

Evaluation OF PNPLA3 level in Liver Cirrhosis and Hepatocellular Carcinoma in Egyptian Patients

Thesis

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List of abbreviations

AASLD	American Association for the Study of Liver Diseases
AAT	Alpha 1 antitrypsin
AFB1	Aflatoxin B1
AFP	Alpha- fetoprotein
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
APASL	Asian Pacific Association for the Study of the Liver disease
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CBC	Complete blood picture
CCC	Cholangiocarcinoma
CDC	Centers for Disease Control and Prevention
CEA	Carcino embryonic antigen
CH	Chronic hepatitis
CLD	Chronic liver disease
Cr	Creatinine
CT	Computed tomography
DALYs	Disability Adjusted Life Years
DNA	Deoxy-ribo nucleic acid
D.bilirubin	Direct bilirubin
T.bilirubin	Total bilirubin
DUS	Doppler ultrasound
ESLC	Egyptien society of liver cancer
GWAS	Genome-wide association studies
GGT	Gamma glutamyl transferase
Hb	Haemoglobin
HFL	Hepatic focal lesion

HDV	Hepatitis D virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIFU	High intensity focused ultrasound.
HIV	Human immunodeficiency virus
INR	International normalized ratio
IVC	Inferior vena cava
IFN	Interferon
MRI	Magnetic resonance imaging
NPD	Nucleotide phosphodiesterase
NAFLD	Non-alcoholic fatty liver disease
NASH	Non alcoholic steatohepatitis
PET	positron emission tomography
PEI	Percutaneous ethanol injection
PS	Performance status
PNPLA	Patatin-like phospholipase
RNA	Ribo nucleic acid
RFA	Radiofrequency ablation
ROS	Reactive oxygen species
SD	Standard deviation
SNP	Single nucleotide polymorphism
TACE	Transcatheter arterial chemoembolization
TARE	Transarterial radio-embolization
TNM	Tumor, Node, Metastasis
US	Ultrasound
WBCs	White blood cells

Acknowledgement

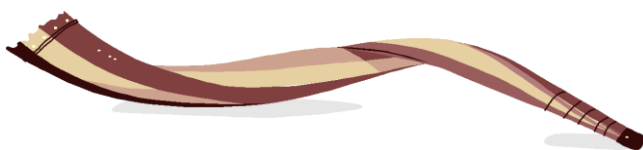
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Introduction

Liver diseases have a significant impact on worldwide mortality and account for 2% of total deaths **(Lozano et al., 2010)**. Like other complex diseases, liver diseases are caused by a combination of environmental and genetic factors and their interactions. **(Visscher et al., 2008)**

Hepatocellular carcinoma (HCC) is the most common form of primary malignancy of the liver, and the second leading cause of cancer-related mortality worldwide. **(Mittal et al., 2013)**

Patatin-like phospholipase domain-containing 3 (PNPLA3), also called adiponutrin, belongs to a novel class of patatin-like phospholipase family proteins, which have emerged as a new biomarker of human hepatic steatosis and nonalcoholic fatty liver disease. **(Romeo et al., 2008)**

A large volume of research data has demonstrated that PNPLA3 is highly expressed in hepatic stellate and hepatoma cells, suggesting a potential role in cirrhosis and hepatocarcinogenesis. **(Min et al., 2014)**

Recently, there is a strong link between a single nucleotide polymorphism (SNP) in the PNPLA3 gene, rs738409, and the development of NAFLD, alcoholic liver disease (ALD), and chronic hepatitis **(Brouwer et al., 2015)**; however, its association with HCC risk is less well-defined, and findings of significant association with HCC related to cirrhosis have been inconsistent. **(Guyot et al., 2013)**

Genetic variants, such as those located in PNPLA3, could be useful for the reclassification of patients. Patients originally assigned to a risk category based on well known risk factors (e.g. older age, male gender,

and BMI for HCC developed on a background of alcoholic cirrhosis) could be reclassified to a higher or lower category after the inclusion of an additional factors such as genetic variants. **(Manolio et al., 2010)**

Aim of the Work

To study the role of PNPLA3 as a novel biomarker in liver cirrhosis and hepatocellular carcinoma in Egyptian patients.

Liver Cirrhosis

Chronic hepatitis C virus (HCV) infection affects about 170 million people worldwide and is the most common cause of chronic liver disease. Of these HCV-infected individuals, 20–30% eventually develops liver cirrhosis (LC) or hepatocellular carcinoma (HCC). **(Imai et al., 2010)**

Histologically, LC is characterized by diffuse nodular regeneration surrounded by dense fibrotic septa with subsequent collapse of liver structures and thus causes pronounced distortion of vascular architecture in the liver. **(Schuppan and Afdhal, 2008)**

Chronic liver diseases (CLDs) comprise several diseases were grouped together because they have common clinical manifestations and characterized by chronic necroinflammatory injury that can lead to liver cirrhosis and end-stage liver disease. **(Rahimi and Rockey, 2011)**

EPIDEMIOLOGY OF HEPATIC CIRRHOSIS

Liver cirrhosis has emerged as a major cause of global health burden. According to the Global Burden of Disease 2010 study, liver cirrhosis caused 31 million Disability Adjusted Life Years (DALYs), or 1.2% of global DALYs, in 2010, and one million deaths, or 2% of all deaths worldwide in that year. **(Murray et al., 2012)**

The epidemiology of liver cirrhosis has been evaluated extensively in several developed countries in Europe and the Americas. There has been much less interest in mortality from the disease, however, in much of the developing world. **(Bosetti et al., 2007)**

The prevalence of CLD continues to rise, especially with the epidemic of obesity and viral hepatitis. CLD and cirrhosis was estimated by the Centers for Disease Control and Prevention (CDC) to be the 12th leading cause of mortality in the United States in 2007 accounting for 29165 deaths, which was 3.4% higher than 2006, resulting in the second largest percentage increase of all-cause mortality. **(National Center for Health Statistics 2010)**

Liver cirrhosis in Egypt

According to the latest WHO data published in April 2011 Liver Disease Deaths in Egypt reached 26,649 or 7.34% of total deaths. The age adjusted Death Rate is 50.91 per 100,000 of population ranks Egypt the third in the world **(World Health Organization 2011)**.

ETIOLOGIES OF HEPATIC CIRRHOSIS

The most common cause of cirrhosis in Egypt is chronic viral hepatitis. Because of high prevalence rate of hepatitis C virus (HCV) in the general Egyptian population, HCV is considered the major risk factor for cirrhosis **(Hassan et al. 2001)**.

Egypt has the highest prevalence of HCV in the world, with 10% of its population 15-59 years of age being chronically infected. **(Egypt. Demographic and Health Survey, 2008)** The chronic infection rate increases with age and goes up to more than 25% for 50-60 year-olds. Among 15-19 year-olds, 4% are chronically infected, demonstrating ongoing HCV transmission. An estimated 150,000 new HCV infections occur each year in Egypt¹ and HCV morbidity and mortality are predicted to double in the coming 20 years. **(Breban et al., 2012)**

Table 1: Etiologies of hepatic cirrhosis (Crawford 2005)

Most common causes 1 Chronic hepatitis B or C 2 Alcohol 3- Primary or secondary biliary cirrhosis 4- Non-alcoholic fatty liver disease (NAFLD)
Less common causes 1- Autoimmune hepatitis 2- Genetic metabolic disease - Hemochromatosis - Wilson's disease - Alpha 1-antitrypsin deficiency - Amino acid disorder (e.g. tyrosinemia) - Lipid disorder (e.g. abetalipoproteinemia) - Carbohydrate disorders (e.g. glycogen storage diseases) 3- Vascular abnormalities - Chronic, passive hepatic congestion caused by right-sided heart failure, pericarditis - Budd-Chiari syndrome - Veno-occlusive disease 4- Idiopathic/ miscellaneous - Granulomatous liver disease (e.g. sarcoidosis) - Idiopathic portal fibrosis - Indian childhood cirrhosis - Polycystic liver disease

COMPLICATIONS OF HEPATIC CIRRHOSIS

The clinical course of patients with advanced cirrhosis is usually complicated by a number of important sequels, which are independent of the underlying liver disease. **(Rahimi and Rockey 2011)**

Portal Hypertension and Variceal Bleeding

Portal hypertension is a clinical syndrome hemodynamically defined by a pathological increase of portal pressure gradient (pressure difference between portal vein and inferior vena cava) and by formation of portal-systemic collaterals that shunt part of the portal blood flow to systemic circulation bypassing the liver **(Garcia-Pagan et al. 2005)**.

Portal hypertension is a frequent complication of liver cirrhosis, which develops in many patients and plays a role in the development of other complications of the disease. Portal hypertension results in the development of esophago-gastric varices which often bleed; and plays a role in the development of ascites, hepatorenal syndrome and hepatic encephalopathy. Portal hypertension and resulting portosystemic collaterals may also be responsible for the cardiopulmonary complications like porto-pulmonary hypertension and hepatopulmonary syndrome. **(Naeije, 2003)**

Normal values of the portal pressure gradient are of 1-5 mm Hg. Clinically significant portal hypertension (CSPH) is diagnosed when clinical manifestations of the disease appear or when portal pressure gradient (PPG) – in case of cirrhosis determined by its equivalent, the hepatic venous pressure gradient (HVPG) exceeds a threshold value of 10 mm Hg. **(Bosch et al. 2006)**

Variceal hemorrhage is the most common complication associated with portal hypertension. Other complications include hepatic

encephalopathy, SBP, ascites, gastric varices, and hepatorenal syndrome **(Genovesi et al. 2009)**.

Variceal hemorrhage is the most common complication associated with portal hypertension. Almost 90% of patients with cirrhosis develop varices, and approximately 30% of varices bleed. The first episode of variceal hemorrhage is estimated to carry a mortality rate of 30-50%. **(Genovesi et al., 2010)**

Bleeding from ruptured esophago-gastric varices is the most severe complication of cirrhosis. In patients with cirrhosis, the overall incidence of variceal bleeding is about 4% per year. This risk increases to 15% per year in patients with medium / large varices. The most important predictive factors related to the risk of bleeding are variceal size, Child-Pugh class and presence of red signs. **(Rodríguez et al. 2007)**

In addition, a combination of portal hypertension and splanchnic arterial vasodilation alters splanchnic microcirculation and intestinal permeability, facilitating the leakage of fluid into the abdominal cavity and hence ascites. Sodium retention and ascites develop and decreased free water excretion leads to dilutional hyponatremia and eventually to impaired renal perfusion and hepatorenal syndrome. Thus portal hypertension effectively plays a role in the development of ascites and the hepatorenal syndrome. **(Qamar et al., 2008)**

Ascites

Ascites is defined as the pathologic accumulation of fluid in the peritoneal space. 20% of cirrhotic patients have ascites at the time of diagnosis, while 30% and 50% will develop ascites by 5 and 10 years, respectively **(Runyon 2004)**.