zhang YS, Chu JH, Cui SX, Song ZY & Qu XJ (2014). Des-γ-carboxy prothrombin (DCP) as a potential autologous growth factor for the development of hepatocellular carcinoma. *Cell Physiol Biochem* 34(3): 903-915.

25. Cui SX, Yu XF & Qu XJ (2016). Roles and signaling pathways of des-γ-carboxyprothrombin in the progression of hepatocellular carcinoma. *Cancer Invest* 34(9): 459-464.

26. Lai Q, lesari S, Sandri GBL, & Lerut J (2017). Des-gamma-carboxy prothrombin in hepatocellular cancer patients waiting for liver transplant: a systematic review and meta-analysis. *Int Bio Markers* 32(4): 370-374.

27. Piñero F, Marciano S, Anders M, Orozco Ganem F, Zerega A, Cagliani J & McCormack L (2016). Identifying patients at higher risk of hepatocellular carcinoma recurrence after liver transplantation in a multicenter cohort study from Argentina. *Eur J Gastroen Hepat* 28(4): 421-427.

28. Fu SJ, Zhao Q, Ji F, Chen MG, Wu LW, Ren QQ, & He XS (2016). Elevated preoperative serum gamma-glutamyltranspeptidase predicts poor prognosis for hepatocellular carcinoma after liver transplantation. *Sci Rep-Uk* 6(1): 1-9.

29. Emdin M, Pompella A & Paolicchi A (2005). "Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. *Circulation* 112(14): 2078-2080.

30. Everhart JE & Wright EC (2013). Association of γ-glutamyl transferase (GGT) activity with treatment and clinical outcomes in chronic hepatitis C (HCV). *Hepatol* 57(5): 1725-1733.

31. Wang L, Yao M, Dong Z, Zhang Y & Yao D (2014). Circulating specific biomarkers in diagnosis of hepatocellular carcinoma and its metastasis monitoring. *Tumor Biol* 35(1): 9-20.

32. Zhao Y J, Ju Q & Li GC (2013). Tumor markers for hepatocellular carcinoma. *MOL Clinic Biol* 1(4): 593-598.

33. Xia Y, Zhang Y, Shen M, Xu H, Li Z & He N (2019). Golgi protein 73 and its diagnostic value in liver diseases. *Cell Proliferat* 52(2): e12538.

34. Hou SC, Xiao MB, Ni RZ, Ni WK, Jiang F, Li XY & Chen BY (2013). Serum GP73 is complementary to AFP and GGT-Ⅱ for the diagnosis of hepatocellular carcinoma. *Oncol Lett* 6(4): 1152-1158.

35. Ba MC, Long H, Tang YQ & Cui SZ (2012). GP73 expression and its significance in the diagnosis of hepatocellular carcinoma: a review. *Int J Clin Exp Patho* 5(9), 874-881.

36. Chaiteerakij R, Zhang X, Addissie BD, Mohamed E A, Harmsen WS, Theobald PJ & Moser CD (2015). Combinations of biomarkers and M ilan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. *Liver Transplant 21*(5): 599-606.

37. Li B, Guo T, Sun Z, Li X & Mao Y (2017). The clinical values of serum markers in the early prediction of hepatocellular carcinoma. *Biomed Res Int* doi.org/10.1155/2017/5358615.

38. Zhou F, Shang W, Yu X & Tian J (2018). Glypican-3: A promising biomarker for hepatocellular carcinoma diagnosis and treatment. *Med Res Rev* 38(2): 741-767.

39. Haruyama Y & Kataoka H (2016). Glypican-3 is a prognostic factor and an immunotherapeutic target in hepatocellular carcinoma. *World J Gastroenterol* 22(1): 275-283.

40. Behne T & Copur MS (2012). Biomarkers for hepatocellular carcinoma. *Int J Hepatol* doi.org/10.1155/2012/859076.

41. Shirakawa H, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S & Nakatsura T (2009). Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 100(8): 1403-1407.

42. Zhou F, Shang W, Yu X & Tian J (2018). Glypican-3: A promising biomarker for hepatocellular carcinoma diagnosis and treatment. *Med Res Rev* 38(2): 741-767.

43. Wang C, Zhang Y, Guo K, Wang N, Jin H, Liu Y & Qin W (2016). Heat shock proteins in hepatocellular carcinoma: Molecular mechanism and therapeutic potential. *Int J Cancer* 138(8): 1824-1834.

44. Jang ES, Jeong SH, Kim JW, Choi YS, Leissner P & Brechot C (2016). Diagnostic performance of alpha-fetoprotein, protein induced by vitamin K absence, osteopontin, Dickkopf-1 and its combinations for hepatocellular carcinoma. *PLoS One* doi.org/10.1371/journal.pone.0151069.

45. Duarte BDP & Bonatto D (2018). The heat shock protein 47 as a potential biomarker and a therapeutic agent in cancer research. *J Cancer Res Clin* 144(12): 2319-2328.

46. Leerapun A, Suravarapu SV, Bida JP, Clark R J, Sanders EL, Mettler TA & LaRusso NF (2007). The utility of lens culinaris agglutinin-reactive α-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol H* 5(3): 394-402.

47. Yang SL, Fang X, Huang ZZ, Liu XJ, Xiong ZF, Liu P & Li CH (2014). Can serum glypican-3 be a biomarker for effective diagnosis of hepatocellular carcinoma? A meta-analysis of the literature. *Dis Markers* doi.org/10.1155/2014/127831.

48. Gruden G, Carucci P, Lolli V, Cosso L, Dellavalle E, Rolle E & Brunello F (2013). Serum heat shock protein 27 levels in patients with hepatocellular carcinoma. *Cell Stress Chaperones* 18(2): 235-241.

49. Jego G, Hazoumé A, Seigneuric R & Garrido C (2013). Targeting heat shock proteins in cancer. *Cancer Lett* 332(2): 275-285.

50. Xiong J, Jiang XM, Mao SS, Yu XN & Huang XX (2017). Heat shock protein 70 downregulation inhibits proliferation, migration and tumorigenicity in hepatocellular carcinoma cells. *Oncol Lett* 14(3): 2703-2708.

51. Milenic DE, Brady ED, Garmestani K, Albert PS, Abdulla A & Brechbiel MW (2010). Improved efficacy of α-particle–targeted radiation therapy: Dual targeting of human epidermal growth factor receptor–2 and tumor-associated glycoprotein 72. *Cancer* 116(S4): 1059-1066.

52. Vasuri F, Visani M, Acquaviva G, Brand T, Fiorentino M, Pession A & de Biase D (2018). Role of microRNAs in the main molecular pathways of hepatocellular carcinoma. *J Gastroenterol* 24(25): 2647-2600.

53. Chien MH, Chang WM, Lee WJ, Chang YC, Lai TC, Chan DV & Hsiao M (2017). A Fas ligand (FasL)-fused humanized antibody against tumor-associated glycoprotein 72 selectively exhibits the cytotoxic effect against oral cancer cells with a low FasL/Fas ratio. *Mol Cancer Ther* 16(6) 1102-1113.

54. Shi W, Hu J, Zhu S, Shen X, Zhang X, Yang C & Zhang H (2015). Expression of MTA2 and Ki-67 in hepatocellular carcinoma and their correlation with prognosis. *Int J Clin Exp Patho* 8(10): 13083-13089.

55. He Y, Liu F, Mou S, Li Q & Wang S (2018). Prognostic analysis of hepatocellular carcinoma on the background of liver cirrhosis via contrast-enhanced ultrasound and pathology. *Oncol Lett* 15(3): 3746-3752.

56. Law P T Y& Wong N (2011). Emerging roles of microRNA in the intracellular signaling networks of hepatocellular carcinoma. *J Gastroen Hepatol* 26(3): 437-449.