Emerging Proteomic and Glycoproteomic biomarkers for Hepatocellular carcinoma

Dalal Kruti[1],

Department of Transfusion Transmitted Diseases,

National Institute of Immunohaematology (ICMR),

Mumbai, INDIA

Dr.Aruna Shankarkumar[2]

PhD, Department of Transfusion Transmitted Diseases,

National Institute of Immunohaematology (ICMR),

Parel, Mumbai 400012

Abstract:

Early diagnosis and proper therapeutics are the most important factors to increasing the survival rate for liver cancer patients in the current medical treatment.To date, the identification of specific and sensitive serological biomarkers to monitor liver disease progression and/or early diagnosis of hepatocellular carcinoma (HCC) is still an unmet medical need.DCP and HGF are promising biomarkers with wide accessibility. Enzymes and isoenzymes such as GGT II, AFU, HCR2 help in early diagnosis and prognosis. Glycoproteomic biomarkers such as AFP and Lens culinaris agglutinin AFP are used in clinical practice with hepatic ecography in screening of cirrhotic patients to discover HCC. GPC3 positive patients show lower survival rate than GPC3 negative patients. Glycomic profiles of haptoglobin and ZAG have provided powerful approach to a better understanding of HCC. OPN is having highest sensitivity and specificity for detecting HCV HCC. Search for new biomarker for HCC is continuous evolving process. This article describes new emerging potentially useful biomarkers.

Keywords: Biomarker,Glycoproteomics, Hepatitis, Hepatocellular carcinoma, Proteomics

Abbreviations:

HCC	:	Hepatocellular carcinoma
HBV	:	Hepatitis B virus
HCV	:	Hepatitis C virus
GP73	:	Golgi protein 73
TGF-β1	:	Transforming Growth Factor β1
SCCA	:	Squamous cell Carcinoma Antigen
DKK1	:	Dickkopf related protein1
DCP	:	Des gamma Carboxyprothrombin
GGT	:	Gamma Glutamyl Transferase
AFU	:	Alpha-L-fucosidase
HCR2	:	Human Carbonyl Reductase-2

AFP	:	Alpha feto protein
ZAG	:	Zinc alpha-2 glycoprotein
OPN	:	Osteopontin
GPC3	:	Glypican-3

Introduction:

Globally, hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of deaths by cancer. Worldwide, there are about 626,000 new HCC cases and nearly 600, 000 HCC related deaths each year with an incidence equal to the death rate [Ferlay J et. al., 2001].

More than half of all HCC in the world are attributed to chronic hepatitis B virus (HBV) [Parkin DM, 2006].The incidence of HCC is 98.4 times higher in HBV carriers than non-carriers [Yang JD et. al., 2010].Geographical correlation exists between the incidence of HCC and the prevalence of chronic hepatitis B and C viruses, suggesting that these two viral infections are the most important risk factors associated with HCC.In addition to the viral infections largely implicated in HCC development, other factors associated with HCC are well documented. These factors include toxins (e.g., alcohol consumption) and drugs (e.g., aflatoxin and anabolic steroid use), cigarette smoking, metabolic liver diseases (e.g., hereditary hemochromatosis, and alpha1- antitrypsin deficiency), and steatosis [Sarma MP et. al., 2012].

Considering Indian scenario, it is at intermediate endemic level of hepatitis B, with hepatitis B surface antigen prevalence 2% - 10% among the population studiedwithout a reference to occult HBV infection in the data. Among tribal populations in Madhya Pradesh and Andaman,the point prevalence was 19.4 in the groups studied and this corresponded to a chronic carrier rate of 15.5 percent [Puliyel J et. al., 2008].According to WHO fact sheets, Cirrhotic patients with HBV have an approximately 10% to 15% annual risk of developing HCC (Figure-1).The seroprevalence rate of HCV among the blood donor population in India is 1.8% - 2.5 %, and the community seroprevalence has been reported to be 0.87% [Pal S et. al., 2005].Among those with chronic HCV, an estimated 20 to 30% will develop cirrhosis, and cirrhotic patients with HCV have an approximately 1 to 4% annual risk of developing HCC and a similar risk of developing end-stage liver disease (Figure-2). These figures cause burden on the occurrence / incidence of hepatocellular carcinoma in the country.

Figure-1

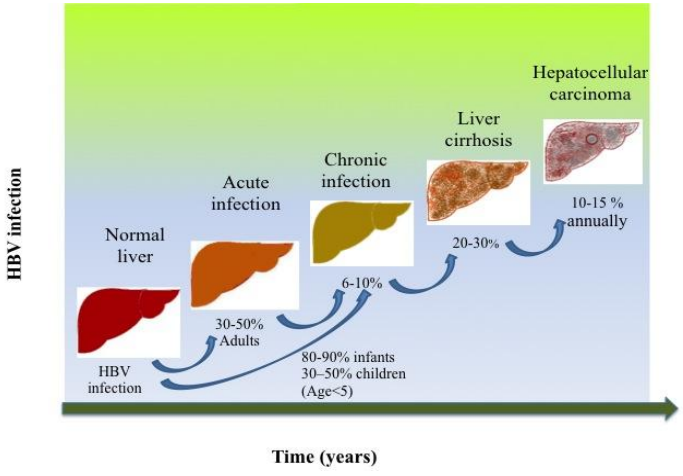

Figure-1

About 80–90% of infants infected during the first year of life and 6-10% adults develop chronic infections; among them an estimated 20 to 30% will develop cirrhosis. Cirrhotic patients with HBV have an approximately 10 to 15% annual risk of developing HCC.

Figure -2

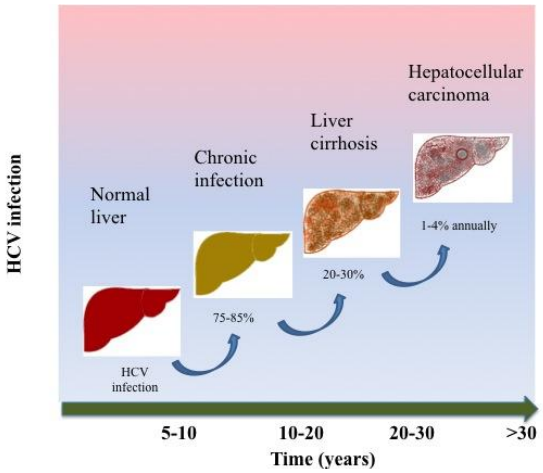

Figure-2

This graphical representation shows that after initial infection with HCV there is typically a lag of 20 to 25 years before cirrhosis develops. Biomarker identification is required at this stage to take preventable or therapeutic measurements for occurrences of HCC.

Definitive diagnosis requires histologicalexamination of biopsy material, however, in many cases conventional pathological analysis of these biopsies is very difficult. The availability of a tumour markermay significantly improve diagnosis of malignancy accurately.Early stages of liver cancer can be treated by resection and/or transplantation, with improved outcomes. However, only 20% to 25% of hepatocellular carcinoma can be managed with a curative intent [Yeung YK et. al., 2005].Symptomatic hepatocellular carcinoma usually presents at an advanced stage [Kumagi T et. al., 2009]. Treatment options for late stage hepatocellular carcinoma are few due to advanced disease. Hepatocellular carcinoma can cause decompensation in previously compensated cirrhosis.Survival at this stage, with only supportive measures, is between three months to seven months [Coon JT et. al., 2007].
Thus, due to high incidence of HCC and poor prognosis when diagnosed at a symptomatic stage, early detection is essential. With the advent of new technologies, a great number of potential biomarkers are identified for HCC. Tumour markers may be useful for detection of HCC at early stages, and also, may help in therapeutic assessment and detection of recurrence.

The FDA Pharmacogenomics guidance defines a valid biomarker as- "A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic or clinical significance of the test results."

The significance of this review is to focus on emerging potential proteomic and glycoproteomic biomarkers for detection of hepatocellular carcinoma.

1.Protein biomarkers:

Golgi Protein 73 (GP 73):

Golgi phosphoprotein 2 (GOLPH2/GP73/GOLM1) is a type II internal membrane protein of the cis and median Golgi. In normal liver, GP73 is expressed by biliary epithelial cells, but minimally by hepatocytes and its expression is significantly increased in liver diseases such as HCC [Kim HJ et. al., 2012].The endosomal trafficking of GP73 allows for proprotein convertase furin-mediated cleavage, resulting in its release into the extracellular space [Bachert C et. al., 2007]. GP73 in the serum of patients with HCC infected by HBV is significantly higher as compared to HBV carriers, patients without hepatic diseases; healthy adults and no increase in the patients with non- malignant liver

lesions.Fucosylated GP73 (FC-GP73) Compared with total GP73, FC-GP73 improves the sensitivity and specificity of diagnosis of HCC from 65-90 to 90-100% [Zhao Y et. al., 2013].

Transforming growth factor β1 (TGF- β1):

TGF-β1, multifunctional pluripotent growth factor plays crucial role in regulation of growth and differentiation of both normal and transformed cells. It induces apoptosis in numerous cell types through the SMAD pathway or the DAXX pathway.

Lee Dhas concluded that plasma TGF-β1 expression is significantlyhigher in patients with HCC compared to normalcontrols and cirrhotic subjects [Lee D et. al., 2012]. Furthermore, it's over expression is associated with invasivenessof HCC and worse prognosis. It can induce microvascular abnormalities through the down regulation of neural cell adhesion molecules in human HCC.In Chinese population study (n=309), the higher incidence of circulating TGF- β1 level was 83.3% in HCC (1.2 ≥ μg/L) and lower incidence was 19.4 % in liver cirrhosis, 7.7% in chronic hepatitis and none in acute hepatitis and healthy controls [Dong ZZ et. al., 2008].Polymorphisms of TGF β1 expression can affect susceptibility to tumor. Hence, TGF β1 signalling pathway has become a hot spot as a tumor therapeutic target.

Hepatocytes Growth factor (HGF):

HGF or scatter factor is the most potent growth factor for hepatocytes and binds to its only known high affinity receptor Met. This receptor tyrosine kinase (RTK) is predominantly expressed on epithelial and endothelial cells. It regulates proliferation, migration, cellsurvival, morphogenesis, angiogenesis, as wellas tissue regeneration [Venepalli NK et. al., 2013].HGF has been shown to be over represented in HCCs as compared to the normal liver. However, it is not expressed by tumor cells themselves instead stellate cells and myofibroblasts are induced to secrete HGF by tumor cell products. HGF in turn stimulates tumor cell invasiveness [Breuhahn K et. al., 2006].HGF concentrations are found higher in patients with diffuse carcinoma or with multiple cancers than in patients with a single cancer [Yim SH et. al., 2010].Invitro Study done by Ogunwobi et. al have found that treatment of BNL cells with HGF led to decreased expression of E-cadherin and increased expression of fibronectin, vimentin, COX-2(cyclooxygenase-2) and direct role of HGF in promoting EMT(epithelial mesenchymal transition) as well as carcinogenic properties in HCC via separate activation of Akt(protein kinase B) and Cox-2 pathways [Ogunwobi OO et. al., 2011].Invivo study by Ding W *et al*., have demonstrated a model of liver cancer EMT, in which mesenchymal tumor cells secrete high levels of HGF resulting in rapid tumor growth and invasion invivo [Ding W et. al., 2010].Specific molecular targeting of these pathways may have clinical therapeutic and chemopreventive benefits in HCC.

Complement C3:

Complement C3a is one of the proteins formed by the cleavage of complement C3. It triggers mast cell degranulation and hence, innate immune response. Tessitore A et. al has seen Protein complement C3a (about 8.9 kDa), elevated both in chronic HCV and HCV-related HCC patients [Tessitore A et. al., 2013]. It is identified as a candidate biomarker and further validated by chip immunoassay and western blot. However this is not the case with HBV related HCC [Lee IN et. al., 2006]. This supports that hepatocarcinogenesis may be different in HBV related and HCV related HCC and hence expression of C3a may be different but results of Mazumdar B *et. al.*, have suggested in study that C3 promoter activity was modestly inhibited by HCV coreprotein and it was significantly reduced in the presence of HCV NS5A protein in dose dependent manner via inhibition

of expression of C/EBP-beta [Mazumdar B et. al., 2012]. so further investigation is needed to understand unique mechanisms of complement regulation by HCV proteins.

Squamous cell Carcinoma Antigen (SCCA):

Squamous cellular carcinoma antigen (SCCA) is a member of the high molecular weight family of **ser**ine **p**rotease **in**hibitors named serpins. It is reported to over express in HCC tissue and in serum from HCC patients. Both SCCA isoforms SCCA1 and SCCA2 protect neoplastic cells from apoptotic death induced by several kinds of stimuli, and in vivo experiments have demonstrated that SCCA1 can promote tumor growth [Beneduce L et. al., 2005].

SCCA is reported to over express in tumoral compared to peritumoral tissue, suggesting a role as a potential marker for histological detection of HCC [Schmilovitz-weiss H et. al., 2011].Hussein MM *et. al.*, have concluded that the levels of SCCA are significantly higher in patients with HCC than in Chronic Liver Disease patients and controls [Hussein MM et. al., 2008].It is reported to be highly sensitive (84.0%) compared to 60% for AFP but has a low specificity (48.9%) for hepatocellular carcinoma [Soyemi OM et. al., 2012].Presently, SCCA is still a research tool and notyet approved for widespread clinical use in the screening of patients with hepatocellular carcinoma.

Dickkopf related protein 1 (DKK1):

Dickkopf-1 (DKK1) is a secretory antagonist of the canonical Wnt signalling pathway. It is hardly expressed in normal human adult tissues, except in placental and embryonic tissues [Tung EK et. al., 2011].Overexpression of DKK1 not only enhances the tumor formation efficiency and tumor growth but also, promotes the cell invasion and metastasis in vitro and in vivo.Knockdown of DKK1 has significantly reduced both migratoryand invasive abilities of HCC cells [Chen L et. al., 2013].DKK1 plays a functional role in cell migration, invasion and tumour growth.Shen Q *et al*have interpreted in large-scale study report that DKK1 can complement measurement of AFP in the diagnosis of HCC and improve identification of patients with AFP-negative HCC and distinguish HCC from non-malignant chronic liver diseases [Shen Q et. al., 2012].

Increase in serum and tissue DKK1 levels in step wise manner in multistep hepatocarcinogenesis may have prognostic significance and as DKK1 down regulation reduces the migration and invasion of HCC cells; it has potential therapeutic strategy for advanced HCC [Chen L et. al., 2013].

1.1 Enzymes and Isoenzymes:

Des gamma Carboxyprothrombin(DCP):

DCP, also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II), is an abnormal prothrombin without carboxylation of 10 glutamic acid residues at its N-terminus and is devoid of coagulation activity. Due to lack of carboxylation of the carbon at the gamma position, it is called DCP. The level of DCP in the serum of patients with HCC is significantly higher than that in patients with chronic hepatitis and cirrhosis [Fujikawa T et. al., 2009]. Suzuki M. *et. al.*have demonstrated that DCP has a mitogenic effect on HCC cell lines. They have found that DCP binds with Met (membrane spanning receptor tyrosine kinase). The transductional apparatus of DCP is identified to activate Janus kinase1 (JAK1) /signal transducers and activators of the transcription3 (STAT3) signaling pathway during cancer proliferation. Their findings provide a description of DCP as novel autocrine/paracrine growth stimulatory mechanism behind the development of HCC [Suzuki M et. al., 2005].

Furthermore, DCP can be used to differentiate between different histopathological stages and grades of HCC, and to evaluate portal vein thrombosis [Zakhary N et. al., 2013]. The level of DCP is closely associated with a larger tumor, vascular invasion. It is considered as a prognostic indicator able to predict rapid tumor progression. The high sensitivity and specificity of DCP may be useful in screening high-risk population and diagnose the disease at early stages when curative treatments are possible [Yamamoto K et. al., 2010].Besides that the diagnostic value of DCP as a biomarker is comparable to that of AFP. Bertino G*et. al.*hasproved that AFP and DCP are not correlated, so the combination of these markers can significantly improve HCC detection, with 61.5% sensitivity and 82.9 % specificity [Bertini G et. al., 2011].

Gamma Glutamyl Transferase (GGT):

GGT, a membrane bound enzyme, in healthy adults is mainly secreted by hepatic kupffer cell and endothelial cell of bile duct. Its physiological role is in counteracting oxidative stress by breaking down extracellular glutathione and making its component amino acids available to the cells. GGT has been widely used as an index of liver dysfunction and marker of alcohol intake. In damaged hepatocytes, particularly in hepatocarcinogenesis, since GGT is released into blood from hepatic tissues, significant changes occur in serum GGT activity [Yao DF et. al., 2004]. Total GGT can be divided into 13 isoenzymes by using polymer acrylamide gradient gel electrophoresis, and some of them can only be detected in the serum of HCC patients. The identification of hepatoma-specific GGT (GGT-II) provides an efficacious serum marker for HCC, which is regarded as an early enzyme marker of precancerous and cancerous processes. The sensitivity of HCC detection using GGT-II was 84.1%. This sensitivity is increased to 92.9 and 93.8% when combined with AFU or AFP, respectively. The combination of three markers has yielded 97.3% detection sensitivity but the lowest diagnostic specificity (64.7%) [Zhu J et. al., 2013].

Alpha-L-fucosidase (AFU):

AFU is a lysosomal enzyme found in all mammalian cells hydrolyses fucose glycosidic linkages of glycoprotein and glycolipids.The activity of AFU is detectable and elevated activities are observed in the sera of HCC patients compared with chronic liver disease and healthy individuals. In addition, AFU can reveal the case 6-9 months earlier than ultrasonography visualization [Pillai AA et. al., 2012]. AFU may be a supplementary marker in HCC detection but the specificity of AFU is relatively poor and is also over expressed in diabetes, pancreatitis and hypothyroidism patients.

Human Carbonyl Reductase-2 (HCR2):

Human Carbonyl reductase-2, cytosolic enzyme is expressed in liver and kidney and plays important role in detoxification of the reactive alpha-dicarbonyl compounds and reactive oxygen species (ROS) deriving from oxidative stress in HCC. In HCC, antioxidant defense system including HCR2 and glutathione-S transferase is repressed. This altered detoxification system is involved in HCC progression. Thus decreased expression of this enzyme in HCC tissues contributes to cancer growth because it increases cellular damage induced by ROS and other carcinogens. Levels of HCR2 are shown to be inversely correlated to the pathological grading of HCC [Liu S et. al., 2006].

2. Glycoprotein biomarkers:

Due to sugars' propensity to form complex structures through monosaccharide types, sequence, branching and isomerism, glyco-conjugates appear to be nature's ideal recognition molecules. Profiling and quantifying glycoprotein expression as well as comprehensive glycan structural analyses may provide information leading to development of biomarkers.

Alpha feto Protein (AFP):

AFP, the primary and classical tumor marker for HCC, is a glycoprotein with molecular weight of approximately 70 kDa, which is synthesized in the endodermal cells of the yolk sac during early foetal development and subsequently in embryonic hepatocytes [Debruyne EN et. al., 2008]. Its level falls rapidly to less than 10ng/mL immediately after birth, but in certain pathological condition it rises again. Pathological elevation of AFP is seen in hepatocyte regeneration, hepatocarcinogenesis and embryonic carcinomas.But it has a reported sensitivity of only 39% to 65% for detection of HCC [Zinkin NT et. al., 2008]. A hepatoma specific AFP subfraction is reported to be superior to total AFP level in both sensitivity and specificity in differentiating benign liver diseases from malignant ones [Lai Q et. al., 2012].Total AFP can be divided in to three glycoforms (AFP-L1, AFP-L2, AFP-L3), according to their binding ability to the lectin lens agglutinin(LCA). L3 isoform has highest affinity for lectin from Lens culinaris, which makes it possible to differentiate L3 from other isoforms.Fucosylation fraction of AFP (AFP-L3) has sensitivity and specificity of 96.9 and 92%,respectively, in detecting HCC [Singhal A, 2012].Lens culinaris agglutinin reactive AFP (AFP-L3) measurement may be of help in diagnosis of HCC as AFP L3 is a product of alpha 1,6 fucosyl transferase; the enzyme is higher in HCC tissues than in peritumoral tissue. Therefore, AFP-L3 is more effective than AFP in clinical practice. Thus AFP-L3% could be complementary to AFP as a marker for HCC.

Glypican-3:

Glypican is a heparin sulphate proteoglycan that is anchored to the plasma membrane through glycosylphostatidylinositol [Filmus J et. al., 2008].Glypican-3 expression is less frequently observed in well-differentiated HCC than in moderately and poorly differentiated HCC. By performing immunohistochemistry with a monoclonal antibody, Cappuro *et. al.*have found that 72% of HCCs express GPC3, whereas this protein is not detected in normal hepatocytes, cirrhotic liver or benign lesions [Capurro Met. al., 2003]. Moreover, GPC3 can be found in the serum of 53% of HCC patients, it is undetectable in normal serum.Capurro MI*et al*. have shown that this glypican promotesthe in vivo and in vitro growth of HCC by stimulatingcanonical Wnt signalling [Capurro MI et. al., 2005].The activation of thissignaling pathway induces the cytosolic accumulationand nuclear translocation of the transcription factorb-catenin. In the nucleus b-catenin associates withmembers of the LEF/TCF family of transcriptionfactors, and induces the expression of genes thatstimulate cell cycle progression and cell survival [Clevers H et. al., 2012, Li L et. al., 2012]. GPC3 immunoreactivity has a reported sensitivity of 77% and specificity of 96% in the diagnosis of small HCC. Study has shown that 14% of the metastases from gastrointestinal and pancreatic tumors are GPC3 positive, suggesting that this marker is not very specific in distinguishing HCC from metastases originating from extra hepatic sources [Mounajjied T et. al., 2013].

Haptoglobin:

Haptoglobin, from Greek word haptein – to bind, is a liver secreted glyprotein which binds with oxygenated, free haemoglobin with a very high affinity. Its major known biological role is the capture of released hemoglobin during intravascular hemolysis and prevention of kidney damage by released iron [Pompach et. al., 2013].It consists of S-S linked alpha and beta subunits.According toShu H *et. al.* beta-haptoglobin protein is differentially expressed in sera of patients with hepatitis B infection, liver cirrhosis and hepatocellular carcinoma. The resultshave clearly indicated that beta-haptoglobin from liver cirrhosis and HCC patients displayed higher glycosylation compared with healthy subjects and HBV patients [Shu H et. al., 2011]. As well as, according to Ang IL *et. al.*a unique pattern of Haptoglobinglycoform is specific to HCC and is associated with tumor progression [Ang IL et. al., 2006]. Mehta A *et. al.* have observed increased levels of a tetra-antennary

glycan in the HCC tissue as compared to the non diseased livers [Mehta A et. al., 2012]. Thus, haptoglobin can be a potential biomarker and needs further investigation is needed.

Zinc alpha-2 glycoprotein (ZAG):

ZAG, a soluble glycoprotein, shows high degree of similarity with class I MHC molecules structurally as well as sequentially. Due to its high homology with lipid mobilizing factor (LMF), it is considered as a novel adipokine [Hassan I et. al., 2008].Zinc-α-2-glycoprotein is down- regulated in HCV-HCC compared to non-B non-C-HCC [Sarvari J et. al., 2014]. Huang Y *et. al.* concluded that ZAG is frequently decreased in HCC; Low expression can be served as a tumor biomarker for poor differentiation and a predictor for poor prognosis of HCC patients [Huang Y et. al., 2012].Whereas, Wang F hasconfirmed that the ZAG protein is over expressed in the HCC group, suggesting it is a novel candidate biomarker for early diagnosis of HCC [Wang F et. al., 2012].Due to these contradictory findings, ZAG requires further investigation as well as validation.

Osteopontin (OPN):

Osteopontin (OPN),chemokine-like extracellular matrix (ECM) protein, is a glycosylated phosphoprotein that acts as a cytokine and binds to αvβ integrins and receptors of the CD44 family to deliver signals to cells to promote cell adhesion, chemotaxis, ECM degradation, angiogenesis, apoptosis prevention, and indolent tumor growth. This protein has also been regarded as a major transcription target induced by hepatocyte growth factor (HGF) and may contribute to HGF-mediated cell-cell dissociation, growth, and invasiveness [Qin L et. al., 2014]. OPN has also a good sensitivity in AFP negative HCC. Abu El Makarem *et al.*, 2011 studied the diagnostic performance of OPN level for discrimination of the HCC patients (n = 113) from chronic liver disease subjects (n = 120) and healthy subjects (n = 120); sensitivity and specificity were 97.7%, 100%. In HCC, an elevated plasma level of OPN is regarded as a potential prognostic biomarker and overexpression of OPN is closely correlated with intrahepatic metastasis, early recurrence and a worse prognosis [Shang S et. al., 2012].Moreover, RNA interference–mediated depletionof osteopontin may be a promising strategy for thetreatment of HCC by sensitizing the chemotherapeutic drugs [Zhao J et. al., 2008].

Future prospects:

Despite of years of research, the number of biomarkers for hepatocellular carcinoma in clinical use is quite small. Although in recent decades, knowledge about the biology of cancers has increased greatly and many candidate biomarkers are reported, very few are sufficiently validated to justify their use in developing drugs or making patient care decisions.

Figure-3

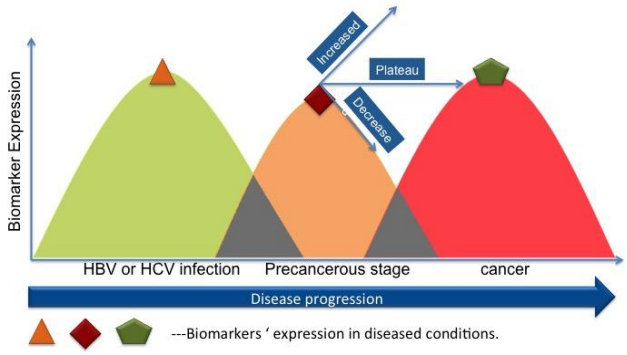

Figure-3

Diagrammatic representation shows that Expression of biomarkers is not uniform through out diseased conditions. As it is shown here some biomarkers show increased, plateau or decreased expression in precancerous stage. So our aim should be to find such novel marker for early diagnosis of HCC.

Figure-3 illustrate that our prime focus should be identification of precancerous biomarkers before occurrence of cancer. Lately, the investigation of new biomarkers for HCC diagnosis has aroused great interest because they can makeit possible to select the most effective therapy for individual patients. As reviewed in this article, many potentially useful molecular biomarkers have been reported, but almost none have been incorporated into the conventional TNM staging system yet. From this viewpoint, biomarkers helping to detect HCC would be a great step forward.Therefore, effective test strategies should be considered to improve the early diagnostic rate of HCC.

1. The diagnosis of HCC may be combined with clinical manifestation, iconography detection (abdominal ultrasonography, abdominal CT scan, abdominal MRI) and histological examination.
2. Selection of biomarkers for different indices based on different detection purposes. Table no- I describes different biomarkers and their clinical use in the different populations. AFP-L3 and DPC is well-establishedtumour marker for HCC in eastern country. GGT-II is an early enzyme marker of precancerous and cancerous processes with considerable sensitivity.Glypican-3 would be a next new and definitive tumour marker for HCC with high specificity.DKK1 attracts much attention as it complements AFP in AFP-negative HCC. Complement C3a biomarker is specific for HCV HCC. Sensitivity and specificity of OPN is beyond traditional tumor biomarkers such as AFP with same expense. It can be used as potential prognostic biomarker.
3. Major drawback includes small sample size in the description of studies having high prevalence of HCC in the country i.e. china where this country alone sees more than 55% of all primary liver cancer worldwide. As consequences description of biomarkers, its sensitivity and specificity as well as possible application in the clinical field has became misleading.
4. Due to heterogeneity of etiology and clinical behaviours of HCC, it would be very difficult to find one biomarker that is both specific and sensitive enough. Combination of biomarkers with high sensitivity and specificity will be more efficient and practical for early diagnosis and prognostication of HCC. (Table no- II) However, combination of markers may increase sensitivity but decrease specificity. GGT II, AFP, AFU combination have highest sensitivity but low specificity. As AFP-L3 and DCP are not correlated, combination of markers GGT II, AFP-L3alongwith DCP, OPN and AFU (supplementary marker) may provide enhanced sensitivity and specificity.
5. Efforts should be directed towards prospective clinical trials in evaluating the prognostic significance of these markers.
6. The future of biomarker research depends on the experiments that are currently being conducted, and retaining stringent requirements for true identification of biomarkers, with sufficient validation. Antibody array based proteomic approach may represents useful tool to explore new serum/plasma biomarkers for early detection of HCC. Moreover, MS based proteome analysis is growing exponentially with search for new and novel biomarkers.
7. Recent advances in genomics and proteomics have provided a novel tool to improve the diagnostic and prognostic prediction of HCC. In this era, exploration for novel biomarkers for the diagnosis of HCC is an evolving process, which will reveal new insights into early HCC diagnosis and personalized therapy. As well as there is need to focus on public health awareness and early detection of the disease to give liver cancer patients the best chance of survival.

Table no-I Diagnostic values of HCC serum biomarkers and their potential clinical use:

Markers	Sensitivity (%)	Specificity (%)	Clinical use	Reference	Population type (subjects enrolled in the study)
AFP	60.0-80.0	70.0-90.0	Early diagnosis, monitoring and recurrence	Carr BI et. al., 2007	United states (99)
DCP	61.0-72.0	90.0-94.0	Early diagnosis and prognosis, portal vein invasion and metastasis	Inagaki Y et. al., 2011	Japan (148)
GP73	75.0	69.0	Diagnosis	Mao Y et. al., 2010	United states (4217)
GPC3	77.0	96.0	Early Diagnosis	Jin GZ et. al., 2013	China (129)
SCCA	84.0	46.0-48.0	Early diagnosis	Schmilovitz Weiss H et. al., 2011, Giannelli G et. al., 2005	Israel, Italy (61, 251)
AFU	81.7	70.7	Early Diagnosis	Malaguarnera G et. al., 2010	China (199)
GGT	84.1	-	Diagnosis	Malaguarnera G et. al., 2010	China (199)
TGF-β1	89.5	94.0	Prognosis and tumour invasiveness	Dong ZZ et. al., 2008	China (309)
AFP-L3	96.9	92.0	Early diagnosis, prognosis, vascular invasion, intrahepatic metastasis	Khien VV et. al., 2001	China (90)
OPN	97.7	100	Prognosis, Intrahepatic metastasis, early recurrence	Abu El Makarem MA et. al., 2011	Egypt (353)

Table no -II Diagnostic values of combination of biomarkers:

Combination of markers	Sensitivity (%)	Specificity (%)	Reference
AFP + DCP	61.5	82.9	Inagaki Y et. al., 2011
AFP+ AFP-L3	73.7	86.6	Abu El Makarem MA et. al., 2012
AFP-L3 +DCP	84.8	97.8	Abu El Makarem MA et. al., 2012
AFP+ AFP-L3+DCP	85.9	59.0	Abu El Makarem MA et. al., 2012
GGT II+ AFU	92.9	-	Zhu J et. al., 2013
GGT II +AFP	93.8	-	Zhu J et. al., 2013
GGT II +AFP +AFU	97.3	64.7	Zhu J et. al., 2013

References:

1. Abu El Makarem M, 2012, An overview of biomarkers for the diagnosis of hepatocellular carcinoma. Hepat Mon, 12, e6122.

2. Abu El Makarem MA, Abdel-Aleem A, Ali A, Saber R, Shatat M, Rahem DA et. al., 2011 Diagnostic significance of plasma osteopontin in hepatitis C virus-related hepatocellular carcinoma. Ann Hepatol, 10, pp 296-305.
3. Ang IL, Poon TC, Lai PB, Chan AT, Ngai SM, Hui AY et. al., 2006, Study of serum haptoglobin and its glycoforms in the diagnosis of hepatocellular carcinoma: a glycoproteomic approach. J Proteome Res, 5, pp 2691-2700.
4. Bachert C, Fimmel C, Linstedt AD, 2007, Endosomal trafficking and proprotein convertase cleavage of cis Golgi protein GP73 produces marker for hepatocellular carcinoma. Traffic, 8, pp 1415-23.
5. Beneduce L, Castaldi F, Marino M,Quarta S, Ruvoletto M, Benvegnù L et. al., 2005, Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. Cancer, 103, pp 2558-65.
6. Bertino G, Neri S, Bruno CM, Ardiri AM, Calvagno GS, Malaguarnera M et. al., 2011 Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxyprothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. Minerva Med, 102, pp 363-371.
7. Breuhahn K, Longerich T, Schirmacher P., 2006 Dysregulation of growth factor signaling in human hepatocellular carcinoma. Oncogene, 25, pp 3787-3800.
8. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E et. al., 2003 Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology, 125, pp 89-97.
9. Capurro MI, Xiang YY, Lobe C, Filmus J., 2005, Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. Cancer Res, 65, pp 6245-54.
10. Carr BI, Kanke F, Wise M, Satomura S., 2007, Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxyprothrombin in histologically proven hepatocellular carcinoma in the United States, Dig Dis Sci., 52, pp 776-82.
11. Chen L, Li M, Li Q, Wang CJ, Xie SQ, 2013, DKK1 promotes hepatocellular carcinoma cell migration and invasion through β-catenin/MMP7 signaling pathway. Mol Cancer, 12, pp157.
12. Clevers H, Nusse R, 2012, Wnt/β-catenin signaling and disease. Cell, 149, pp 1192-1205.
13. Coon JT, Rogers G, Hewson P, Wright D, Anderson R, Cramp M, 2007, Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. Health Technology Assessment, 11, pp 1–206.
14. Debruyne EN, Delanghe JR, 2008, Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. Clin Chim Acta, 395, pp 19-26.
15. Ding W, You H, Dang H, LeBlanc F, Galicia V, Lu SC, Stiles B, Rountree CB, 2010, Epithelial-to-mesenchymal transition of murine liver tumor cells promotes invasion. Hepatology, 52, pp 945-53.
16. Dong ZZ, Yao DF, Yao M, Qiu LW, Zong L, Wu W et. al., 2008, Clinicalimpact of plasma TGF-beta1 and circulating TGF-beta1 mRNA in diagnosis of hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int.,7, pp 288-95.
17. Ferlay J, Bray F, Pisani P and Parkin D. Cancer Incidence Mortality and Prevalence Worldwide Version 1.0, no. 5, IARC Press, Lyon, France, 2001.
18. Filmus J, Capurro M, Rast J, 2008, Glypicans. Genome Biol, 9, pp 224.
19. Fujikawa T, Shiraha H, Yamamoto K, 2009, Significance of des-gamma-carboxyprothrombin production in hepatocellular carcinoma, Acta Med Okayama, 63, pp 299-304.

20. Giannelli G, Marinosci F, Trerotoli P, Volpe A, Quaranta M, Dentico P et. al. 2005, SCCA antigen combined with alpha-fetoprotein as serologic markers of HCC. Int J Cancer, 117, pp 506-09.
21. Hassan I, Waheed A, Yadav S, Singh T, Ahmad F, 2008, Zinc A2-Glycoprotein: A Multidisciplinary Protein,Mol Cancer Res, 6, pp 892-906.
22. Huang Y, Li LZ, Zhang CZ, Yi C, Liu LL, Zhou X et. al., 2012, Decreased expression of zinc-alpha2-glycoprotein in hepatocellular carcinoma associates with poor prognosis.J Transl Med,10, pp 106.
23. Hussein MM, Ibrahim AA, Abdella HM, Montasser IF, Hassan MI, 2008, Evaluation of serum squamous cell carcinoma antigen as a novel biomarker for diagnosis of hepatocellular carcinoma in Egyptian patients. Indian J Cancer, 45, pp167-72.
24. Inagaki Y, Tang W, Makuuchi M, Hasegawa K, Sugawara Y, Kokudo N, 2011, Clinical and molecular insights into the hepatocellular carcinoma tumour marker des-γ-carboxyprothrombin. Liver Int, 31, pp 22-35.
25. Jin GZ, Dong H, Yu WL, Li Y, Lu XY, Yu H., 2013, A novel panel of biomarkers in distinction of small well-differentiated HCC from dysplastic nodules and outcome values. BMC Cancer13, pp 161.
26. Khien VV, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV et. al., 2001 Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. Int J Biol Markers, 16, pp105-11.
27. Kim HJ, Lv D, Zhang Y, Peng T, Ma X, 2012, Golgi phosphoprotein 2 in physiology and in diseases. Cell Biosci, 2, pp 31.
28. Kumagi T, Hiyasi Y, Hirschfield GM, 2009, Hepatocellular carcinoma for the non-specialist.BMJ (Clinical Research Ed.), 339, pp 5039–44.
29. Lai Q, Melandro F, Pinheiro RS, Donfrancesco A, Fadel BA, Levi Sandri GB,Rossi M, Berloco PB, Frattaroli FM., 2012, Alpha-fetoprotein and novel tumor biomarkers as predictors of hepatocellular carcinoma recurrence after surgery: a brilliant star raises again. Int J Hepatol , 893130.
30. Lee D, Chung YH, Kim JA, Lee YS, Lee D, Jang MK et. al., 2012, Transforming growth factor beta 1 overexpression is closely related to invasiveness of hepatocellular carcinoma. Oncology, 82, pp 11-8.
31. Lee IN, Chen CH, Sheu JC,Lee HS, Huang GT, Chen DS et. al, 2006, Identification of complement C3a as a candidate biomarker in human chronic hepatitis C and HCV-related hepatocellular carcinoma using a proteomics approach. Proteomics,6, pp 2865-73.
32. Li L, Jin R, Zhang X, Lv F, Liu L, Liu D et. al., 2012, Oncogenic activation of glypican-3 by c-Myc in human hepatocellular carcinoma. Hepatology, 56, pp 1380-90.
33. Liu S, Ma L, Huang W, Shai Y, Ji X, Ding Let. al., 2006, Decreased expression of the human carbonyl reductase 2 gene HCR2 in hepatocellular carcinoma, Cell Mol Biol Lett, 11, pp 230-41.
34. Malaguarnera G, Giordano M,Paladina I, Berretta M, CappellaniA, Malaguarnera M., 2010,Serum markers of hepatocellular carcinoma. Digestive Diseases and Sciences, 55, pp 2744-55.
35. Mao Y, Yang H, Xu H, Lu X, Sang X, Du S et. al., 2010, Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. Gut, 59, pp 1687-93.
36. Mazumdar B, Kim H, Meyer K, Bose SK, Di Bisceglie AM, Ray RB, Ray R., 2012, Hepatitis C virus proteins inhibit C3 complement production, J Virol., 86, pp 2221-8.

37. Mehta A, Norton P, Liang H, Comunale MA, Wang M, Rodemich-Betesh L et. al., 2012, Increased levels of tetra-antennary N-linked glycan but not core fucosylation are associated with hepatocellular carcinoma tissue., Cancer Epidemiol Biomarkers Prev21, pp 925-33.
38. Mounajjed T, Zhang L, Wu TT, 2013, Glypican-3 expression in gastrointestinal and pancreatic epithelial neoplasms. Hum Pathol, 44, pp 542-50.
39. Ogunwobi OO, Liu C., 2011, Hepatocyte growth factor upregulation promotes carcinogenesis and epithelial-mesenchymal transition in hepatocellular carcinoma via Akt and COX-2 pathways. ClinExp Metastasis,28, pp 721-31.
40. Pal S, Choudhuri G., 2005, HEPATITIS C: THE INDIAN SCENARIO. Gastroenterology, 27, pp 46-53.
41. Parkin DM, 2006, The global health burden of infection-associated cancers in the year 2002. Int J Cancer, 118, pp 3030-44.
42. Pillai AA, Fimmel CJ, 2012, Emerging serum biomarkers of HCC. Clin Gastroenterol, 27, pp 247-62.
43. Pompach P, Brnakova Z, Sanda M, Wu J, Edwards N, Goldman R, 2013, Site-specific glycoforms of haptoglobin in liver cirrhosis and hepatocellular carcinoma. Mol Cell Proteomics, 12, pp 1281-93.
44. Puliyel J, Rastogi P, Mathew J., 2008, Hepatitis B in INDIA: Systematic review & report of the 'IMA sub-committee on immunization'. Indian J Med Res, 127, pp 494-97.
45. Qin L., 2014, Osteopontin is a promoter for hepatocellular carcinoma metastasis: a summary of 10 years of studies. Front Med, 8, pp 24-32.
46. Sarma MP, Asim M, Medhi S, Bharathi T, Diwan R, Kar P., 2012, Viral genotypes and associated risk factors of hepatocellular carcinoma in India.Cancer Biol Med, 9, pp172-81.
47. Sarvari J, Mojtahedi Z, Kuramitsu Y, Fattahi M, Ghaderi, Nakamura K, ErfaniN., 2014, Comparative Proteomics of Sera From HCC Patients With Different Origins. Hepat Mon., e13103.
48. Schmilovitz-Weiss H, Tobar A, Halpern M, Levy I, Shabtai E, Ben-Ari Z, 2011, Tissue expression of squamous cellular carcinoma antigen and Ki67 in hepatocellular carcinoma-correlation with prognosis: a historical prospective study, Diagn Pathol, 6, pp 121.
49. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajrang S et. al., 2012, Identification of osteopontin as a novel marker for early hepatocellular carcinoma. Hepatology, 55, pp 483-90.
50. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y et. al. 2012, Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Onco, 13, pp 817-26.
51. Shu H, Zhang S, Kang X, Li S, Qin X, Sun C et. al., 2011, Protein expression and fucosylated glycans of the serum haptoglobin-{beta} subunit in hepatitis B virus-based liver diseases. Acta Biochim Biophys Sin, 43, pp 528-34.
52. Singhal A, Jayaraman M, Dhanasekaran DN, Kohli V., 2012, Molecular and serum markers in hepatocellular carcinoma: predictive tools for prognosis and recurrence. Crit Rev Oncol Hematol, 82, pp 116-40.
53. Soyemi OM, Otegbayo JA, Ola SO, Akere A, Soyemi T, 2012, Comparative diagnostic efficacy of serum squamous cell carcinoma antigen in hepatocellular carcinoma. BMC Res Notes, 5, pp 403.
54. Suzuki M, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, Koike K et. al., 2005 Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. J Biol Chem, 280, pp 6409-15.

55. Tessitore A, Gaggiano A, Cicciarelli G,Verzella D, Capece D, Fischietti M, et. al., 2013, Serum biomarkers identification by mass spectrometry in high-mortality tumors. Int J Proteomics, 125858.
56. Tung EK, Mak CK, Fatima S, Lo RC, Zhao H, Zhang C et. al, 2011, Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatocellular carcinoma. Liver Int., 31, pp 1494-1504.
57. Venepalli NK, Goff L, 2013, Targeting the HGF-cMET Axis in Hepatocellular Carcinoma. Int J Hepatol, 341636.
58. Wang F, Geng Y, Zhang WM, Geng X, 2012, Identification of ZAG protein as a novel serologic biomarker candidate for liver cancer. Adv Mater Res,340, pp 383-89.
59. Yamamoto K, Imamura H, Matsuyama Y, Kume Y, Ikeda H, Norman GL et. al., 2010, 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, 45, pp 1272-82.
60. Yang JD, Roberts LR, 2010, Hepatocellular carcinoma: A global view. Nat Rev GastroenterolHepatol, 7, pp 448-58.
61. Yao DF, Dong ZZ, Yao DB, Wu XH, Wu W, Qiu LW et. al. 2004, Abnormal expression of hepatoma-derived gamma-glutamyltransferase subtyping and its early alteration for carcinogenesis of hepatocytes. Hepatobiliary Pancreat Dis Int., 3, pp 564–570.
62. Yeung YK, Lo CM, Liu CL, Wong BC, Fan ST, Wong J, 2005, Natural history of untreated non-surgical hepatocellular carcinoma. Am J Gastroenterol, 100, pp 1995-2004.
63. Yim SH, Chung YJ., 2010, An Overview of Biomarkers and Molecular Signatures in HCC. Cancers, 2, pp 809-23.
64. Zakhary N, Khodeer S, Shafik S, Camlia A, Malak A, 2013, Impact of PIVKA-II in diagnosis of hepatocellular carcinoma. J of Adv Res,4, pp 539-46.
65. Zhao J, Dong L, Lu B, Wu G, Xu D, Chen J et. al. 2008, Down-regulation of osteopontin suppresses growth and metastasis of hepatocellular carcinoma via induction of apoptosis. Gastroenterology, 135, pp 956-68.
66. Zhao Y., Ju Q. and Li G., 2013, Tumor markers for hepatocellular carcinoma. Mol Clin Oncol, 1, pp 593-98.
67. Zhu J, Jiang F, Ni HB, Xiao MB, Chen BY, Ni WK et. al., 2013, Combined analysis of serum γ-glutamyl transferase isoenzyme II, α-L-fucosidase and α-fetoprotein detected using a commercial kit in the diagnosis of hepatocellular carcinoma. Exp Ther Med,5, pp 89-94.
68. Zinkin NT, Grall F, Bhaskar K, Otu HH, Spentzos D, Kalmowitz B et. al., 2008, Serum proteomics and biomarkers in hepatocellular carcinoma and chronic liver disease. Clin Cancer Res, 14, pp 470-77.