Evaluation of Serum Thioredoxin Level as a New Diagnostic Marker for Hepatocellular Carcinoma

Thesis

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Abb.	Full term
A1ATD	Alpha 1 antitrypsin deficiency
AASLD	American Association for the Study of Liver Diseases
AFB1	Aflatoxin B1
AFP L1, L2, L3	Alpha Fetoprotein L1, L2, L3
AFP	Alpha Fetoprotein
AFU	Alpha-l-fucosidase
AJCC	American Joint Committee on Cancer
ALT	Alanine aminoTranferase
ASN 233	A single asparanin linked 233
AST	Aspartate aminotransferase
BCLC	Barcelona clinic liver cancer
BMI	Body Mass Index
CD	Cluster of Differentiation
CD166	Cluster of Differentiation 166
CECT	Contrast enhanced CT
CEMRI	Contrast enhanced magnetic resonance imaging
CEUS	Contrast Enhanced Ultrasound
CK 7	Cytokeratins 7
CLIP	Cancer of the Liver Italian Program score
CRP	C- Reacive Protein
CT	Computed tomography
CTP	Child-Turcotte –pugh classification.
DCP	Des-gamma-carboxyprothrombin
DKK1	Dickkopf-1
DM	Diabetes Mellitus
DUS	Doppler Ultrasound
EASL	European Association for Study of Liver
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
ESLC	Egyptian Society of Liver Cancer.
EUS	Endoscopic US

List of Abbreviations cont…

Abb.	Full term
FBG	Fasting blood glucose
FDG	Fluorodeoxyglucose
FGF	Fibroblast growth factor
GEP	Granulin epithelin precursor
GGT mRNA	Gamma-Glutamyl Transferase mRNA
GGT	Gamma-Glutamyl Transferase
GLOBOCAN	Global cancer statics estimate project of WHO.
GOLPH2	Golgi phosphoprotein 2
GP73	Golgi Protein 73
GPC3	Glypican-3
Hb	Haemoglobin
HBeAg	Hepatitis B envelope antigen
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C virus
HCV-Ab	Hepatitis C antibody
HDV	Hepatitis D virus
HFL	Hepatic Focal Lesion
HGF	Hepatocyte growth factor
HGF/SF	Hepatocyte Growth Factor/ scatter factor
HIV	Human Immune Deficiency
HR	Hepatic resection
HSP-70	Heat Shock Protein 70
HTERT mRNA	Human telomerase reverse transcriptase mRNA
HTERT	Human telomerase reverse transcriptase
IGF-II	Insulin-like growth factor-II
IGFR	Insulin Growth Factor Receptor
IL-6	Interleukin- 6
INR	International Normalized ratio
LC	Liver Cirrhosis
LCA	Lectin Lens Agglutinin

List of Abbreviations cont…

Abb.	Full term
LKM	Liver kidney microsome
LT	Liver transplantation
M	Metastasis classification
MDCT	Multi Detector CT
MDK	Midkine
MELD	Model for End Stage Liver Disease
MiRNAs	MicroRNAs
MRI	Magnetic resonance imaging
MRP2	Multi-drug resistance associated protein 2
MWA	Micro wave ablation
N	Node Classification
NAFLD	Nonalcoholic fatty liver disease
NASH	Non-Alcoholic steato Hepatitis
NCI	National Cancer Institute
NO	Nitric oxide
PAI	Percutaneous acetic acid injection
PEI	Percutaneous ethanol injection
PIG3 P53	Inducible Gene-3
PIVIKA	Protein induced by vitamin K absence
PLT	Platelet
PPBG	Post prandial blood glucose
PS	Performance Score
PST	Performance Score test
PVT	Portal vein thrombosis
PVTT	Portal Vein Tumour Thrombosis.
RFA	Radiofrequency ablation
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
RT-PCR	Reverse transcription –polymerase chain reaction
SF	scatter factor
SLA	Soluble liver antigen
TAC	Trans arterial chemoembolization

List of Abbreviations cont…

Abb.	Full term
TACE	Transarterial chemoembolisation
TAE	Trans arterial embolization
TARE	Trans arterial radio-embolization
TGF-β1	Transforming growth factor-beta 1
TLC	Total leucocyte count
TNM	Tumor, node, metastasis staging
TPO	Thrombopoietin
TRX	Thioredoxin
TSGF	Tumor – Specific growth factor
TSGF	Tumor-specific growth factor
USA	United Sates of America
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WBC	White Blood Cells
WHO	World health organization

Abstract

AFP was significantly higher in HCC group than in cirrhotic and control groups ($p<0.001$) with median levels (186.5), (9.3), (3.5) ng/ml respectively, and insignificantly higher in cirrhotic group than in control group while **CRP level** was significantly different among studied groups being highest in HCC group followed by cirrhotic group and lowest in control group ($p<0.001$) with mean levels (10.5±3.7), (6.1±2.2), (2.4±1.4) mg/dl respectively.

Laboratory results as regard Hb, platelet, albumin, AST, ALT and bilirubin revealed insignificant difference between HCC and cirrhotic group ($p > 0.05$).

TRX has better diagnostic performance than AFP in differentiating HCC from other groups at a cut off point **≥ 100 ng/ml** for **TRX** where sensitivity and specificity with positive predictive value and negative predictive value (81.8%, 88.9%, 90%, and 80%) respectively and at a cut off point **≥ 25.6 ng/ml** for **AFP** where sensitivity and specificity with positive predictive value and negative predictive value (60%, 76.7%, 72%, and 65.7%) respectively. **Combined use of TRX and AFP** revealed higher diagnostic performance than using one of each markers alone with sensitivity, specificity, positive predictive value, and negative predictive value (85.4%, 91.3%, 87.8, and 82.4%) respectively.

Keywords: Transforming growth factor-beta 1- Thrombopoietin- Thioredoxin- Percutaneous ethanol injection

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common forms of cancer in the world and is the third leading cause of cancer related death ***(Jiang et al., 2014).***

More than 80% of cases occur in the developing countries; rates are more than twice as high in men compared to women. Among primary liver cancers occurring worldwide, HCC is the most common, accounting for 70– 85% of liver tumors ***(Castello et al., 2010).***

Major risk factors for HCC include infection with HBV or HCV, alcoholic liver disease, and most probably nonalcoholic fatty liver disease (NAFLD). Less common causes include hereditary hemochromatosis, alpha1-antitrypsin deficiency, autoimmune hepatitis, and Wilson's disease. Most of these risk factors lead to the formation and progression of cirrhosis, which is present in 80 to 90% of patients with HCC ***(El-Serag, 2011).***

Although tumor marker levels are not included in the diagnostic criteria for HCC or in the screening recommendations in the guidelines of the American Association for the Study of Liver Diseases (AASLD) or the European Association for the Study of the Liver (EASL), they provide valuable supportive information for diagnosing HCC ***(Toyoda et al., 2015).***

Ultrasound examination of the liver and detection of AFP level in serum are commonly used to screen for HCC. Although detection of AFP level is easy and less expensive, but it shows less sensitivity, since elevation in AFP level is common in patients with chronic liver disease, pregnancy and germ cell tumors. AFP titers also rise with flares of active hepatitis, and may be persistently elevated in patients with cirrhosis. Ultrasound is better, but is more expensive, operator dependent and less reliable in the presence of cirrhosis. Thus, new markers with high sensitivity and specificity are required ***(Zakhary et al., 2013).***

Current treatment strategies, such as liver transplantation, surgical resection or regional therapy for advanced HCC, are unsatisfactory. Chemotherapy is commonly used for the treatment of various malignancies. However, systemic cytotoxic chemotherapeutic agents have not significantly improved the survival of HCC patients because of the resistance of HCC to anticancer drugs. Tumor recurrence after curative liver resection remains high, and most patients die within several months of diagnosis ***(Jiang et al., 2014).***

Thioredoxin (Trx) is an ubiquitous antioxidant enzyme that is found in organisms ranging from archae to mammals. The first thioredoxin was originally discovered in 1964 in Escherichia coli as an electron donor for ribonucleotide reductase, an enzyme required for DNA synthesis ***(Collet and Messens, 2010).***

The Trx system plays a key role in regulating the overall intracellular redox balance. It basically comprises the small redox protein thioredoxin, nicotinamide adenine dinucleotide phosphate, in its reduced form (NADPH), and thioredoxin reductase (Trx R). Thioredoxin exerts many of its biological activities by reducing a variety of protein thiols, usually having a relatively low molecular weight disulfide. The activity of thioredoxin is regulated by NADPH, which in turn is produced by Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the oxidative hexose monophosphate shunt cycle (HMPS). Two thioredoxins have been cloned: (thioredoxin 1) that is found predominantly in the cytoplasm and (thioredoxin 2) that contains a mitochondrial import sequence ***(Li et al., 2015).***

Thioredoxins (Trxs) play multivalent cellular roles. They act as reductases in redox control, protect proteins from oxidative aggregation and inactivation, help the cells cope with various environmental stresses (reactive oxygen species (ROS), peroxynitrite and arsenate), and regulate programmed cell death. Some thioredoxins also act as growth factor, modulate the inflammatory response, promote protein folding, or play important roles in the lifecycles of viruses and phages ***(Collet and Messens, 2010).***