A Study of Cyclase-Associated Plasma Protein 2 as a Novel Biomarker for Detection of Hepato-Cellular carcinoma in HCV Patients.

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By

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*Last but not least, I dedicate this work to **my family**, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.*

Michael Yousri Shaker

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List of Abbreviations

A1ATD	:	Alpha 1 antitrypsin deficiency
AASLD	:	American Association for the Study of Liver Diseases
AFB1	:	Aflatoxin B1
AFP mRNA	:	AFP messenger RNA
AFP	:	Alpha Fetoprotein
AFU	:	Alpha-l-fucosidase
ALT	:	Alanine aminoTranferase
AST	:	Aspartate aminotransferase
BCLC	:	Barcelona clinic liver cancer
CAMP	:	Cyclic adenosine monophophate
CAP	:	Cyclase associated plasm protein
CAP2	:	Cyclase associated plasm protein 2
CD	:	Cluster of Differentiation
CECT	:	Contrast enhanced CT
CEMRI	:	Contrast enhanced magnetic resonance imaging
CEUS	:	Contrast Enhanced Ultrasound
CK 7	:	Cytokeratins 7
CRP	:	C- Reacive Protein
CT	:	Computed tomography
CTLA-4	:	Cytotoxic T-lymphocyte-associated protein 4
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
EASL–EORTC	:	European Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer
eDFR	:	estimated Glomerular Filtration Rate
EGFR	:	Epidermal growth factor receptor
EHIS	:	Egyptian Health Issues Survey
ELISA	:	Enzyme-Linked Immunosorbent Assay
ERK	:	Extracellular signal-Regulated Kinases

List of Abbreviations (Cont.)

ESLC	:	Egyptian Society of Liver Cancer.
EUS	:	Endoscopic US
FBG	:	Fasting blood glucose
FDA	:	Food And Drug Administration
GGT mRNA	:	Gamma-Glutamyl Transferase mRNA
GGT	:	Gamma-Glutamyl Transferase
GLOBOCAN	:	Global cancer statics estimate project of WHO
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
HFD	:	Helical folded domain
HFL	:	Hepatic Focal Lesion
HGDN	:	High-grade dysplastic nodule
HGF	:	Hepatocyte growth factor
HGF/SF	:	Hepatocyte Growth Factor/ scatter factor
HIV	:	Human Immune Deficiency
HR	:	Hepatic resection
HSP-70	:	Heat Shock Protein 70
IDU	:	Intravenous drug use
IGF-II	:	Insulin-like growth factor-II
IGFR	:	Insulin Growth Factor Receptor
INR	:	International Normalized ratio
JNK	:	Jun N-terminal kinases consist
KLCSG-NCC	:	Korean Liver Cancer Study G, National Cancer Center
LC	:	Liver Cirrhosis
LCA	:	Lectin Lens Agglutinin
LT	:	Liver transplantation
MDCT	:	Multi Detector CT
MDK	:	Midkine
MELD	:	Model for End Stage Liver Disease
MiRNAs	:	MicroRNAs
MRI	:	Magnetic resonance imaging

List of Abbreviations (Cont.)

MWA	:	Micro wave ablation
NAFLD	:	Nonalcoholic fatty liver disease
NASH	:	Non-Alcoholic steato Hepatitis
NO	:	Nitric oxide
NSCLC	:	Non small cell lung cancer
OPN	:	Osteopontin
PAI	:	Percutaneous acetic acid injection
PBMCs	:	Peripheral blood mononuclear cells
PCR	:	Polymerase chain reaction
PDGF	:	Platelet-derived growth factor
PEIT	:	Percutaneous ethanol injection Therapy
PLT	:	Platelet
PVT	:	Portal vein thrombosis
RFA	:	Radiofrequency ablation
ROC	:	Receiver operating characteristic
ROS	:	Reactive oxygen species
RT-PCR	:	Reverse transcription –polymerase chain reaction
SCCA	:	Squamous Cell Carcinoma Antigen
SEER	:	Surveillance Epidemiology End Result
SIRT	:	Selective internal radiation therapy
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
TSGF	:	Tumor – Specific growth factor
VEGF	:	Vascular endothelial growth factor
VEGFR	:	Vascular endothelial growth factor receptor
WBC	:	White Blood Cells
WHO	:	World health organization

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Introduction

Hepatocellular carcinoma (HCC) is the dominant form of primary liver cancer and is histologically and etiologically distinct from other forms of primary liver cancer (**Yu et al., 2008**).

Approximately 70%–90% of patients with HCC have an established background of chronic liver disease and cirrhosis, with major risk factors for developing cirrhosis including chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease, and nonalcoholic steato-hepatitis (NASH) **(El-Serag et al., 2007**).

Additional risk factors for developing HCC include intake of aflatoxin-contaminated food, diabetes, obesity, certain hereditary conditions such as hemochromatosis, and some metabolic disorders (**Montalto et al., 2002**).

HCC is unusual among human cancers in that the causative agent is often clear. However, there are multiple etiologic factors affecting HCC, all of which vary by geographic location, have a direct impact on the characteristics of these patients, and influence the disease course, making HCC an extremely complex condition associated with a poor prognosis **(Venook et al., 2010).**

HCC carcinogenesis is a complex process that can involve various modifications to a number of molecular pathways as well as genetic alterations, and ultimately leads

to malignant transformation and HCC disease progression **(Shekih et al., 2008).**

In molecular biology, the Cyclase-associated protein family (CAP) is a family of highly conserved actin binding proteins present in a wide range of organisms and mammals. CAPs are multifunctional proteins that contain several structural domains. CAP is involved in species-specific signalling pathways (**Deeks et al., 2007**).

CAPs family includes CAP1 and CAP2. They are firstly identified in yeast and mediate thecAMP-dependent pathway whichis necessary for many different cell responses as the increase in heart rate, cortisol secretion, and breakdown of glycogen and fat (**Effendi et al., 2013).**

It has been recently reported that CAP2 (cyclase-associated protein 2) is up-regulated in HCC patients and its expression correlates to the multistage development of HCC, CAP2 is also closely associated with cellular invasion and metastasis of HCC. In addition, CAP2 can be detected in tumor cells invading the stromal area in liver tissue (**Chuma et al., 2013**).

Aim of the Work

The aim of this study is to assess the value of plasma cyclase-associated protein 2 level in diagnosis of hepatocellular carcinoma among the Egyptian patients with chronic hepatitis C virus.

Chapter (I)

HEPATOCELLULAR CARCINOMA

Epidemiology of HCC:

HCC is one of the major malignant tumors in the world and considered one of the biggest causes of cancer related death. There is a great increase in the number of cases per year and has a bad fulminant course with a bad prognosis.it has a high recurrence rate after resection and a poor response to anti cancer drugs and radiotherapy (***Kew, 2014)***.

Primary liver tumors are considered the second largest cause of tumor mortality worldwide, Hepatocellular carcinoma is the most common histological pattern (around 80%) from liver cancers which is a parenchymal cell malignancy of the liver.in the same time Intrahepatic cholangiocarcinoma is considered the second commonest type (approximately 15%) ***(McGlynn et al., 2015)***.

HCC is the fifth most common tumor worldwide (approximately 600, 000 new cases per year) and there are some variations as follows;

- Areas with high numbers of cases of hepatitis C and B (East Asia, sub-Saharan Africa) have highest incidence
- Males are more affected than females, with incidence ratios between 2:1 and 4:1.
- Peak incidence: fifth and sixth decades in Western countries, earlier in areas with perinatal transmission of hepatitis B (***Ferri, 2017).***

The incidence and prevalence of HCC include many variations among geographic locations, racial and ethnic groups ***(Al-Mahtab et al., 2014).***

There is a wide variation in HCC incidence with geographic variations more obviously, in Europe it is approximately 8.6/100, 000 people. in certain regions of Africa and Asia this rate levels up to 120/100, 000 people. and it is related to the different level of exposure to many specific risk factors ***(Lopez et al., 2015).***

In Egypt, liver tumors especially HCC forms 11.75% of malignancies of all digestive system tumors and 1.68% of the all malignancies. HCC is approximately 70.48% of all liver tumors in Egypt. HCC represents the main complication of Liver cirrhosis, and shows a growing numbers in Egypt ***(Holah et al., 2015).***

A retrospective analysis study was done reviewing 1313 Egyptian patients with HCC. The majority in the studied patients were males {1035 (3.7) males: 278 (1) females}, of which 75% were living in rural areas. Most of the cases were between 50 and 60 years of age on presentation. Underlying HCV related liver disease was responsible for 91% of the cases (***Shaker et al (2013)***.

In Egypt, HCC is the first commonest malignancy in males (**figure 1)** and the second commonest malignancy in females **(figure 2)** and HCC has a growing peak, rising from 4.0% in 1993 to 7.2% in 2002, being obviously more prevalent in rural residents and in patients with previous history of Schistosomiasis and/or patients with blood transfusion ***(Ferlay et al., 2013).***

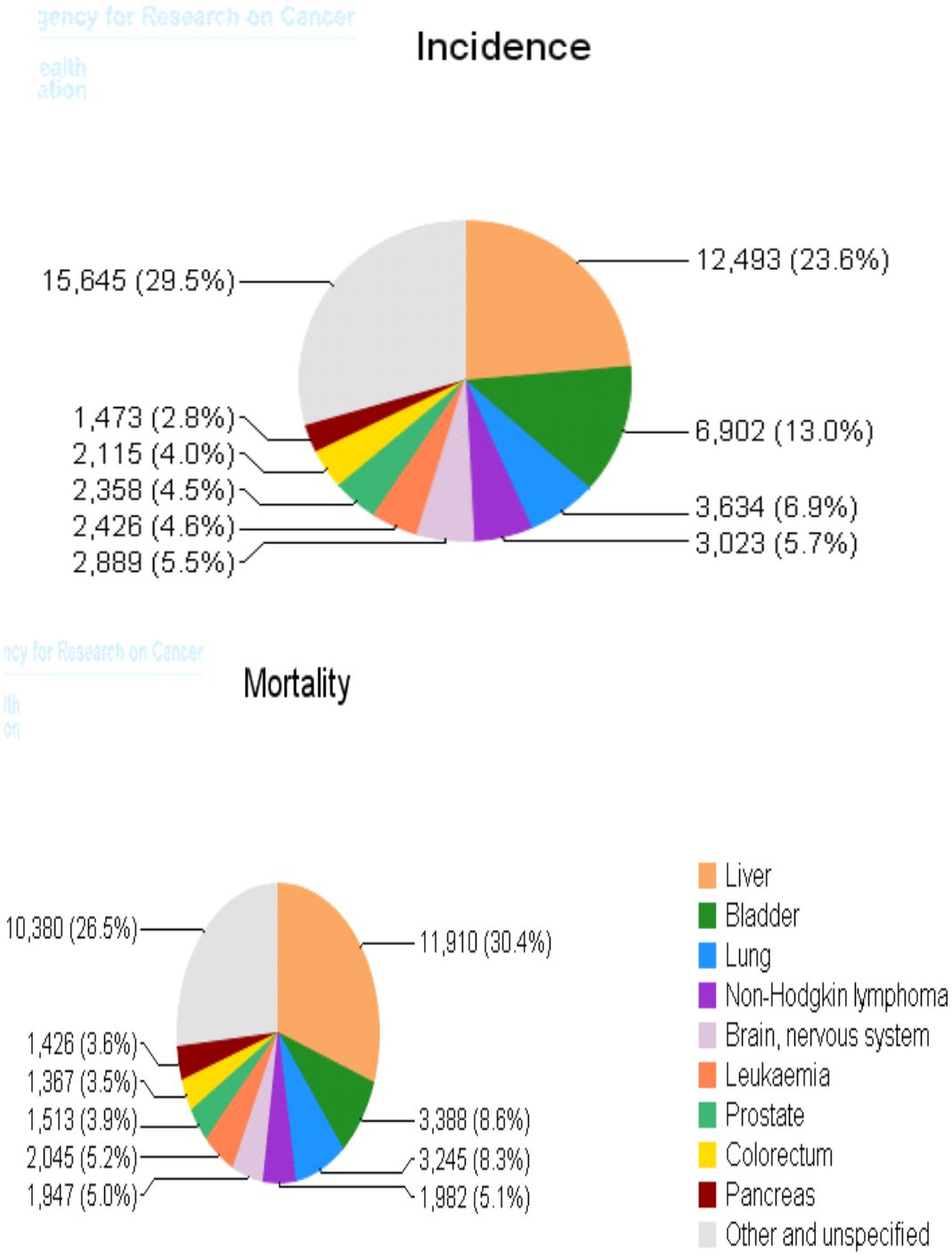

Figure (1): Incidence and mortality of HCC in Egyptian men by ***(GLOBOCAN, 2012).***

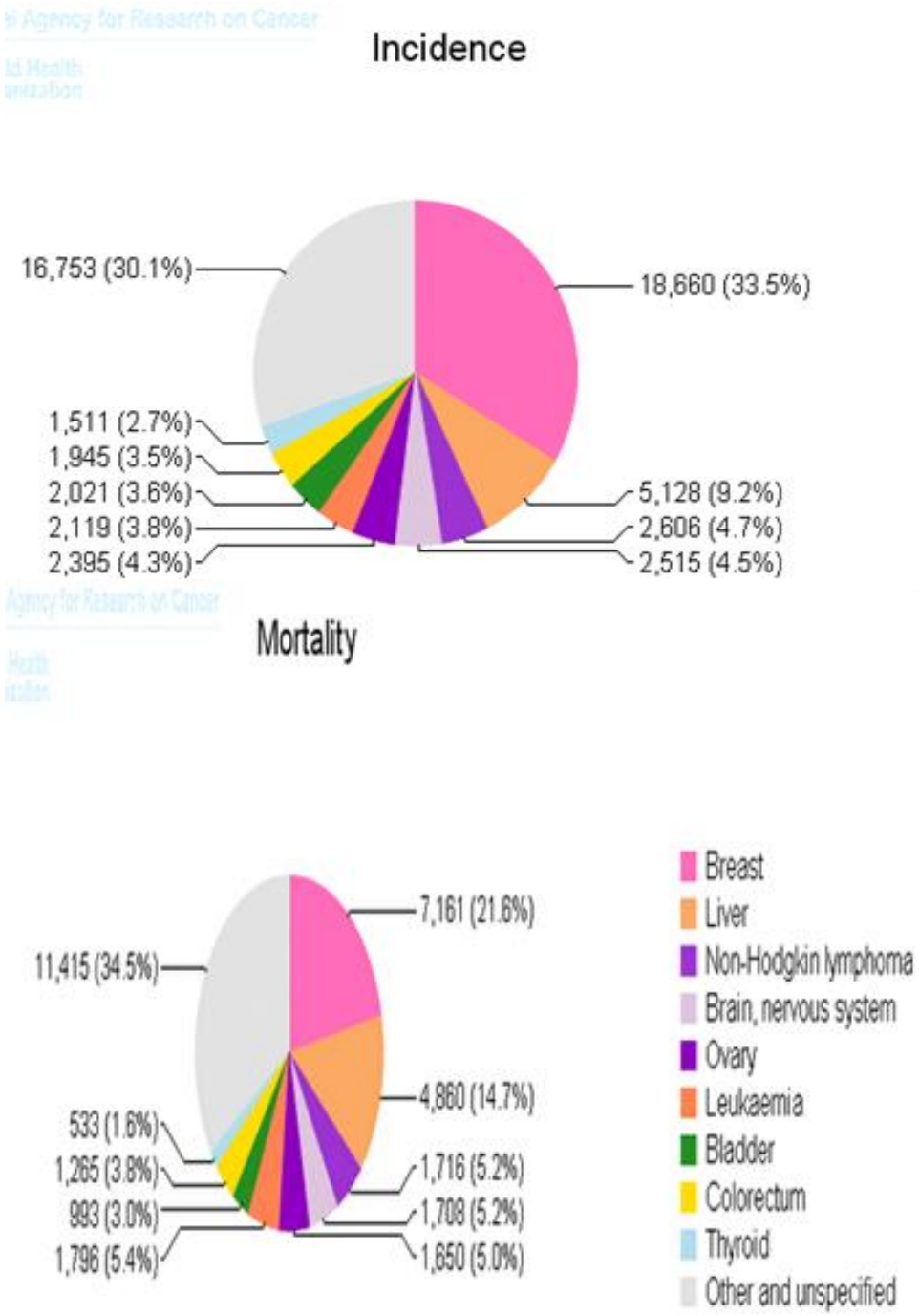

Figure (2): Incidence and mortality of HCC in Egyptian women by ***GLOBOCAN (2012)***