Study of Retinoic Acid Level in Accessing Degree of NAFLD

Thesis

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بسم الله الرحمن الرحيم

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللهِ عَلَيْكَ عَظِيمًا﴾

صدق الله العظيم

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First of all, I would like to express my deep gratitude to **Allah**, *the most merciful and the most grateful. Thanks for all gifts and thanks for love of all people I am living with.*

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List of Contents

Title Page No.

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term

BMI.....Body mass index.
CAP.....Controlled Attenuation Parameter.
CEU .....Contrast Enhanced Ultrasound.
DDP4 .....Dipeptidyl Peptidase 4.
GLP1..... Glucagon like Peptide 1.
HDL .....High Density Cholesterol.
HSC .....Hepatic Stellate Cells.
HU .....House field Unit.
IGB.....Intragastric Balloon.
KP ..... Kilo Pascal.
LDL.....Low Density Cholesterol.
MR .....Magnetic Resonance.
MRI.....Magnetic Resonance Imaging.
MS.....Metabolic Syndrome.
NAFLD .....None-Alcoholic Fatty Liver Disease.
NASH.....None-Alcoholic Steatohepatitis.
NGT .....None Glucose Tolerance.
NPPV ..... Negative Predictive Value.
RAR..... Retinoic Acid Receptor.
ROS..... Reactive Oxygen Species.
RXR.....Retinoic acid X Receptor.
SGT2.....Sodium Glucose Transporter 2.
TE .....Transient Elastography.
TZDS .....Thiazolidnedionees.
VLDL ..... Very low Denisty Lipoproteins

Abstract

In our study, ALT was significantly highest in NASH group, followed by NAFLD and lowest in control group with no significant difference between control and NAFLD groups. But, there was no significant difference between study groups regarding AST.

Our study showed that retinoic acid was significantly different among studied groups, was lowest in NASH group, followed by NAFLD and high in control group. Also, NAFLD score was significantly highest in NASH group, followed by NAFLD and lowest in control group with no significant difference between control and NAFLD groups. NAFLD grade was significantly different among study groups; was highest in NASH group, followed by NAFLD and lowest in control group.

Correlations revealed significant negative correlations between retinoic acid and NAFLD score among NAFLD and NASH groups. Receiver operating characteristics (ROC) curve was used to define the best cutoff value of serum retinoic acid level.

Retinoic acid had significantly high diagnostic performance in differentiation between NAFLD group and control group with 80% sensitivity and 100% specificity and also in differentiation between NASH group and NAFLD group with 82.5% sensitivity and 77.5% specificity.

Keywords: Retinoic Acid Receptor- Reactive Oxygen Species- Retinoic acid X Receptor- Transient Elastography- Thiazolidnedionees- Dipeptidyl Peptidase 4

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the abnormal accumulation of lipids, primarily in the form of triglycerides in individuals who do not consume significant amounts of alcohol (≤ 20 g ethanol/d). It is characterized by a spectrum of disease varying from simple steatosis through to steatohepatitis with fibrosis and scarring which can lead to cirrhosis ***(Hazlehurst and Tomlinso, 2013).***

NAFLD is the most common liver disease of the Western world, largely due to obesity induced by readily available high energy food and sedentary lifestyle of the modern society. As a consequence, the incidence of NAFLD is rising in parallel with the rising rates of obesity worldwide. Currently, the prevalence of NAFLD stands at 24.4% globally ***(Zhu et al., 2015).***

Retinoic acid is a metabolite of vitamin A (retinol) that mediates the functions of vitamin A required for growth and development. Retinoic acid is required in chordate animals which includes all higher animals from fish to humans. During early embryonic development, retinoic acid generated in a specific region of the embryo helps determine position along the embryonic anterior/posterior axis by serving as an intercellular signaling molecule that guides development of the posterior portion of the embryo. It acts through Hox genes, which ultimately control anterior/posterior patterning in early developmental stages ***(Duester, 2008).***

Retinoic acid (RA) is a morphogen derived from retinol (vitamin A) that plays important roles in cell growth, differentiation, and organogenesis. The production of RA from retinol requires two consecutive enzymatic reactions catalyzed by different sets of dehydrogenases. The RA interacts with retinoic acid receptor (RAR) and retinoic acid X receptor (RXR) which then regulates the target gene expression ***(Kam et al., 2012).***

Bonet et al. (2012) revealed that RA effectively reduced adiposity not only in fat but also in liver through increased triglyceride hydrolysis and fat oxidation.

Liu et al. (2015) investigated the clinical relevance of RA in patients with NAFLD and NASH. They showed that circulating RA concentrations were lower in subjects with NAFLD and was associated with insulin resistance.

Aim of the Work

The aim of this work is to study the role of retinoic acid in development and progression of NAFLD from simple steatosis to NASH to prevent progression to chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC).

Chapter One

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) is defined as the abnormal accumulation of lipids, primarily in the form of triglycerides in individuals who do not consume significant amounts of alcohol (≤ 20 g ethanol/d). It is characterized by a spectrum of disease varying from simple steatosis through to steatohepatitis with fibrosis and scarring, which can lead to cirrhosis ***(Hazlehurst and Tomlinson, 2013).***

Currently, NAFLD is the most common liver disease of the Western world, largely due to obesity induced by readily available high energy food and sedentary lifestyle of the modern society. As a consequence, the incidence of NAFLD is rising in parallel with the rising rates of obesity worldwide. Currently, the prevalence of NAFLD stands at 24.4% globally ***(Zhu et al., 2015).*** The mortality rate and the number of liver transplantations due to NAFLD are also on the rise, and non-alcoholic steatohepatitis (NASH) is now the second leading indication for liver transplantation in the US ***(Wong et al., 2015).***

Simple steatosis usually follows a benign clinical course, while NASH is considered to be a potentially health threatening that may progress to cirrhosis, liver cell failure and HCC ***(Naschimbeni et al., 2013).*** Therefore physicians are required

to accurately differentiate NASH from simple steatosis and evaluate the severity of liver fibrosis in order to determine the prognosis and optimal treatment.

At present, the gold standard technique for the diagnosis of NASH is the liver biopsy which is an invasive procedure with possible serious complications and limitations. A reliable non-invasive test as a replacement for liver biopsy would allow definite diagnosis in NAFLD patients and enables us to feasibly re-evaluate patients during follow up ***(Bell et al., 2010).***

Natural history:

The natural history of patients with NAFLD has a mixed picture. In a large cohort study, it was demonstrated that liver related illness was the third leading cause of death in liver patients, and the hazard ratio for general mortality and liver related mortality was 1.038 and 9.32, respectively**.** As in the general population, the leading cause of death in patients with NAFLD is cardiovascular disease ***(Ong et al., 2008).***

This highlights the need for patients with NAFLD to have extensive risk management therapy for the prevention of cardiovascular disease. However, no concrete guidelines have been made for the prevention of adverse cardiac events in these patients ***(Monsour et al., 2012)***.

Diagnosis:

The diagnosis of NAFLD requires that there is hepatic steatosis by imaging or histology, there is no significant alcohol consumption, no competing etiologies are present for hepatic steatosis, and there are no coexisting causes for chronic liver disease ***(Chalasani et al., 2012).***

Patients with NAFLD are typically asymptomatic, and when present, manifest with vague symptoms such as fatigue and abdominal discomfort ***(Machado and Cortez-Pinto., 2013).*** On physical examination, it is useful to examine risk factors for NAFLD such as an increased body mass index (BMI), weight, and elevated blood pressure ***(Wilkins et al., 2013).*** Furthermore, as there exists an association between metabolic syndrome and NAFLD ***(Ratziu et al., 2010).***

Presence of insulin resistance should raise suspicion for NAFLD. It is important to rule out common causes of liver injury, such as alcohol, drug use, and viral hepatitis as well as other co-existing etiologies for chronic liver disease including alpha-1 antitrypsin deficiency, autoimmune liver disease (types 1 and 2), chronic viral hepatitis, and Wilson's disease ***(Wilkins et al., 2013).***

Laboratory tests for alpha-1 antitrypsin deficiency, antinuclear antibody, smooth muscle antibody, anti-liver/kidney microsomes antibody, anti-liver cytosol antigen, serum ferritin

and transferrin levels, HFE genetic testing, as well as ceruloplasmin levels should be obtained ***(Loria et al., 2010).***

Elevated alanine aminotransferase(ALT) and aspartate aminotransferase level(AST) may indicate the presence of hepatic steatosis, inflammation, or fibrosis, however their utility in the diagnosis of NASH is limited because of their low specificity, sensitivity, and prognostic value ***(Musso et al., 2011).***

Serum markers:

There have been a number of serum markers that have been proposed for the diagnosis of NAFLD, but very few that have been extensively researched. In particular, cytokeratin-18 fragments have shown the most promising the diagnosis of NASH ***(Machado and Cortez-Pinto., 2013).***

Scoring systems for identifying NASH from NAFLD:

Scoring systems using one or several clinical and/or laboratory parameters to identify patients with NASH from the larger pool of NAFLD patients also have been assessed ***(Mitry et al., 2007)***.

Palekar et al. (2005) developed a clinical model that sums 5 risks factors for NASH identified on multivariate logistic regression. These factors include age 50 or older, female sex, aspartate aminotransferase (AST) level of 45 or