INTRODUCTION

Chronic liver diseases represent a major public health problem, accounting for significant morbidity and mortality worldwide. The prevalence of chronic infection is estimated to be 2 million persons their prognosis and management greatly depend on the amount and progression of liver fibrosis with the risk of developing cirrhosis ***(Armstrong et al., 2006)***.

In the year 2000, ***Frank et al.*** found that Egypt had the highest countrywide prevalence of hepatitis C virus (HCV) in the world, with an estimated 8–10 million among a population of 68 million having been exposed to the virus and 5–7 million active infections. An important cause for the high exposure to HCV was the establishment of a large reservoir of infection as a result of extensive schistosomiasis control programs that used intravenously administered tartar emetic 20–50 years ago.

Only 15–20% of people infected with HCV have an acute viral hepatitis syndrome, but the majority develops chronic hepatitis that is usually asymptomatic and undetected for many years. Over a course of 20–40 years approximately 20% of those with HCV-caused chronic hepatitis progress to cirrhosis, and a proportion of these (possibly 2–3% per year) die as a result of complications of cirrhosis or hepatocellular carcinoma. Other causes for chronic liver disease, but less frequent than HCV, include HBV infection, Budd Chiari syndrome, portal hypertension and alcohol intake ***(Hoofnagle, 2002).***

Natriuretic peptides consist of the three family members namely, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). C-type natriuretic peptide is widely expressed in the vasculature, especially on the endothelium ***(Barr et al., 1996)***, and many inflammatory cytokines including interleukin-1, TNF-α and endotoxins are known to trigger the release of C-type natriurectic peptide from endothelial cells in animal models ***(Suga et al., 1993)***. Therefore, circulating NT-proCNP was suggested to be a possible biomarker for inflammatory conditions ***(Bahrami et al., 2010)***.

Many studies showed that circulating NT-proCNP was inversely correlated to parameters reflecting hepatic biosynthetic capacity in critically ill patients, namely albumin and pseudocholinesterase activity ***(Koch et al., 2011)***. Because sepsis and cirrhosis are both characterised by a hyperdynamic circulation associated with a low systemic vascular resistance and the release of many pro-inflammatory mediators, it was speculated that NT-proCNP might be involved in the progression of chronic liver disease and portal hypertension ***(Vincent and Gustot, 2010).***

AIM OF THE WORK

The aim of this work is to study serum level of N- terminal pro C- type natriuretic peptide in chronic liver disease patients with and without ascites and to correlate its level with the progression of liver diseases in such patients.

Chapter (1)

CHRONIC LIVER DISEASE

A. Introduction:

The liver aids greatly in the maintenance of metabolic homeostasis by processing dietary amino acids, carbohydrates, lipids, and vitamins; metabolizing cholesterol and toxins; producing clotting factors; and storing glycogen ***(Friedman et al., 2004)***.

B. Definition:

Chronic liver diseases (CLDs) are characterized by progressive destruction and regeneration of the liver parenchyma. This injury is associated with an influx of acute or chronic inflammatory cells leading to fibrosis and cirrhosis. Clinically, it is defined as any inflamation lasting for 6 months or longer ***(Goldberg, 2009)***.

C. Pathogenesis:

Liver fibrosis is a dynamic process consisting of the chronic activation of the wound healing reaction in response to sustained liver damage, leading to the excessive deposition of extracellular matrix (ECM) into the liver and eventually, if the cause of injury is not removed, liver cirrhosis occurs ***(Friedman, 2003)***.

Whatever the etiology, the fibrogenic processes within the liver share similar features including the presence of an inflammatory state due to infiltrating leukocytes and macrophages and activation of ECM-producing cells and leads to progressive scarring and liver cirrhosis ***(Bertolani and Marra, 2008)***.

The activated (myo) fibroblast is the cell type responsible for wound closure and fibrosis (reflecting persistent wound-healing activity attributable to chronic damage) in any CLD. Several potential sources for this critical mediator exist, including bone-marrow-derived fibrocytes or circulating mesenchymal cells, which can pass through the injured liver and become myofibroblasts ***(Friedman, 2008)***. Resident cells, e.g. tissue fibroblasts located in the portal tract of the liver or quiescent hepatic stellate cells (HSC) located in the Space of Disse, might also be activated to become myofibroblasts ***(Popov and Schuppan, 2010)***.

Any chronic insult to the liver can cause progression to cirrhosis. Although numerous pathophysiologic mechanisms of injury exist, the final common pathway is persistent wound healing resulting in hepatic parenchyma fibrosis. In most persons, approximately 80 to 90 percent of the liver parenchyma must be destroyed before liver failure is manifested clinically. Cirrhosis refers to a progressive, diffuse, fibrosing, nodular condition that disrupts the entire normal architecture of the liver *(Figures 1 through 4*) ***(Friedman et al.,***

2004). When complications of cirrhosis occur, they typically are related to impaired hepatic function or actual physical disruption and reorganization of the liver parenchyma ***(Crawford, 2005)***.

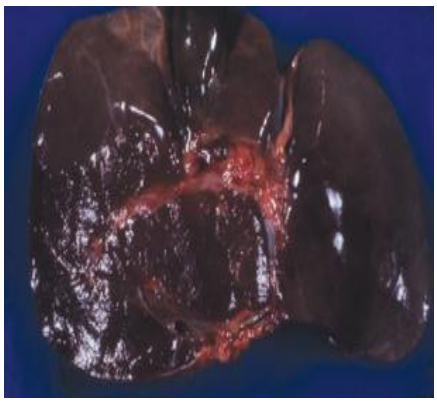

Figure (1): Inferior surface of liver, biliary tree, and gallbladder (gross) revealing normal hepatic tissue and structure

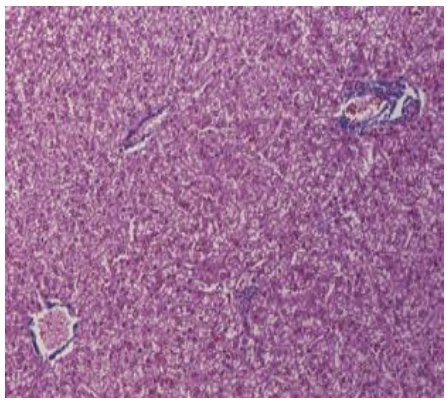

Figure (2): Normal hepatic tissue (microscopic, 10X, trichrome stain).

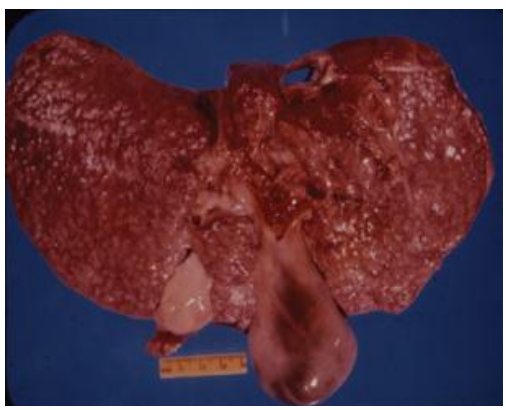

Figure (3): Inferior surface of liver and gallbladder (gross) revealing cirrhotic liver.

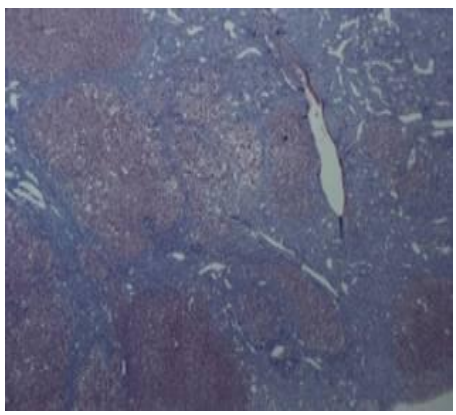

Figure (4): Cirrhosis (microscopic, 10X, trichrome stain).

(Friedman et al., 2004)

D. Etiologies Of Chronic Liver Disease:

Causes of chronic liver disease can be classified into most common causes and less common ones (table 1) ***(Wiegand and Berg, 2013)***.

Table (1): **Causes of chronic liver disease *(Wiegand and Berg, 2013)***.

Most common causes
hepatitis B, C or D
Alcohol (60 to 70 percent)
NAFLD (NASH) (10 percent)—most commonly resulting from obesity
Hemochromatosis (5 to 10 percent)
Biliary obstruction (5 to 10 percent)
Biliary atresia/neonatal hepatitis
Congenital biliary cysts
Cystic fibrosis
Primary or secondary biliary cirrhosis
Less common causes
Autoimmune chronic hepatitis types 1, 2, and 3
Drugs and toxins
Alpha-methyldopa (Aldomet)
Amiodarone (Cordarone)
Isoniazid (INH)
Methotrexate

Table (1) Causes of chronic liver disease (Cont…)

Vitamin A
Genetic/ metabolic diseases
a1-Antitrypsin deficiency
Amino acid disorders (e.g., tyrosinemia)
Bile acid disorders
Carbohydrate disorders (e.g., fructose intolerance,
galactosemia, glycogen storage diseases)
Lipid disorders (e.g., abetalipoproteinemia)
Porphyria
Urea cycle defects (e.g., ornithine carbamoyltransferase
deficiency)
Wilson's disease
Idiopathic/miscellaneous
Schistosomiasis
Veno-occlusive disease
Granulomatous liver disease (e.g., sarcoidosis)
Idiopathic portal fibrosis
Polycystic liver disease
Brucellosis
Congenital or tertiary syphilis
Vascular abnormalities
Chronic, passive hepatic congestion caused by right-sided heart failure, pericarditis
Hereditary hemorrhagic telangiectasia

In addition, causes of chronic liver disease can also be classified according to its etiology into infectious causes including viruses & parasites, alcohol intake, vascular causes as well as autoimmune causes.

1. Viral causes:

a. Hepatitis B:

i. Virology:

Hepatitis B virus (HBV) was recognized as a member of hepadnaviridae family which may cause persistent infections in its natural hosts ***(Howard, 1986).***

ii. Genome:

Hepatitis B virus is the smallest human DNA virus. It is double stranded and contains 3200 nucleotides with overlapping coding regions, leading to several major open reading frames. The S gene codes for several different length variant of surface protein. The smallest forms, HBsAg, is produced independently of and in excess of the amount needed for viral replication; the largest form (S1) makes up the surface coat of circulating viral particles. The C gene encodes the hepatitis B core antigen (HBcAg), which is part of the infectious core of the virus. The X gene codes for trans-activating factor that may be involved with viral replication and the development of malignancy. The precore and basal core

promoter regions code for production of hepatitis B e antigen (HBeAg), a protein found only in those with circulating viral particles. The final major viral protein is a polymerase, which has several different enzymatically active sites ***(Dufour, 2012).***

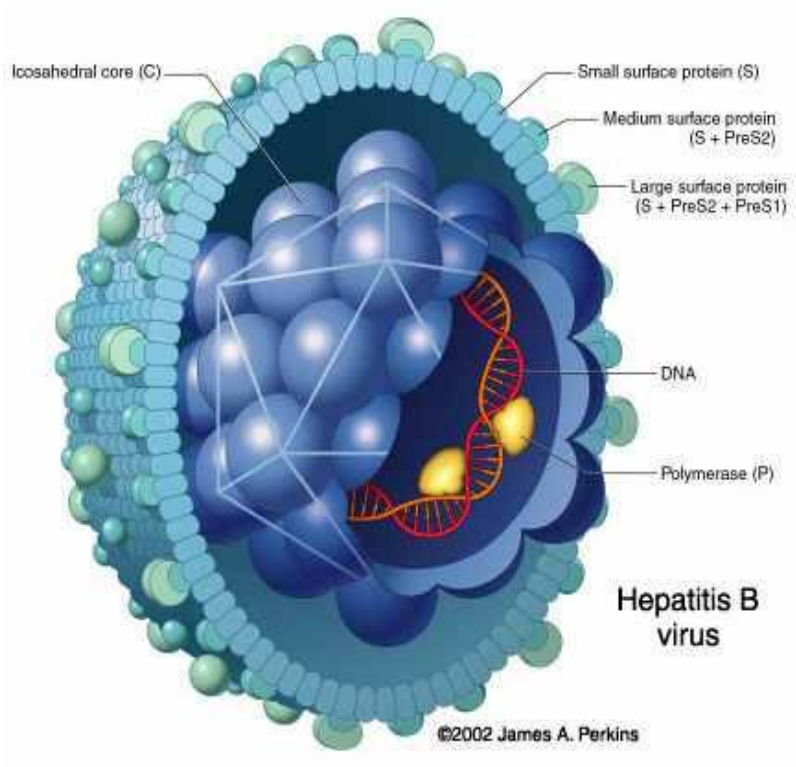

Figure (5): Hepatitis B Virus structure ***(James, 2002)***.

iii. Routes of transmission:

Hepatitis B virus is transmitted through body fluids; primarily by parenteral or sexual contact and also through the skin, by inoculation with contaminated blood or blood products, by transplantation of organs from infected donors, and perinatally from infected mothers to their babies ***(Locarnini, 2004).***

iv. Epidemiology:

It is estimated that about 2 billion people have been infected with HBV worldwide; more than 350 million are chronic carriers. Approximately 15-40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC). HBV infection accounts for 500,000 to 1.2 million deaths each year and is the 10th leading cause of death worldwide ***(Locarnini, 2004).*** Studies in the Middle East showed that the prevalence of HBsAg ranged from 3 to 11% of population in Egypt ***(Awadalla et al., 2011).***

v. Pathology:

Liver damage in chronic hepatitis B results mainly from the direct interaction between the host's immune system and HBV-infected hepatocytes. The antiviral cytokines, such as interferon alpha, beta, and gamma (IFN-α, IFN-β, IFN-γ) as well as tumor necrosis factor alpha (TNF-α), have been implicated as the major contributors to viral clearance, whereas destruction of infected hepatocytes by cytotoxic T lymphocytes contributes to both viral clearance and the development of liver disease ***(Guidotti et al., 1999).***

In patients with chronic HBV infection, the peripheral cytotoxic T-lymphocyte response is usually weak or undetectable and narrow in focus. An activated humoral response develops, characterized by the production of

interleukins 4,5&10 (IL-4, IL-5, and IL-10) secreted by type-2 helper T lymphocytes; this response promotes antibody production rather than viral clearance. Low levels of intrahepatic HBV-specific cytotoxic T lymphocytes have been detected in such patients and are probably responsible for the hepatic flares that occur in patients with chronic disease. However, these activated cytotoxic T lymphocytes are unable to clear HBV ***(Locarnini, 2004).***

b. Hepatitis C virus:

Discovered in 1989, the hepatitis C virus (HCV) continues to cause significant morbidity and mortality world-wide despite a huge research commitment to defining and understanding the virus and the disease it causes ***(Batey, 2007)***.

i. Virology:

Hepatitis C virus is a member of the flaviviridae family. It is enveloped in a lipid bilayer in which two or more envelope proteins (E) are anchored. The envelope surrounds the nucleocapsid, which is composed of multiple copies of a small basic protein (core or C), and contains the RNA genome ***(Thurner et al., 2004).***

ii. **Genome:**

Hepatitis C virus has a single strand RNA genome that consists of 9600 nucleotide bases ***(Kato, 2000).*** As an RNA

virus, HCV is subject to a high rate of spontaneous mutation, these results in six major genotypes. These mutations are unique to the infected individual and play a role in establishing chronic inflammation and contribute to the fluctuating nature of chronic HCV infection.

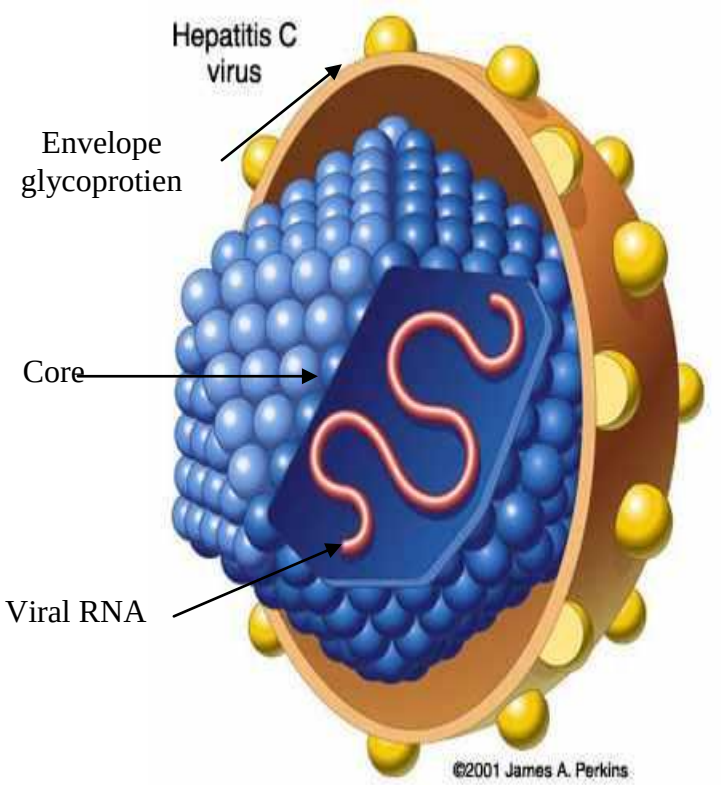

Figure (6): Hepatitis C Virus structure ***(James, 2001)***.

iii. Routes of transmission:

The most efficient transmission of HCV is through large or repeated direct percutaneous exposures to infected blood (e.g., transfusion or transplantation from infectious donors, injection drug use) ***(Alter, 2007)***. HCV infection is also

prevalent in patients undergoing hemodialysis and is associated with greater mortality ***(Gordon et al., 2008)***. Mother to infant transmission may be intrauterine, intrapartum or postnatal ***(Yeung et al., 2001).***

iv. Epidemiology:

Hepatitis C virus is the most common cause of chronic hepatitis in north America, Europe and Japan and it estimated to affect 170 million individuals worldwide (2% of the world's population). Eleven major genotypes and more than 50 subtypes of HCV have been described. In general, HCV genotype 4 (HCV4) is predominant in Africa and the Middle East. In Egypt, where hepatitis C is highly endemic (up to 15% of the population), 91% of the patients were infected with HCV4. Although the genotype is a strong predictor of response to therapy and can affect the duration, however in a country like Egypt, where more than 90% of HCV cases are of genotype 4, genotyping may be revaluated for its cost-effectiveness ***(Kamal and Nasser, 2008)***.

The last published Egyptian Demographic Health Survey (EDHS) in 2008 estimated an overall anti-HCV antibody prevalence of 14.7%. The number of Egyptians estimated to be chronically infected was 9.8% (more than 500,000 new HCV infections occur every year). Egypt has been

widely regarded as having an epidemic, with the highest recorded prevalence of HCV in the World ***(Mahmoud et al., 2013)***.

v. Pathology:

Spontaneous viral clearance rates are highly variable. Ten to sixty percent of persons infected with HCV clear the virus from their bodies during the acute phase. Anti-HCV antibodies indicate exposure to the virus, but cannot determine if ongoing infection is present. All persons with positive anti-HCV antibody tests must undergo additional testing for the presence of the hepatitis C virus itself to determine whether or not current infection is present. Most patients develop chronic hepatitis which is usually associated with evidence of liver injury. Only 15 to 20% of chronically infected cases progress to cirrhosis after 20-30 years of exposure ***(Caruntu and Benea, 2006).*** Expected HCC risk exceeds 1.5% per year in patients with HCV and 0.2% per year in patients with HBV ***(Bruix and Sherman, 2011).***

c. Hepatitis D:

Hepatitis D virus (HDV) was first discovered in HBV-infected patients by an Italian physician, Mario Rizzetto, in 1977. It was originally thought to be a new nuclear antigen associated with HBV. However, it was later proved to be a new virus that requires the surface antigens of HBV (HBsAgs) to