Serum Laminin And Syndecan -1: Novel Biomarkers In Predicting Liver Fibrosis Stage In Patients With Hepatocllular Carcinoma.

Thesis

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ABBREVIATIONS

AFB1: Aflatoxin B1 .

BCLC:The Barcelona Clinic Liver Cancer Staging.
bFGF: basic fibroblast growth factor.
BM: Basement membrane.
CD138: syndecan-1.
c-erbB-2: erythroblastic leukemia viral oncogene homolog 2.
CLIP: Cancer of the Liver Italian Program.
DG: dystroglycan.

EASL: European Association for the Study of the Liver.
ECM: extracellular matrix.
EGF: Epidermal Growth Factor
GalNAc: *N*- acetylgalactosamine.
GlcNAc: *N*- acetylglucosamine.

HBsAg: Hepatitis B surface antigen.
HBV: Hepatitis B Virus.
HCC : Hepatocellular carcinoma.
HCV: Hepatitis C Virus.

HDV: Hepatitis D Virus.

HIFU: High intensity focused ultrasound.
HIV: Human immunodeficiency virus.
HPV: Human papilloma virus.
ILP: Interstitial laser photocoagulation.
JIS Score: Japan Integrated Score .
kDa: Kilo Dalton.
Ln-5: Laminin-5.
MMPs: matrix metalloproteinases.

NAFLD: non-alcoholic fatty liver disease .

NASH: non- alcoholic steatohepatitis.
PAI: Percutaneous acetic acid injection.
PDGF: platelet-derived growth factor.
PDZ = PSD-95, Dlg, ZO-1
PEI: Percutaneous ethanol injection.
PMC: Percutaneous microwave coagulation.
PSA: Prostate Specific Antigen.
RT: Radiotherapy.
TACE: transarterial chemoembolization.
TAE: Transarterial embolization.

ABSTRACT

Background and Aim: Worldwide, HCC is the cause of thousand deaths per year. The aim of this study was to test whether syndecan-1 and laminin could serve as a non-invasive marker for detection of liver fibrosis and thereby reduce the need for liver biopsy in HCC patients.

Subjects and Methods: The study included 30 patients with HCC and 20 healthy subjects serving as control. Patients were staged according to liver biopsies (Metavir fibrosis staging, stage F2 n = 3, F3, n = 7; F4, n = 20).Laminin and syndecan-1 was measured with an enzyme-linked immunosorbent assay.

Results. Laminin and syndecan-1 levels were significantly higher in the F4 than F2 and F3 ,meanwhile there was no significant difference between F3 and F2. Laminin and syndecan-1 levels were significantly higher in the HCC patients than in controls. The diagnostic sensitivity of laminin at a cutoff value of 213.3903 ng/ml was 100% and specificity was 75%. The diagnostic sensitivity of syndecan-1 at a cutoff of 19.4487 ng/dl was 100% and the specificity was 75%.

Conclusion. Combination of laminin and syndecan-1 as non invasive markers of liver fibrosis in HCC would improve the diagnostic power of these markers.

Key words: HCC, Laminin, syndecan-1, liver fibrosis.

INTRODUCTION

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health problem worldwide, with more than 500,000 cases diagnosed annually (**Parkin et al., 2001**). While the incidence of HCC has reportedly risen over the last 5-8 years, the survival of those affected has not changed significantly in the last two decades (**Bosch et al., 2004)**. This is related to both its late detection the lack of effective therapies for advanced stage disease (**Bruix and Llovet, 2002)**. Up to 80% of HCCs develop against a background of cirrhosis of the liver and while we believe that surveillance of the at risk cirrhotic population could aid earlier detection of the disease and decrease the cancer related mortality rate, the present success is limited by the lack of sensitive biomarkers. Currently, standard surveillance includes a combination of 6 monthly abdominal ultrasound scan (USS) and serum alphafetoprotein (AFP) measurement, but this strategy does not reliably detect early disease. The diagnostic performance of AFP is inadequate (**Sherman, 2001)** as it is only elevated in 40-60% of cases, while abdominal USS is difficult in cirrhotic nodular livers and notoriously user dependent (**Bruix, et al., 2001**). Alternative serum biomarkers are being actively sought and proposed candidates include laminin and syndecan-1.

Laminin is one of the main glycoproteins of the basement membrane and participates in a series of such biological phenomena as adhesion, migration, cellular differentiation and growth, inflammatory response and the maintenance of the cytoskeleton upon its binding to several components of the matrix, such as collagen type IV heparan-sulphate and entacin **(Kershenobich Stalnikowitz and Weissbrod, 2003)**. Not only have serum laminin levels been studied in patients with liver diseases, but also in patients with cancer, especially in cases where tumor proliferation

and invasion are found. Serum values tend to increase significantly with the emergence of metastases, irrespective of tumor lineage or the organ originating the neoplasm **(AbouFarha et al., 1992)**. Hence, serum laminin could be regarded as a tumor marker in cases of alterations in the basement membrane, proliferation and tumor invasion **(Saito et al., 2005)**. In fact serum laminin concentration is increased in metastatic cancer of different origins as melanoma, gastric adenocarcinoma, hepatocellular carcinoma, colorectal cancer, epithelial ovarian tumor **(Gao et al., 2007)**.

The syndecans are a family of transmembrane heparan sulfate proteoglycans, which, together with the lipid-linked glypicans, are the major source of heparan sulfates at cell surfaces **(Bernfield et al., 1999)**. The syndecan family is composed of four closely related proteins (syndecan-1, syndecan-2, syndecan-3, and syndecan-4) encoded by four different genes. All adhesive cells express at least one syndecan but most express multiple syndecans **(Kim et al., 1994)**. Syndecan-1 and syndecan-4 are expressed in a variety of cell types, including epithelial, endothelial, and vascular smooth muscle cells, syndecan-2 (fibroglycan) is expressed high levels in cultured lung and skin fibroblasts, and syndecan-3 N-syndecan) expression is largely restricted to central nervous system and peripheral nerves. All syndecans have an extracellular domain capable of carrying heparan sulfate and chondroitin sulfate chains, a transmembrane domain, and a cytoplasmic domain containing four universally conserved tyrosines and a conserved serine **(Volk et al., 1999)**. Unlike syndecans, glypicans carry essentially only heparan sulfate side chains **(Aviezer et al., 1994)**. Previously, it had been revealed that the expression of syndecan-1 was reduced in human hepatocellular carcinomas with high metastatic potential and speculated

that syndecan-1 played an important role in inhibition of invasion and metastasis **(Watari et al., 2004)**.

Aim of the work

The objective of the present study is to test soluble syndecan-1and laminin in plasma of patients with hepatocellular carcinoma in order to determine whether syndecan 1 and laminin may serve as markers for liver fibrosis and may also correlate with the stage of fibrosis of these patients.

Review of Literature

HEPATOCELLULAR CARCINOMA

Epidemiology

Hepatocellular carcinoma (HCC) is the commonest primary cancer of the liver. Incidence is increasing and HCC has risen to become one of the commonest malignancy worldwide and a leading cause of cancer related death, exceeded only by cancers of the lung and stomach (***Gomaa et al., 2008***). The estimated incidence of new cases is about 500,000-1000,000 per year, causing 600,000 deaths globally per year (***Yeh et al., 2007***).

In Egypt, between 1993 and 2002, there was an almost two folds increase in HCC amongst chronic liver patients (***El-Zayadi et al., 2005***).

The World Health Organization report (***Jong-wook, 2003***) indicated a total of 714,600 new cases of HCC worldwide, with 71% among men. HCC is the 4th commonest cause of death due to cancer, after cancers of the respiratory system, stomach, and colon/rectum. Liver cancer ranked 3rd for male subjects and 5th for women. Geographically, there were 45,000 liver cancer deaths in Africa, 37,000 in the Americas, 15,000 in the eastern Mediterranean, 67,000 in Europe, 61,000 in South-East Asia, and 394,000 in the western Pacific region, including China and Japan **(*Seeff et al., 2006***).

The incidence of HCC increases with age, reaching its highest prevalence among those aged over 65 years (***El-Serag, 2007***). Although HCC is rare before the age of 50 years in North America and Western Europe (***Bosch et al., 2004***), a shift in incidence towards younger persons has been noted in the last two decades. HCC tends to occur in the background of cirrhosis of the liver. In western countries, this holds true in

over 90% of cases, whereas in Asia and Africa the percentage of cases of HCC is higher in individuals with non-cirrhotic livers, compared to those with cirrhotic livers (***Montalto et al., 2002***).

Risk Factors

The major risk factor for the development of HCC is cirrhosis of the liver. The major known risk factors for HCC are viral (chronic hepatitis B and hepatitis C), toxic (alcohol and aflatoxins), metabolic (diabetes and non-alcoholic fatty liver disease, hereditary haemochromatosis) and immune-related (primary biliary cirrhosis and autoimmune hepatitis) (***Parikh et al., 2007***).

Hepatitis C Virus (HCV)

HCV is the most important risk factor for HCC in western European and North American countries, since epidemiological studies have shown up to 70% of patients with HCC have anti-HCV antibody in the serum (***Montalto et al., 2002***). The prevalence of HCV infection varies considerably by geographical region. African and Asian countries reported high HCV infection prevalence rates, while rates in North America, Europe and Australia have usually reported lower rates (***Suruki et al., 2006***).

Egypt has the highest prevalence of HCV in the world (predominantly genotype 4), which has been attributed to previous public health eradication schemes for schistosomaisis. Even higher HCV infection rates, up to 60%, have been reported in older individuals, in rural areas such as the Nile delta, and in lower social classes (***Hassan et al., 2001***).

The natural history of HCV infection has been investigated in several studies (***Montalto et al., 2002***). A Japanese study by ***Kiyosawa et al., 1990***, reported a time lag of 13 years from infection by transfusion of HCV