Review Article

**Serum biomarkers of hepatocellular carcinoma in patients with liver cirrhosis: a narrative review**

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**Abstract**

**Background:**Hepatocellular carcinoma (HCC) is the sixth leading cause of cancer-related mortality worldwide and the most frequent type of primary liver cancer. HCC is diagnosed in the laboratory using either fine-needle cytology, which is invasive and has intra- or inter-observer variability, or measuring circulating biomarkers. In combination with ultrasonography and computed tomography, several prognostic and diagnostic indicators are helpful in the clinical practice of screening and diagnosing HCC. **Aim of work:** To give an overview of serum biomarkers of HCC.

**Keywords:** Hepatocellular Carcinoma, Cirrhosis, Serological Markers, alpha-fetoprotein.

**Introduction**

**Epidemiology:**

Hepatocellular carcinoma is the sixth leading cause of cancer-related mortality worldwide and the most frequent type of primary liver cancer **[1]**.

Egypt is Africa's third and the world's fifteenth most populated country. Across the past decade, the proportion of HCC among chronic liver disease patients substantially doubled in Egypt, where 48 % of HCC cases were ascribed to hepatitis C virus (HCV) associated with cirrhosis. Indeed, it is acknowledged that HCC almost often develops after cirrhosis has been established in chronic HCV. HCC is Egypt's most common kind of cancer, accounting for 2.3 % of all cancers **[2].**

**Risk Factors:**

Because the majority of HCC patients are linked with cirrhosis caused by chronic hepatitis B virus (HBV) or HCV infection, they should be recruited in ultrasonography and serum-fetoprotein surveillance programs (AFP) **[3].**

**Pathogenesis:**

The specific mechanism of HCC development is still unknown, despite recent improvements in cancer biology and genetic profiling. However, many processes, including gene mutations, epigenetic modification, copy number variations, and gene rearrangements, have been examined as critical phases in HCC development **[4].**

HCC has a moderate number of '20-100' mutations/genome, but this is greatly affected by the underlying cause, e.g., HBV has a higher rate of mutations because it proliferates using an RNA-dependent reverse transcriptase that lacks proofreading capacity and produces direct integration into the host genome. On the other hand, HCV is linked to mutations in the immunoglobulin gene -catenin and TP53. Furthermore, many gene mutations in HCC are telomerase promoter mutations, which keep telomere length above the level that activates DNA damage. Inherited genetic variations also influence this effect in the telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC) genes, which keep telomere length above the level that activates DNA damage **[5].**

Epigenetics refers to inherited states of gene expression that occur in the absence of changes in DNA sequences, causing chromosomal stability, gene transcription, and cell differentiation to be affected. This happens through various processes, including histone methylation and acetylation and gene control by non-coding RNAs. MicroRNAs (miRNAs) are short non-coding RNAs that operate many genes. For example, MiRNA-122 is a member of this family that has been downregulated in 70% of instances of liver disease. In addition, it acts as a tumor suppressor by inducing apoptosis in HCC cells**[6].**

Copy number variation is a change in the structure of the genome caused by amplification or deletion of chromosomal segments; localized amplification was detected in 32% of HCC cases, whereas deletion was established in 40%. Another kind of genetic variation seen in HCC is chromosomal rearrangements, which include combining two genes by chromosome translocation, inversion, or deletion; a chromosomal rearrangement has a 400-kilobase loss in chromosome 19 is tailored explicitly for fibrolamellar HCC**[7].**

**Diagnosis:**

1. ***Clinical manifestations***:

HCC is generally asymptomatic in patients with compensated hepatic disease and thus escapes early detection, particularly in areas with poor surveillance programs. In contrast, symptoms in advanced liver disease are usually emphasized in the pattern of unspecific upper abdominal pain, hepatomegaly, jaundice, fever, lethargy, and weight loss. In contrast, cases with compensated liver disease show liver decompensation. In addition, patients with HCC may develop the paraneoplastic syndrome. HCC rupture, portal vein invasion, and hepatic vein obstruction, culminating in Budd-Chiari syndrome, are some of the other consequences**[8].**

1. ***Radiology and Laboratory Diagnosis:***

Ultrasonography (US), triphasic computerized tomography (triphasic CT-scan), and dynamic magnetic resonance imaging (d MRI) are the mainstays of radiological diagnostics. Because it is cheap, has wide availability, and is non-invasiveness, the US is typically the initial imaging modality employed in the examination of parenchymal organs of the abdomen. HCC is diagnosed in the laboratory using either fine-needle cytology, which is invasive and has intra- or inter-observer variability, or measuring circulating biomarkers**[9].**

During surveillance, finding a suspicious lesion using ultrasound in the cirrhotic liver is followed by diagnostic confirmation using contrast-enhanced helical computed tomography (CT) or (dMRI). Also, non-pathological confirmation of HCC diagnosis is achieved by AFP testing combined with previously mentioned imaging techniques**[8].**

1. Radiology:

The US, Triphasic CT, and MRI are imaging examinations utilized in diagnosing, staging, therapy, and follow-up of HCC. In addition, small HCC and regenerating nodules can be distinguished with contrast-enhanced ultrasonography, which is also used to facilitate diagnostic needle biopsy**10].**

Arterial enhancement with washout in the portal and delayed phases is a characteristic of HCC diagnosis on CT. HCC is fed mainly by arterial blood. Therefore, it looks brighter than adjacent liver tissue in the arterial phase but becomes less optimistic in the venous phase because it contains contrast-free arterial blood while surrounding liver tissue has venous blood with contrast. This dynamic pattern of amplification is almost unique to HCC**[11].**

MRI is the optimal radiographic technique for the characterization and diagnosis of hepatic focal lesions in cirrhotic patients. T1 weighted MRI results in HCC are hypointense, while T2 weighted MRI findings are hyperintense. Diffusion-weighted MRI (DWI) is an alternate MRI technique that enhances the characterization of minor hepatic lesions by using the diffusion characteristics of water molecules in living tissues. The hyperintensity on DWI is strongly predictive of hepatocellular cancer**[12].**

HCC is not a tumor that responds well to Positron emission tomography with 18F-fluorodeoxyglucose '18F-FDG-PET', with uptake occurring in fewer than 40% of patients. However, uptake appears to be prognostic since it is linked to higher AFP, vascular invasion, and poor prognosis**[8].**

1. Laboratory**:**

Alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP), also termed as prothrombin induced by vitamin K absence II 'PIVKA II,' the ratio of glycosylated AFP 'L3 fraction' to total AFP, alpha-fucosidase, and glypican is among the serological tests utilized in the early identification of HCC. However, although AFP is the most extensively used serological marker for HCC diagnosis, its monitoring is limited. Furthermore, its sensitivity for detecting tiny lesions is low, and higher values indicate a more advanced stage with a bad prognosis**[13].**

Laboratory diagnosis of HCC can be achieved via assessing some diagnostic and prognostic markers as the following:

1. ***Protein Biomarkers:***
* Alpha-fetoprotein (AFP):

AFP has a limited sensitivity because it can be expected in up to 40 % of HCC cases, particularly in the initial stages of the tumor, and a low specificity because its levels can be raised in cases other than HCC, such as cirrhosis, chronic hepatitis exacerbation, and even cholangiocarcinoma in some cases. Nevertheless, AFP is beneficial in HCC screening and diagnosis in conjunction with the US, HCC staging in the Cancer of the Liver Italian Program (CLIP) staging method, and tumor progression assessment in clinical practice. A drop in AFP level to less than ten ng/ml during 30 days of therapy suggests an acceptable response in HCC patients monitored with CT/MRI**[14].**

In chronic liver diseases, the value of AFP is 20 ng/ml (above which investigations for HCC are recommended)**[15].**

High AFP serum levels are associated with a poorer prognosis in HCC patients, and serum AFP concentrations ≥400 ng/mL denote poorer prognosis in different clinical settings**[16].**

* Des-γ-carboxyprothrombin (DCP):

DCP, also termed as PIVKA 'prothrombin induced by vitamin K deficiency, is aberrant prothrombin that results from an acquired error in prothrombin precursor carboxylation in HCC cells. DCP is specific for HCC than AFP since it does not increase in other liver disorders although it, is influenced by intrahepatic cholestasis, obstructive jaundice, and warfarin use. Furthermore, higher levels in HCC are related to tumor angiogenesis and the release of angiogenesis factors such as vascular endothelial growth factor (VEGF) and epithelial growth factor (EGF)**[17].**

* Glypican-3 (GPC-3):

It is an oncofetal protein that serves as a tumor suppressor and is generally responsible for cell proliferation and survival during embryonic life. It is downregulated in ovarian, breast, and lung adenocarcinoma, whereas it is elevated in HCC. Normal hepatocytes and non-malignant hepatic disorders lack GPC-3**[18].**

* α-l-fucosidase (AFU):

It's a lysosomal enzyme that hydrolyzes L-fucose sugars and is overexpressed in HCC patients. It's utilized in conjunction with AFP to diagnose HCC in its early stages**[19].**

* Squamous cell carcinoma antigen (SCCA):

SCCA1 and SCCA2 are two isoforms of a serine protease found in many normal squamous epithelial cells. It's more common in head and neck cancers and other epithelial tumors, including lung and cervix. Despite the absence of squamous epithelium in the liver, it is overexpressed in HCC due to a shared embryonic source**[20].**

* E-Cadherin, b-Catenin:

E-cadherin prevents tumor cells from forming tight junctions linked to metastasis and poor tumor differentiation. b-catenin is overexpressed in HCC and is related to activating a carcinogenic signaling cascade**[21].**

* Human Carbonyl Reductase 2:

Reduced expression in HCC is connected with cellular damage and promotes cancer progression. It is an enzyme coding gene expressed in the liver and kidney and detoxifies oxidative stress products**[22].**

* Transforming Growth Factor-b1 (TGF-b1):

TGF-b1 levels are higher in HCC patients and are linked to hepatic carcinogenesis, angiogenesis, and tumor growth**[23].**

* Golgi protein-73 (GP-73):

The biliary epithelium generally produces GP-73. Its level rises in hepatocytes in liver disease, and it is more significant in HCC than in liver cirrhosis**[24].**

* Vascular Endothelial Growth Factor (VEGF):

VEGF is an essential angiogenic agent that increases vascular permeability and promotes endothelial cell proliferation and migration. High levels of VEGF in the blood are linked to poorly encapsulated tumors, portal vein infiltration, and tumor metastasis, and it's used to predict tumor aggressiveness and overall survival following therapeutic tumor excision**[25].**

* Vitronectin:

Vitronectin is a glycoprotein of the hemopexin family, found in serum, the extracellular matrix, and bone. Vitronectin (VTN) is synthesized in the liver. Vitronectin is involved in malignancy. Vitronectin was restricted to the stroma of portal tracts and the subendothelial matrix of central veins in the normal liver. Intense vitronectin staining was detected in the tumor stroma in HCC**[26].**

1. ***Genetic Biomarkers:***

In patients with HCV-induced HCC, the study identified 40 up-regulated genes in HCV triggered HCC cases compared to the controls, including RYBP, ATP1B3, TMC, ZNF567, GPR108, and CD19.23 These studies identified potential biomarkers for HCV induced HCC, which are crucial to the design of treatment strategy for precision medicine**[27].**

1. ***ctDNA &ctRNA :***

Liquid biopsy has been extensively developed and put into clinical practice over the past decades. It detects circulating tumor DNA (ctDNA), circulating tumor cell (CTC), exosomes, and circulating tumor RNA (ctRNA) in body liquid, including plasma, urine, and cerebrospinal fluid. Among them, ctDNA is a widely applied genetic biomarker. It is derived from tumor tissue and carries somatic mutations, DNA methylations, viral sequences, and physical characteristics associated with carcinogenesis**[28].**

1. ***GALAD and BALAD Scores :***

The GALAD score was used as a statistical model for estimating the likelihood of HCC in patients with chronic liver disease. The GALAD score is derived from **g**ender, **a**ge, AFP-**L**3, **A**FP, and **D**es-carboxy-prothrombin (DCP) and was shown to be a highly accurate model for detecting HCC**[29].**

The BALAD score is applicable in the population with HCC. It is derived from five serum markers: albumin, bilirubin, alpha-fetoprotein (AFP), agglutinin-reactive alpha-fetoprotein (AFP-L3), and des-γ-carboxy prothrombin. It helps diagnose HCC and predict patient survival**[29].**

Furthermore, in cirrhotic patients, there are non-invasive indicators of HCC. The HCC-ART score could be another diagnostic indicator that was based on age, AFP, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), alkaline phosphatase (ALP), and serum albumin and had greater sensitivity and specificity than AFP, particularly in early HCC**[30].**

HCC is usually diagnosed based on a typical contrast enhancement pattern in patients with liver cirrhosis. Therefore, liver biopsy is only required when radiology is inconclusive, especially in less than 2 cm lesions. In contrast, imaging alone in non-cirrhotic patients is insufficient to diagnose HCC, and histopathological evaluation is required**[31].**

HCC is comparable to hepatic parenchyma; well and moderately differentiated tumors have a trabecular development pattern with sinusoidal capillarization, whereas poorly differentiated tumors have a compact structure without sinusoids. Therefore, as a vital phase in the management approach, after an HCC diagnosis has been established, a staging system should be used for prognostic evaluation**[32].**

Although there are many blood tumor markers for HCC, no one has superiority over alpha-fetoprotein, as it is still the standard and widely used worldwide**[33].**

We can conclude that in Egypt, the proportion of HCC among chronic liver disease patients increased approximately twofold over a decade. In combination with US and CT, several prognostic and diagnostic indicators are helpful in the clinical practice of screening and diagnosing HCC. These indicators may aid in the improvement of patient management and prognosis. So far, no tumor marker is superior to alpha-fetoprotein. In the future, new tumor markers with high sensitivity and specificity may be available.

**Footnotes.**

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