

**CLINICAL SIGNIFICANCE OF GLYPICAN- β IN
EARLY DIAGNOSIS OF HEPATOCELLULAR
CARCINOMA PATIENTS**

Thesis

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LIST OF ABBREVIATIONS

| | |
|--------------------------|---|
| AFP | Alpha fetoprotein |
| AFP-L¹ | Alpha fetoprotein – lectin ¹ |
| AFB¹ | Aflatoxin B ¹ |
| ALP | Alkaline phosphatase |
| AUC | Area under curve |
| BMPs | Bone morphogenetic proteins |
| CEA | Carcinoembryonic antigen |
| CT | Computed tomography |
| cDNA | Complementary DNA |
| dNTP | Deoxynucleotide triphosphate |
| DCP | Des gamma carboxyprothrombin |
| DN | Dysplastic nodule |
| ELISA | Enzyme linked immunosorbent assay |
| ECM | Extracellular matrix |
| EXTs | Extension genes |
| FGF | Fibroblast growth factor |
| FGFR | Fibroblast growth factor receptors family |
| FNAB | Fine needle aspiration biopsy |
| Fz | Frizzled |
| GAGs | Glycosaminoglycan |
| GPCs | Glypicans |
| GPC³ | Glypican ³ |
| GPC¹ | Glypican ¹ |
| GPI | Glycosylphosphatidylinositol |

| | |
|--------------------------------|--|
| GGT | Gamma glutamyl transferase |
| GIT | Gastrointestinal tract |
| HAART | Highly active antiretroviral therapy |
| HSPGs | Heparan sulfate proteoglycans |
| HS | Heparan sulfate |
| HME | Hereditary Multiple Exostoses |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| Hh | Hedgehog |
| HAART | Highly active antiretroviral therapy |
| hTERT | Human telomerase reverse transcriptase |
| IGF-II | Insulin growth factor II |
| IL-Λ | Interleukin Λ |
| IEP | Immunoelectrophoresis |
| LD | Lactate dehydrogenase |
| LDL | Lower detection limit |
| MIS | Magnetic immunosorbent |
| MRI | Magnetic resonance imaging |
| m-RNA | Messenger RNA |
| MRN | Macroregenerative nodule |
| PBS | Phosphate buffered saline |
| PCNA | Proliferating cell nuclear antigen |
| PIVKA-II | Protein induced by vitamin K antagonist II |
| PCR | Polymerase chain reaction |
| RT-PCR | Reverse transcriptase PCR |

| | |
|--------------------------|---|
| SGBS | Simpson Golabi Behmel Syndrome |
| SHF | Shistosomal hepatic fibrosis |
| TGF-β¹ | Transforming growth factor β ¹ |
| TSGF | Tumor specific growth factor |
| US | Ultrasonography |
| VCAM-1 | Vascular adhesion molecule |
| VEGF | Vascular endothelial growth factor |
| WHO | World Health Organization |
| Wg | Wingless |

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer related death (*Liovet et al., 2003*). The most common cause of hepatocellular carcinoma (HCC) is chronic hepatitis or liver cirrhosis caused by hepatitis C virus infection (*Wands, 2004 and Tang, 2002*).

Surgical resection remains the treatment of choice for these tumors, but only 10-20 % of primary HCCs are resectable at the time of diagnosis. Therefore early detection of HCC is a critical goal to improve the outcome of treatment of hepatocellular malignancy, and hence screening programs of patients at risk, such as chronic carriers of hepatitis B and C, are justified (*Capurro et al., 2005*).

Currently, imaging techniques including ultrasonography, computed tomography scanning and magnetic resonance are used for the diagnosis of HCCs. However, these techniques can not adequately differentiate benign hepatic lesions from HCC (*Befeler et al., 2002 and Louis et al., 2006*). Moreover, the detection rate by imaging techniques is only 33% in HCC lesions less than 1cm in diameter (*Krinsky et al., 2001*).

Serum α -fetoprotein (AFP) is the only marker that has been widely used for screening and diagnosis of HCCs. However, development of false-negative or false-positive rates with (AFP) was as high as 30% and 40% respectively for patients with small hepatocellular carcinomas (*Wei et al., 2006*).

Histopathological examination is considered the most reliable method to diagnose HCC. However, it is an invasive technique that carries the risk of bleeding and tumour spreading along the needle track (*Durand et al., 2001*). Thus the identification of novel biochemical markers for HCC remains an important goal for many laboratories around the world (*Nakatsura et al., 2003*).

Glypican-3 (GPC3), a heparan sulfate proteoglycan anchored to the plasma membrane is an oncofetal protein overexpressed in HCC at both the mRNA and protein levels (*Blackhall et al., 2001*, *Lozzo, 2001* and *Filmus et al., 2004*).

Capurro et al. (2003) reported that glypican-3 (GPC3) can be considered a serum and histochemical marker for HCCs. They showed that this protein was expressed in HCCs, whereas it was not detectable in hepatocytes from normal liver or benign liver diseases.

The role of GPC γ as biomarker for HCC has been suggested by many authors. They established that whereas GPC γ was undetectable in the serum of healthy donors and patients with hepatitis, its levels were significantly increased in >80% of patients with HCC. In addition, only 0% of patients with liver cirrhosis displayed elevated levels of serum GPC γ (*Nakatsura et al., 2002, Hippo et al., 2004 and Jiang et al., 2006*).

AIM OF THE WORK

The aim of the present study is to investigate the clinical utility of glypican γ (GPC γ) quantitated by ELISA in peripheral blood of an adequate number of hepatocellular carcinoma (HCC) patients for early diagnosis of this malignancy as well as evaluating its sensitivity and specificity.

I. HEPATOCELLULAR CARCINOMA

Primary epithelial tumors in the liver are either: benign, borderline, or malignant hepatocellular tumors (*Kumar et al., 2004*). Malignant epithelial tumors account for about 98% of all primary hepatic malignancies, with HCC representing (80%-90%); the single most common histological type (*Ishak et al., 2001, Monto and Wright, 2001 and El-Serag, 2003*).

1. EPIDEMIOLOGY

A. Geographic distribution

The epidemiology of HCC exhibits two main patterns, one in North America and Western Europe and the other in non-Western countries, Sub-Saharan Africa, central & South-east Asia and the Amazon basin (*Kumar et al., 2004*).

In non-Western countries the largest numbers of cases are found in Asia (76% of all HCC), followed by Africa. The highest annual incidence rates are found in Korea, Taiwan, Mozambique, and Southeast China, approaching 36 per 100,000 within each geographic area. Blacks have rates approximately three-fold higher than Caucasians. An increase in the prevalence of viral-induced cirrhosis especially from hepatitis C virus is the likely explanation. This trend may continue, owing to the large

pool of chronically HCV-infected persons (*Poynard et al., 2003 and Kumar et al., 2004*). Food infected with *Aspergillus flavus* stored during wet seasons poses another risk factor for HCC in China.

Hepatitis C virus (HCV) related HCC represents 50% of all HCC in Japan. The incidence of HCC accounted for 15% of HCC in Japan. Geographically, HCC is more frequent in Western than Eastern Japan. The death rates from HCC in each country correlate with the prevalence of anti-HCV, but not with HBsAg prevalence (*El-Serag and Rudolph, 2004 and Umemura and Kiyosawa, 2004*).

In North America and Western Europe the most malignant tumor of the liver discovered is metastases from elsewhere. HCC is mainly seen in those with pre-existing liver diseases (hepatic porphyrias, Tyrosinemia, chronic HBV, or HCV, alcoholism, liver cirrhosis or hemochromatosis (*WHO, 2004*). However, documented incidence of HCC is rising in the United States, tripling from 1.5 per 100,000 during 1976 to 1980 to 4.7 per 100,000 during 1996 to 1997 (*Kumar et al., 2004*). Interferon therapy for chronic hepatitis C has reduced the risk for development of HCC, especially among patients with sustained response (*Umemura and Kiyosawa, 2004*).

In Egyptian patients both HCV and HBV virus infection increase the risk of HCC, whereas isolated *Schistosoma* infection does not (*Sayed et al., 2005*). On
