STUDY OF HEPATITIS C & B VIRUS CO-INFECTION IN HEPATOCELLULAR CARCINOMA WITH OR WITHOUT OCCULT B

THESIS

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By

REHAB BADAWY MOHAMED EL SHESHTAWY
M.B.B.Ch., Tanta University

SUPERVISORS

Prof. Dr.
ABDEL RAOUF ABDEL BARY ABO AL-AZM
Professor of Tropical Medicine
and Infectious Diseases
Faculty of Medicine
Tanta University

Prof. Dr. **HALA AHMED FOUAD ISMAIL** ***Professor of Microbiology and Immunology*** ***Faculty of Medicine*** ***Tanta University***	**Prof. Dr.** **MONA AHMED HELMY SHEHATA** ***Professor of Tropical Medicine and Infectious Diseases*** ***Faculty of Medicine*** ***Tanta University***

Faculty of Medicine
Tanta University
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LIST OF ABBREVIATIONS

AMA	Adeno-associated viruses.
ALT	Alanine transferase
AJCC	American Joint Committee on Cancer
AMA	Antimitochondrial antibodies
Anti-HBe	Hepatitis Be antibody.
ASA	Antismooth muscle antibodies.
AST	Aspartate transferase.
BCLC	Barcelona Clinic Liver Cancer
CLIP	Cancer of the Liver Italian Program.
CCL4	Carbon tetrachloride.
CCC DNA	Covalently closed circular DNA.
CP	Cryoprecipitate.
DNA	Deoxyribonucliec acid.
DCP	Des-gamma carboxyprothrombin.
DDB	Dimethyl Dimethoxy Biphenyl Dicarboxylate.
ELISA	Enzyme- linked immunosorbent assay.
EMC	Essential mixed cryoglobulinemia.
FGF-B	Fibroblastic growth factor- beta.
FDA	Food and Drug Administration.
HBcAg	Hepatitis B core antigen.
HBsAg	Hepatitis B suface antigen.
HBV	Hepatitis B virus.
HCC	Hepatocellular carcinoma.
HCV	Hepatitis C virus.
HDV	Hepatitis D virus
HIV	Human immunodeficiency virus.
IFN	Interferon.
IL	Interleukin.
LKMA	Liver kidney microsomal antibodies.
NK	Natural killer
PEI	Percutaneous ethanol injection
PEIPELIT	Percutaneous ethanol lipiodol injection therapy
PCR	Polymerase chain reaction.
RIBA	Recombinant immunoblot assay
r-IFN	Recombinant interferon.
RT-PCR	Reverse transcriptase polymerase chain reaction.
RF	Rheumatoid factor.
RNA	Ribonucleic acid.
TAE	Transarterial embolization
TGF	Transforming growth factor.
TNF	Tumour necrosis factor
NFκ B	Nuclear factor kappa B

mRNA	Messenger ribonucleic acid.

CONTENTS

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are primarily hepatotropic. Chronic infection with those viruses causes progressive liver disease and hepatocellular carcinoma **(Janaki et al., 2006)**. HBV/HCV co-infection further increases the risk of HCC **(Shi et al., 2005)**.

HCC is a common cancer worldwide **(Mboto et al., 2005)**. HCC affects approximately half a million persons each year world-wide making it the fifth most common malignancy in men and the ninth most common in women **(El-Serag, 2001)**.

HCC is a rapidly fatal cancer that mostly affects persons in developing countries where HBV and HCV are endemic. Recently, however, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia **(Yoshizawa, 2002)**. Development of most HCC results from normal liver to cirrhosis and to cancer that may involve a number of successive and additional genetic and epigenetic events **(Paradis et al., 2003)**.

Carcinogenesis is believed to be a multistage process, occurring through a sequence of steps termed initiation, promotion and progression. This process may evolve over several or many years. Tumor initiation begins in cells through mutations induced by exposure to carcinogens. DNA changes, maintained during successive cell divisions, activation of oncogenes and inactivation of suppressor genes lead to dysregulation of the cell division **(Michielsen et al., 2005)**.

Several studies have shown that HBV and HCV interact with each other and affect immune responses. HCV infection can suppress HBV replication however other studies had reported that HBV can reciprocally inhibit HCV replication as well **(Crockett and Keeffe, 2005)**. Co-infection of HBV and HCV leads to aggravated course of disease and faster progression to HCC **(Yates et al., 1999)**.

Several studies found that patients co-infected with HBV and HCV have increased risks of HCC compared to those with mono-infection. Recently it has been thought that occult HBV infection may result in cirrhosis and HCC **(Lok, 2004)**. Occult HBV infection can be a co-factor in the

development of HCC among patients with alcoholic liver disease or cirrhosis due to other etiologies **(Tagger et al., 1999)**.

Aim of the Work

Is to study patients with hepatitis B and C virus co-infection in hepatocellular carcinoma with or without occult B.

Review of Literature

Functional anatomy of liver

The liver has right and left lobes separated along the line of insertion of the falciform ligament, this separation, however, does not correlate with blood supply or biliary drainage **(Portmann et al., 1999).**

The main portal vein divides into the right and left branches, and each of these supplies two further subunits (sectors). The sectors on the right side are anterior and posterior, and in the left lobe are medial and lateral giving a total of four sectors **(Sherlock and Dooley, 2002a)**.

Using this definition, the right and left sides of the liver are divided not along the line of falciform ligament, but along a slightly oblique line drawn from the inferior vena cava above to the gallbladder bed below. The right and left sides are independent with regard to portal and arterial blood supply and bile drainage. Three plains separate the four sectors and contain the three major hepatic vein branches **(Fausto, 2000)**.

These four hepatic sectors are further divided into segments. The right anterior sector contains segments V and VIII, the right posterior sector contains segments VI and VII, the left medial sector contains segment IV and the left lateral sector contains segments II and III.

Segment I, the equivalent of the caudate lobe is separated from the other segments and does not derive blood directly from the major portal branches or drain by any of the three major hepatic veins **(Van Leeuwen et al., 1994)**.

Hepatitis B Virus Infection

Epidemiology:

The hepatitis B virus (HBV) was discovered in 1966, It is the most prevalent chronic infectious disease and is widely spread throughout the world. The global prevalence of HBV is more than 400 million people worldwide **(Crovari, 2003)** and is varying widely from low: <2% as in Western Europe, North America and Japan to high: >8% as in Africa, Southeast Asia and China **(El-Khouri and Dos- Santos, 2004)**. Egypt is considered to be a region of intermediate prevalence (4.5%) for HBV infection **(MOHP, 1999)**. Abo Al-Azm and EL-Sheikh in (1996) reported that prevalence of HBV was 3.4% in Gharbia governorate rural area. **Kamel et al., (1994) and Darwish et al., (1996)** reported that, HBV is hyperendemic in Egypt with seroprevalence rates ranging from 24% in general population to 66% in persons 40-67 years of age. **Ezzat et al., (2005)** reported that rural male and female cases had twice to three times the rate of HBsAg compared to their control counterparts ranging from 0% to 10.8%. The most important epidemiologic factor affecting the chronic carrier rate is age of infection. The earlier in life an infection occurs, the higher the probability that this infection will result in chronic carriage; 90% of infants, 25-50% of children 1-5 years and less than 5% in adults who acquire the infection became chronic carriers **(Crovari, 2003 and Reda et al., 2003)**. Only 2% spontaneously seroconvert annually. Ongoing vaccination programs appear to be promising in attempt to decrease the prevalence of this disease **(Lok and Mc Mahon, 2004)**. Hepatitis B is a leading cause of chronic hepatitis, cirrhosis, and may be hepatocellular carcinoma, approximately accounting for l million deaths annually in the world **(Mboto et al., 2005)**.

Virologic Characteristics

HBV belongs to the Hepadnaviridae family of viruses. Its genome consists of partially double-stranded circular DNA of 3.2 Kilo base pairs **(Zacharakis et al., 2005)**. The DNA is enclosed in a nucleocapsid; or core antigen, which is surrounded by a spherical envelope (surface antigen). The entire virion is known as the Dane particle. In addition to the core and surface proteins, the HBV genome encodes a DNA polymerase that also acts as a reverse transcriptase **(Lin and Kirchner, 2004)**.

Replication

The life cycle of the virus begins with its attachment to the appropriate hepatocyte receptor, which still remains unknown. In contrast, the region between amino acids 21 and 47 of the Pre-Sl has long been known to be involved in virus binding to the hepatocyte membrane **(Perrillo, 2001)**. Recently, it has been suggested that a domain within the small S protein may also be involved in attachment to the hepatocyte also, bringing the virus particle into close contact with the cell membrane, and thus facilitating the specific interaction of the Pre-Sl domain with its receptor **(Kahn, 2002)**. The virion is internalized and uncoated in the cytosol, whence the genome translocates to the nucleus, where it is converted into a double- stranded covalently closed circular DNA (cccDNA) molecule, In this form, cccDNA serves as the template for viral transcript synthesis by host RNA polymerase II. This cccDNA is the template for messenger RNA (mRNA). The mRNA transcribes viral proteins as well as "pre-genomic" RNA that is reverse transcribed into the HBV DNA of new virions. Without the reverse transcriptase, new virions cannot be produced, and replication ceases. The core gene also produces a circulating peptide, the "e" antigen that is associated with high levels of viral replication. These antigens, as well as corresponding antibodies produced by the immune system, serve as useful laboratory markers of past, current, or chronic infection (Lin and Kirchner, 2004). Most antiviral agents so far have been unable to prevent the replenishment of the cccDNA pool from genomic HBV-DNA recycled from the cytoplasm, or to effect efficient clearance of cccDNA-containing hepatocytes **(Hunt and Sharara, 1999)**. This explained the rather rapid rebound in serum HBV-DNA after cessation of antiviral treatment.

Viral kinetic studies have indicated that whereas virion half-life is about a day, the half-life of infected cells is much longer and variable, ranging from 10 to 100 days **(Malik and Lee, 2000 and Hill et al., 2002)**. This

biphasic response pattern, however in the case of HBV is unlike hepatitis C virus (HCV) or human immunodeficiency virus (HTV), may not be universal. Recent findings suggest that viral decay patterns may be more complex or multiphasic **(Lewin et al., 2002)**, possibly representing both cytolytic and non-cytolytic mechanism involvement in loss of infected hepatocytes (Karayiannis, 2003).

Life Cycle of HBV in the Human Host:

Under most circumstances, HBV is not cytopathic (i.e., it does not kill hepatocytes). An intact immune system is vital to cell injury and viral clearance **(Jung et al, 1994, and Moradpour and Wands, 1995)**.

It is useful to consider the life cycle of HBV in four stages, **(Lin and Kirchner, 2004)**.

The first stage is characterized by *immune tolerance*. In the healthy adult, this incubation period lasts about two to four weeks. In contrast, with neonatal infection, this period often lasts for decades. In most cases of HBV infection throughout the world, active viral replication continues despite little or no elevation in the aminotransferase levels and no symptoms of illness **(Lin and Kirchner, 2004)**.

In the second stage, an immunologic response develops or improves, leading to cytokine stimulation and direct cell lysis and the inflammatory process. Secretion of HBeAg still occurs in stage 2, but HBV DNA levels in serum drop as the number of infected cells declines. In patients with acute HBV infection, stage 2 is the period of symptomatic hepatitis and typically lasts three to four weeks. In patients with chronic disease, stage 2 may persist for 10 or more years, leading to cirrhosis and its complication **(Befeler and Di-Bisceglie, 2000)**.

The third stage; When the host is able to mount a response that eliminates infected cells or greatly diminishes their number, active viral replication ends. In this stage, HBeAg is no longer present, and antibody to HBeAg becomes detectable. A marked decrease in viral DNA is observed, although many patients remain positive for HBV DNA as detected by PCR. Practically speaking, the infection has cleared by stage 3, and aminotransferase levels become normal. However, patients remain positive for HBsAg, presumably because of the integration of the S gene

into the host's hepatocyte genome **(Lee, 1997 and Perrillo, 2001)**.

The fourth stage; Most patients eventually become negative for HBsAg and positive for antibody to HBsAg, marking the fourth, or immune, stage in the HBV life cycle. HBV DNA can no longer be detected by any means, and the patient is unlikely to become reinfected or to have a reactivated infection. Factors affecting the evolution through the four stages, in addition to the genetic predisposition of the host, noted above, include the presence of other viruses, treatment with immunosuppressive agents, sex, and the appearance of HBV mutants **(Lee, 1997)**.

Clinical picture:-

Table (1): Groups at increased risk for HBV infection **(Centers for Disease Control and Prevention, 2002)**.

Persons with a history of sexually transmitted disease
Household contacts of HBV-infected persons
Health care workers
Hemodialysis patients
Intravenous drug users
Infants born to HBV-infected mothers
Immigrants and children of immigrants from hyperendemic areas
Men homosexuality.
More than one sexual partner in a six-month period
Sexual partners of HBV infected persons

Acute Infection:

Acute HBV infection is subclinical in 70 percent of adults and 90 percent of children younger than five years **(Lin and Kirchner, 2004)**.

The incubation period: **lasts one to four** months **(Lin and Kirchner, 2004).**

Symptoms: include nausea, anorexia, fatigue, low-grade fever, and right upper quadrant or epigastric pain. Clinical jaundice appears as constitutional symptoms are resolving. Extrahepatic manifestations of acute HBV infection include myalgias, joint pain, and urticaria. Symptoms of acute disease resolve by one to three months, although

some persons have prolonged fatigue. Treatment for acute infection is generally supportive, although some patients require hospitalization **(Lin and Kirchner, 2004).**

Hepatic transaminases levels (alanine transaminase [ALT] and aspartate transaminase [AST] reflect hepatocellular injury and range from several hundred to 20.000 U per L. These values tend to rise one to two weeks before the onset of jaundice. Serum bilirubin values are usually less than 20 mg per dL. Mild anemia is common, leucopenia with relative lymphocytosis. More severe disease results in an elevation in the prothrombin time and a decrease in the serum albumin level. HBV is not cytopathic, and liver injury is caused by the host's immune response against infected hepatocytes **(Befeler and Di-Bisceglie, 2000).**

Acute HBV infection leads to fulminant hepatic failure from massive hepatocellular necrosis in about 1 percent of infections. Rarely, patients with an "exuberant" immune response present with clinical symptoms but progress to hepatic decompensation, including encephalopathy and coagulopathy. Mortality is high, and liver transplantation is often necessary **(Befeler and Di-Bisceglie, 2000).**

Chronic Infection:

Symptoms of chronic hepatitis range from asymptomatic infection to non specific complaints (fatigue, right upper quadrant pain, arthralgias) and, in advanced disease, to complications of cirrhosis (variceal bleeding, encephalopathy, ascites, jaundice, and hepatocellular carcinoma **(Befeler and Di-Bisceglie, 2000).**

Extrahepatic Diseases

Hepatitis B is frequently detected in patients with polyarteritis nodosa and less commonly in those with membranous or membranoproliferative glomerulonephritis or leukocytoclastic vasculitis, all are immune-complex-mediated diseases **(Bonkovsky et al, 1995).**

Diagnosis:

There are several diagnostic tools that are used to identify the status of HBV infection. These include serological, virological, biochemical and

histological tests **(Park and Keeffee, 2004.)**

Serological markers:

(Table 2): Showed Laboratory markers for HBV infection (Lee, 1997).

Hepatitis B surface antigen (HBsAg): present in acute or chronic infection Hepatitis B surface antibody (anti-HBs): marker of immunity acquired through natural HBV infection, vaccination, or passive antibody (immune globulin)
Hepatitis B core antibody (anti-HBc):
IgM—indicative of infection in the previous six months
IgG—indicative of more distant HBV infection that may have been cleared by the immune system or that may persist; positive HBsAg and anti-HBc IgG—indicative of persistent chronic HBV infection
Hepatitis B e antigen (HBeAg)*: correlates with a high level of viral replication; often called a "marker of infectivity"
Hepatitis B e antibody (anti-HBe): correlates with low rates of viral replication HBV DNA: correlates with active replication; useful in monitoring response to treatment of HBV infection, especially in HBeAg-negative mutants

*** A small but significant number of persons infected with a mutant strain of HBV cannot synthesize HBeAg; nevertheless, HBeAg is associated with a high rate of viral replication and infectivity**

Virological markers:

Virological assays used to measure serum HBV DNA quantitatively, include polymerase chain reaction (PCR) and non-PCR methods. With current PCR assays, HBV DNA levels as low as 102 copies/ml can be detected **(Chu et al., 2002)**. In addition, PCR assays have shown that HBV DNA can be detected in patients with cirrhosis and those with HCC who have undetectable DNA using non-PCR based methods **(Brechot et al., 2001)**. Significant intrahepatic HBV DNA and covalently closed circular DNA levels (cccDNA) have also been detected in patients with undetectable HBV DNA levels by non- PCR assays **(Werle et al., 2002).** Continued study of more sensitive PCR assays are advancing knowledge of the state of viral replication in the different phases of HBV infection and leading to modification of current treatment guidelines **(Keeffe et al., 2004).**

Biochemical markers:

The main biochemical markers used in the diagnosis and management of chronic HBV infection is serum ALT. An elevated ALT level, particularly if greater than 2 times the upper limit of normal (ULN), is a marker of necroinflammatory activity in the liver. Patients who are chronically infected with HBV and have persistently normal ALT or minimally

elevated levels tend to have mild or no inflammation on histologic evaluation. The ALT level plays an important role in guiding initiation of treatment. In general, the higher the baseline ALT level in patients with chronic infection, the more effective is the response to antiviral therapy. Patients with persistently normal ALT levels are not routinely treated because of their poor response to antiviral therapy **(De Franchis et al., 2003 Keeffe et al., 2004 and Lok and Mc Mahon, 2004)**.

Histological markers:

A liver biopsy can confirm the diagnosis of chronic hepatitis B, grade the severity of necroinflammation and stage the amount of fibrosis **(Ferrell et al., 2002)**. When ALT and HBV DNA levels are discordant, a biopsy may be helpful to clarify the diagnosis. Liver biopsy may also be useful to exclude other coexistent causes of liver disease, particularly fatty liver or alcoholic liver disease. Although invasive and limited by sampling variation, a liver biopsy can establish a baseline status of the severity of liver disease and help guide the decision regarding initiation of treatment **(Buckley and Petrunia, 2000)**.

Treatment:

Table (3): Indications for Treating Patients with Chronic HBV infection **(Malik and Lee, 2000 and Lok and Mc Mahon, 2001)**.

Presence of HBeAg plus a serum alanine transaminase level greater than twice the normal level.
Presence of HBV DNA plus a serum alanine transaminase level greater than twice the normal level.
Moderate to severe hepatitis on liver biopsy. Presence of HBV DNA plus cirrhosis*

* If the patient has decompensated cirrhosis, treatment with interferon is contraindicated.

Table (4): FDA-approved treatments for chronic HBV infection. **(Malik and Lee, 2000 and Lok and Mc Mahon, 2001).**

Recombinant interferon alfa-2b (Intron A)

Recommended dosage: adults~5 million IU SC per day, or 10 million IU SC three times per week; children~3 million IU per m^2 SC three times in the first week, 6 million IU per m^2 SC three times per week after that (not recommended for children younger than one year)
Recommended duration of therapy: HBeAg-positive patients—16 weeks; HBeAg-negative patients—one year
Cautions: Interferon alfa-2b may cause or aggravate fatal or life-threatening neuropsychiatric, autoimmune or ischemic disorders. Patients should be monitored closely with periodic clinical and laboratory evaluations.
Lamivudine (Epivir)
Recommended dosage: adults—100 mg orally per day; children--3 mg per kg orally per day (maximum: 100 mg per day)
Recommended duration of therapy: HBeAg-positive patients—one year but may be longer in those with HBeAg seroconversion; HBeAg-negative patients with chronic HBV infection—longer than one year, although duration has not been established
Cautions: Lactic acidosis and severe, possibly fatal, hepatomegaly have been reported. If lamivudine is prescribed for patients with unrecognized or untreated HIV infection, rapid emergence of HIV resistance is likely.
Adefovir dipivoxil (Hepsera)
Recommended dosage: adults—10 mg orally per day
Recommended duration of therapy: one year
Cautions: Discontinuation of adefovir dipivoxil therapy may result in severe acute exacerbation. Chronic use may result in nephrotoxicity in patients at high risk for underlying renal dysfunction. Lactic acidosis and severe hepatosplenomegaly with steatosis have been reported. Rapid emergence of HTV resistance may occur in patients with unrecognized or untreated HTV infection. This drug is not FDA-approved for use in children.

FDA = U.S. Food and Drug Administration; HBV = hepatitis B virus; SC = subcutaneously; HBeAg = hepatitis B e antigen; HTV = human immunodeficiency virus.

Hepatitis D virus

HDV infection occurs either as a simultaneous co-infection with HBV (acute HBV and HDV infections), which is usually self-limited because of the eradication of HBV, or as a superinfection in an HBV carrier,

typically an injection-drug user **(Rizzetto et al., 1997).**
The remarkable discovery in 1977 of a passenger virus termed delta, or hepatitis D virus (HDV), added to our understanding of HBV **(Rizzetto et al., 1997)**. HDV is a defective, RNA-containing virus requiring the helper functions provided by HBV, including nucleocapsid assembly and provision of an HBsAg-derived envelope **(Govindarajan et al., 1984)**.

Fulminant Hepatitis B

Acute liver failure with coagulopathy, encephalopathy, and cerebral edema develops in 1 percent of patients with acute hepatitis B Fulminant infection occasionally develops after the withdrawal of immunosuppressive agents — e.g in patients receiving chemotherapy for cancer. The cause of the fulminant infection is also due to acceleration of immune response to viral infection, provided HDV or HCV infection was added as co- infection or super infection **(Lee, 1993)**.
Patients with acute liver failure have early clearance of HBsAg, which may obscure the diagnosis, but a positive test for IgM antibody to HBeAg should clarify the situation. Rapid clearance of HBV is favorable, since HBV reinfection seldom develops in the allograft, transplantation should be required **(Lee, 1993)**.

Hepatitis C Virus (HCV)

HCV can cause persistent infection in susceptible hosts after parenteral or percutaneous transmission **(Poynard et al., 2003)**. Progression to chronic hepatitis C occurs in most people acutely infected with HCV, and persistent infection is an important cause of cirrhosis, end stage liver disease, and hepatocellular carcinoma **(Kim, 2002)**.

Life cycle of HCV:

HCV has been classified as the Hepacivirus genus of the Flaviviridae family **(Van Regenmortel et al., 2000)**. The single stranded RNA genome (of approximately 9600 nucleotide length) encodes a single polyprotein precursor (approximately 3000 amino acids), which is cleaved into several small structural (core, envelope 1, 2) and non-structural (NS1, NS3, NS4, NS5) proteins **(Szabb et al., 2003)**. The

virus has no RT activity and does not integrate into the cell genome. The low fidelity of the RNA-dependent RNA polymerase is partly responsible for genetic heterogenecity **(Rosen and Gretch, 1999)**.

Prevalence of hepatitis C:

HCV infection is an important public health problem in both developing and developed countries **(Kim, 2002)**. Despite the introduction of laboratory tests to screen national blood supplies, HCV remains the most common cause of post-transfusion hepatitis worldwide. WHO estimates that 200 million people, or about 3% of the world's population, have been infected with HCV, making this one of the most common blood borne infections globally. However, most people are asymptomatic and unaware that they are infected. About 170 million people are estimated to be living with chronic HCV and are at risk of advanced liver disease **(WHO 2000)**. Three to four million new HCV infections occur each year, and about 250 000 annual deaths throughout the world resulting from HCV associated chronic liver disease **(Kim, 2002)**.

HCV infection is a major health problem in Egypt where the seroprevalence is 10-20 times higher than that in United States **(Ray et al., 2000)**. The overall prevalence among the general population ranges from 15-20% **(Frank et al., 2000)**. Seroprevalence of anti-HCV was reported to be 12.1% among rural primary school children, 22% of army recruits, 16.4% among children with hepatosplenomegaly **(Abdel-Wahab et al., 1994)** and 11.8% of population in a rural area in Gharbia Governorate **(Abo Al-Azm and El-Sheikh, 1996)**. Among blood donors HCV is carried by about 0.01-2% worldwide **(Alter, 1995). Bassily et al., 1995a** stated that the prevalence of anti HCV among Egyptian blood donors was 26.6% by using the second generation recombinant immunoblot assay. **Darwish et al., 2001** noticed high seroprevalence of HCV in persons ten years of age and over, peaking at 51% for persons aged 40-67 years **Darwish et al., 2005** reported that, Egypt has one of the highest (16-18%) prevalence rates of HCV infection in the world. Despite the fact that HCV genotype 4 is sometimes found in Europe, this genotype is the predominant one in the middle East and Africa, 96% of Egyptian HCV strains were genotype 4 **(Yates et al., 1999). Bakr et al., 2006,** reported that prevalence of HCV is very high reaching 45% among

adult older than 40 years in rural areas.

CLINICAL FEATURES OF HCV:

In more than 70%-90% of HCV infections, the infection persists and leads to chronic hepatitis **(Yates et al., 1999).** HCV accounts for approximately 20% of cases of acute hepatitis, 70% of chronic hepatitis and 30% of end stage liver disease **(Alter et al., 1995).**

Within 1 to 3 weeks of exposure, HCV RNA can be detected in blood. The mean incubation period until the onest of symptoms is 7 weeks **(Strickland, 2000).** The illness generally lasts for 2-12 weeks. Acute hepatitis can result in fulminant hepatitis but this is rare **(Hoofnagle, 2002).**

Chronic HCV is the rule and usually characterized by a lack of symptoms or only fatigue or vague abdominal pain, arthragia. Extrahepatic manifestations of chronic HCV may be identified, and these are associated primarily with autoimmune or lymphoproliferative states **(Zignego and Brechot, 1999).** Essential mixed cryoglobulins, membranous and membranoproliferative glomerulonephritis are highly associated with HCV infection **(Kristiansen and Florholmen, 2001).** Paoletti et al., 2000 reported the presence of peripheral neuropathy in HCV patients without detectable cryoglobulins especially in patients infected with genotype (lb). Systemic vasculitis in patients with HCV has different types, polyarteritis nodos a or mixed cryoglobulinaemia **(Cacoub et al., 2000).**

Several other conditions are associated with chronic HCV infection as autoimmune thyroiditis, lichen planus, and idiopathic pulmonary fibrosis **(Salem, 1996).** Involvement of salivary and lacrimal glands and rheumatological complication as myalgia, arthritis and fibromyalgia are common in HCV infected subjects **(Buskila, 2000).**

Increases in serum alanine aminotransferase (ALT) reflect hepatocyte injury, but these values typically fluctuate over time and may be even normal on occasion **(Poynard et al., 2003).**

The major complication of chronic HCV infection is progressive hepatic fibrosis leading to cirrhosis, which develops in about 20% of those with chronic HCV **(Conry-Cantilena et al., 1996).** The natural history of chronic HCV is variable, and progression of chronic liver disease is insidious in most patients. About one third of patients with chronic HCV develop hepatic cirrhosis 15 to 20 years after infection ("rapid fibrotic

progressors"), one third develop cirrhosis 20 to 30 years after infection ("intermediate fibrotic progressors"), and one third develop it only after 30 years of HCV infection ("slow fibrotic progressors") **(Alberti et al., 1999)**. Chronic HCV infection is associated with an increased risk of hepatocellular carcinoma, but this occurs primarily in patients with cirrhosis **(Liang et al., 2000 & El- Serag, 2002 and Poynard et al., 2003)**. Death from chronic HCV typically occurs because of decompensated cirrhosis or hepatocellular carcinoma.

Risk factors for HCV infection:

HCV is transmitted primarily through percutaneous exposure to blood **(Centers for Disease Control and Prevention (CDC), 1998; National Institutes of Health Consensus Development Conference Statement (NIHCDCS), 2002 and Strader et al., 2004)**

Risk factors associated with transmission of HCV include:

- *Injection and other illicit drug use*—currently, most new HCV infections are associated with injection drug use, and this accounts for about 60% of HCV transmission **(Flamm et al., 1998)**. About 65% of injection drug users are infected within one year of initiation of injection behaviour **(Garfein et al., 1996).** Intranasal cocaine use has also been associated with acquisition of HCV **(Conry-Cantilena et al., 1996).**
- *Transfusion and organ transplantation* **(Schreiber et al., 1996).**
- *Haemodialysis*— the prevalence of HCV antibodies among haemodialysis patients is about 8% **(Tokars et al., 2002).**
- *Health care workers*— the incidence of infection after needle stick injury is 3% to 4% **(Lauer and Walker, 2001).**
- *Sexual activity*—sexual transmission may reflect differences in sexual risk behaviors **(Terrault, 2002).** Monogamous couples do not need to use barrier protection but should be advised that condoms may reduce the already low risk of HCV transmission **(CDC, 1998)**
- *Tattooing/body piercing*—contaminated equipment or supplies **(Ko et al., 1992 and Conry-Cantilena et al., 1996).**
- *Vertical transmission*—the incidence of HCV infection is 5% to 6% among infants born to HCV infected women **(Conte et al., 2000),** but the incidence rises to about 20% among children born

to mothers co-infected with both HCV and HIV **(Gibb et al., 2000).** Infants born to HCV infected women should have their blood tested for either HCV RNA at six months of age or HCV antibody at 15 months of age (after maternal antibodies have waned) **(NIHCDCCS, 2002).** Breast feeding does not seem to transmit HCV **(Kumar et al., 1998).**

- *Alternative routes of transmission*—there is no evidence that casual contact, such as kissing, hugging or sharing eating utensils, is associated with HCV transmission. However, sharing household items that may be contaminated with blood, such as razors, tooth brushes, or nail grooming equipment, should be avoided **(CDC, 1998 & Booth et al., 2001 & NIHCDCS, 2002 and Strader et al., 2004).**

Laboratory evaluation:

A number of tests are useful in the evaluation of HCV infection:

- *HCV antibody*—the detection of HCV antibodies is recommended as the initial test for the identification of HCV and is useful for screening at risk populations **(NIHCDCS, 2002).**
- *HCVRNA assays*—assays are based on the molecular detection of HCV using polymerase chain reaction or other gene amplification techniques are available. A qualitative HCV RNA assay can confirm viraemia in patients with a positive enzyme immunoassay result as well as in those with a negative test in whom infection is still suspected **(Lauer and Walker, 2001).** The quantitative HCV RNA assay can predict treatment response. However, HCV RNA values provide no information about disease severity or risk of progression, so serial monitoring of HCV viral loads in untreated patients is unnecessary **(NIHCDCS, 2002).**
- *ALT and assessment of liver function*—ALT estimation may be useful in monitoring HCV infection but are insensitive in predicting disease progression to cirrhosis. ALT may be normal or fluctuate in those with HCV infection, and a single normal value does not exclude active infection, progressive liver

disease, or cirrhosis **(Pradat et al., 2002).** Liver function tests, which include prothrombin time, bilirubin, and albumin, should also be performed.

- *Genotype*—worldwide, six genetically distinct groups of HCV isolates, called genotypes (numbered 1 through 6), have been identified **(Rosenberg, 2001).** There is little difference in the mode of transmission or natural history of infection among the different genotypes. However, cure rates with antiviral therapy are notably higher with genotypes 2 and 3, and the duration of HCV therapy is shorter for these genotypes. Genotype lb is associated with poor response to interferon **(Hadziyannis et al., 2004).** Thus, HCV genotype is an important parameter to be determined in the evaluation of chronic HCV infection. Genotype does not change during the course of infection and should only be evaluated once **(Poynard et al., 2003).**
- *Liver biopsy*—Histological evaluation of a liver biopsy specimen remains the gold standard for reliably estimating the stage of hepatic fibrosis and degree of hepatic inflammation in patients with chronic HCV **(Strader et al., 2004)**. Thus, the liver biopsy can determine the relative urgency of HCV therapy. The most validated method for evaluating the degree of hepatic fibrosis is the METAVIR classification system, which divides liver fibrosis into five discrete stages (0 = no fibrosis; 1 = mild fibrosis (portal fibrosis without septae); 2 = moderate fibrosis (a few septae); 3 = severe fibrosis (numerous septae without cirrhosis), 4 = cirrhosis) **(Bedossa and Poynard, 1996).** A liver biopsy may identify concurrent disease processes (for example, steatosis, and iron overload) that can contribute to hepatic injury. It aids in the selection of chronic HCV patients for treatment and helps to correctly time therapeutic interventions **(Rosenberg, 2001).** The liver biopsy can also determine the presence of cirrhosis.
- *HIV screening*—many risk factors for HCV transmission are shared by HIV infection **(Conry-Cantilena et al., 1996).**
- *Hepatitis A and B screening*.
- *Hepatocellular carcinoma screening*.
- *Antinuclear antibody*—patients with chronic HCV infection have been found to have a higher rate of autoantibodies in the serum

(Clifford et al., 1995). As a result, determination of the antinuclear antibody has been recommended before starting HCV therapy **(Herrine, 2002).**

- *Thyroid function tests*—thyroid disorders are common in patients with chronic HCV, and interferon induced thyroid disease **(Marazuela et al., 1996).** Thyroid stimulating hormone concentrations should therefore be checked before starting HCV therapy **(Herrine, 2002).**

Treatment of chronic HCV infection.

- Support and education
- Treatment of neuropsychiatry disorders
- Imunisation against hepatitis A and B
- Avoidance of hepatotoxins:e.g,alcohol.
- Pegylated interferon and ribavirin therapy:-

Combination therapy with pegylated interferon (alfa-2a & alfa- 2b) and ribavirin is currently the standard of care for treating patients with chronic HCV. The primary goal of treatment is to eradicate chronic HCV **(Strader et al., 2004).** But therapy can also decrease hepatic inflammation and fibrosis, slow disease progression, and risk for cirrhosis and hepatocellular carcinoma even in the absence of cure **(NIHCDCS, 2002).** Two types of pegylated interferons, pegylated interferon alfa-2a (Pegasys, Hoffmann-La Roche) (optimal dose: 180 mg/weak) and pegylated interferon alfa-2b (optimal dose: 1.5mg/kg/weak) (Peg-Intron, Schering-Plough) in patients with genotype I infection, which differ in their pharmacokinetic and chemical properties, have been developed **(Karnam and Reddy, 2003).** Both formulations are given weekly via subcutaneous injection . Ribavirin is dosed by weight for genotype 1 (1000 mg per day if <75 kg and 1200 mg per day if 3=75 kg), whereas 800 mg per day is sufficient for genotypes 2 and 3, regardless of weight. Combination pegylated interferon plus ribavirin for 48 weaks will optimize response in those with genotype I infection **(Hadziyannis**

et al., 2004).

Hepatocellular Carcinoma

Prevalence

Hepatocellular carcinoma (HCC) is usually diagnosed in the setting of chronic liver disease with cirrhosis and has been estimated to result in between 500,000 and 1,000,000 deaths per year **(Parkin et al., 2001 and Befeler and Di- Bisceglie, 2002)**. In the West, HCC is becoming the first complication, and the most frequent cause of death, in patients with viral-associated cirrhosis **(Benvegnu et al., 2004)**. The incidence and mortality associated with HCC, unlike most other cancers, are increasing in the United States.

In the United States, population-based studies showed that the incidence of HCC increased to 11.4% between 1975 and 1998. Even more concerning, is the finding that patients, aged 45 to 55 years, are the fastest growing group **(El-Serag et al., 2003).** An analysis of the Surveillance, Epidemiology and End Results (SEER) database showed that the hepatitis C virus (HCV) infection is a major contributor to the recent increase in the incidence and mortality due to HCC **(El-Serag and Mason, 1999).**

Although there is no available confirming data, death rates from HCC in Egypt appear to be increasing over the last decade **(Bedwani et al., 1996 and Abo Al-Azm et al., 2005).**

HCC is strongly associated with HCV infection in Egypt. In one study it was found that HCV antibodies were detected in 76% of HCC patients **(Yates et al., 1999).** In another study of the prevalence and epidemiological features of HCC in Egypt, it was mentioned that HCV antibodies were detected in 51% of HCC in Egypt, HBsAg was detected in 21.3% and aflatoxin B1 was found in 17% of HCC patients **(El-Zayadi et al., 2001)**. In another study on 33 Egypian patients with HCC conducted at the Cairo National Institute of Cancer, it was noticed that HCV seroprevalence (anti-HCV antibodies) were detected in 75.8% of patients, whereas, hepatitis B surface antigen was detected in 15.2% of patients **(Hassan et al., 2001). Zekri et al., (2006)** reported that, HCC

account for about 4.7% of chronic liver disease patients in Egypt.

Risk Factors:

1- Role of cirrhosis:

Cirrhosis may be a premalignant condition irrespective of the etiology. The chronic cirrhotic state appears to carry a threefold increased risk of cancer. It seems probable that malignant changes occur as a progression from liver cell dysplasia **(Macias et al., 2000).**

2- Role of hepatitis viruses:

The relation of the virus in the development of HCC is through chronic hepatitis and cirrhosis. Almost all patients with viral related HCC have underlying cirrhosis. In a recent study, it was found hat 81% of HCC patients were attributable to viral hepatitis; 58% HBV, 23% HCV and the remaining 19% likely represent misclassification of HBV infected HCC patients that have lost HBsAg **(Kirk et al., 2004).**

3- Role of aflatoxins:

Aflatoxins are mycotoxins produced by the fungi Aspergillus flavus. These toxins may contaminate food stored in humid conditions. Animal studies suggest that aflatoxin B (AFB1) is carcinogen **(Okuda, 2000)**. Areas with high aflatoxin contamination overlap areas with high rates of HBV infection **(Park et al., 2000)**. In liver cells, aflatoxin is metabolized by the microsomal mixed function oxidase. Aflatoxin B1-2-3-epoxide, a highly reactive metabolite of aflatoxin Bl, it is capable of binding to DNA and RNA and is thought to be the main carcinogenic metabolite of aflatoxin. Aflatoxin Bl is known to induce a mutation at codon 249 of P53.This mutation could be one of the mechanisms leading to HCC **(Ming et al., 2002).** However, **Mokhles et al., 2006** noticed that, there is higher aflatoxin prevalence and concentration in urine of cirrhotics than HCC patients and reported higher aflatoxin concentration in urine of patients from Upper than Lower Egypt.

4- Role of iron and copper:

Iron and copper are potentially mutagenic through a number of mechanisms related to the oxidative stress **(Vautier et al., 1999).**

Savas et al., 2006 reported that HCC may complicate Wilson's disease regardless of the age of the patient.

5- Role of estrogen:

Oral contraceptives pills (OCP) were reported to be associated with the development of hepatic cells carcinomas, benign hepatomas, focal nodular hyperplasia, hamartomas, and HCC. The relative high risk of estrogen-induced liver cancer is two to four times higher in regions where hepatitis is not endemic than in areas where hepatitis is endemic **(Akrivadis et al., 1998). Sun et al., 2003** found a significant relation between OCP and HCC development especially in women received OCP for more than 5 years.

6- Role of androgen:

Several lines of evidence in animals' studies suggest a relationship between androgenic-anabolic steroids and HCC. Several additional cases of hepatic tumors in androgen treated boys or men were reported **(Agnew and Gardner, 1992).** There is no evidence that biologically malignant liver tumors have yet resulted from androgen use inspite of the malignant histologic appearance. However, the relationship between androgenic anabolic steroids and HCC seems stronger than contraceptives **(Bernstein, 1995).**

7- Role of alcohol:

In northern Europe and North America there is a four fold risk of primary hepatocellular cancer in alcoholics particularly in older patients. Alcohol may be a co-carcinogen with HBV so it was found that hepatitis B markers are highly prevalent in alcoholic cirrhotic patients complicated by hepatocellular cancer **(Wang et al., 2003).** However, **Di-Costanzo, 2004** noticed that, in patients with cirrhosis and a known duration of HCV infection, alcohol & cigarette smoking

do not increase the risk of HCC development.

8- Diabetes mellitus:

Several reports have suggested that persons with diabetes mellitus are at an increased risk for developing HCC **(Adami et al., 1996 and Coughlin et al., 2004).** Diabetes is also associated with increased level of insulin-like factors that are potential carcinogenic factors **(Moor et al., 1998).**

9- Smoking:

There is a significant dose-response and positive association between cigarette smoking and HCC risk (>2 packs per day). This association was stronger in individuals without chronic HBV or HCV **(Kuper et al., 2000). Abdel Aziz et al., (2006)** reported that smoking may play an important additive role in pathogenesis of HCC in Egypt. **Munaka et al. (2003)** explained the carcinogenic effect of tobacco by possible induction of genetic polymorphism for glutathione S- transferase through a study on 78 Japanese patients with HCC, while **Goa et al. (2003)** explained this carcinogenic effect by slowing acetylation genotype of N'acetyl transferase.

10- Other carcinogenic factors:

- **Parasites:**

The parasites implicated in hepatocarcinogenesis include echinococcal cysts, Schistosoma japonicum, however, evidence for the etiologic association of these parasites with HCC is not clear **(Okuda and Nakashima, 1985)**.

- **Chemicals:**

Vinyl chloride **(Engstrom et al., 1997),** propachlor and radiation **(Moore et al., 1996),** are claimed to be associated with higher incidence of HCC. **Ezzat et al., (2005)** found that, two classes of pesticides, carbamates and organophosphates, were used most commonly in Egypt had significant additive risks for development of HCC among rural males. In contrast, household applications of insecticides and rodenticides were not associated with cancer risk among either urban or rural residents. Due to the high prevalence of HCV RNA in this population, the attributable risk of HCV to the burden of HCC is 90.4%.

Pathology of HCC:

Gross pathology:

A liver involved by HCC usually weights between 2 and 3 kg but may be of normal size and weight. The right lobe is more frequently involved than the left lobe. HCC appears as solitary bulky mass or multiple small nodules scattered throughout the liver **(Anderson et al, 1986).**

The growth pattern of HCC has some epidemiologic and clinical significance. A reasonable classification would be to emphasize the boundary between tumour, parenchyma and the number of discrete tumour masses, putting the size of the tumour and co existing cirrhosis as separate modifiers **(Halpern et al, 1991).**

Such a classification as follows: **(Halpern et al, 1991).**

- ***Expanding type:*** The boundary between tumour and parenchyma is discrete; the tumour is expanding, compressing and distorting the surrounding parenchyma. An "encapsulated" expanding tumour is one with a grossly distinct thick capsule of several millimeters formed by the expanding tumour nodule followed by collagenization of the remaining reticulin fibres. This type tends to affect the non-cirrhotic livers.
- ***Spreading type (infiltrative):*** This type of tumour is poorly defined, particularly when the liver is cirrhotic. The growth pattern of this type of tumour may be further subdivided into "nodular", "pseudolobular", and "invasive".
- ***Multifocal type:*** In this type, multiple small, often indiscrete, tumour nodules diffusely involve the entire liver. This type is usually confined to cirrhotic livers.
- ***Minute HCC:*** Occasionally, HCC invades the portal vein or the bile duct system when the primary tumours are still small. Such early spread is responsible for the patient's symptoms. This minute HCC may be mainly intravascular or mainly intraductal

depending upon whether ducts or vessels were invaded.

Histopathology:

The most important criterion in histologic diagnosis is similarity of the cancer cells to normal hepatocytes, except in cases of anaplastic HCC. The degree of differentiation varies from case to case and from area to area in the same liver. Pleomorphism in the broad sense is common in HCC. The following classification is based on the WHO publication on international classification of tumours (Omata et al, 1991).

(1) Trabecular (sinusoidal) pattern:

Tumour cells grow in cords of variable thickness separated by prominent sinusoids lined by endothelial cell. This type is typically characterized by lack of stroma (Omata et al, 1991).

(2) Pseudoglandular (acinar):

Tumour cells may assume a gland-like structure in which canaliculi are recognised and may be dilated into gland-like spaces that may contain bile. The content of gland-like spaces may be fat- laden macrophages, fibrinous exudates, cellular debris or colloid like material, and in that case resemble thyroid follicles (Nagato et al, 1991).

(3) Compact:

This is a trabecular pattern, but the tumour cells grow in apparently solid masses and sinusoids are rendered inconspicuous by compression (Omata et al, 1991).

(4) Schirrhous:

Abundant fibrous stroma separating tumour cell are seen in sclerosing HCC and should be distinguished from cholangiocarcinoma. This type of histology may be also seen following chemotherapy, radiation or infarction (Won et al, 1991).

Degree of differentiation:

Grade I:

The difference between the tumour cells and hyeprplastic liver is so minor that diagnosis of carcinoma rests upon demonstration of more aggressive growths in other parts of the neoplasm (Hood et al, 1990).

Grade II:

The cells resemble normal hepatocytes. The nuclei are large and more hyperchromatic than normal, but the cytoplasm is abundant and acidophlic, the cell borders are often sharp. Acini are frequent; their lumens vary in size from tiny canaliculi to large thyroid-like spaces. The lumens are often filled with bile (Hood et al, 1990).

Grade III:

The nuclei are usually larger and more hyperchromatic than those of grade II, and occupy a relatively greater proportion of the cell. The cytoplasm is granular and acidophlic as in grade II, but is usually less. Some breaking or distortion of the usually trabecular pattern is present. Bile and acinar formation are noted less frequently. Giant tumour cells are most numerous in this group (Hood et al, 1990).

***Grade* IV:**

The nuclei are intensely hyperchromic and occupy the grater part of the cell. The cytoplasm is variable in amount, often scanty, and contains fewer granules. The trabeculae are difficult to find. Grading of cell differentiation is of clinical significance because prognosis may be related to the grade (Okuda and Nakashima, 1985).

Production of alpha-fetoprotein by anaplastic tumour cells is usually less than that by differentiated tumour cells. HCCs in highly cirrhotic liver tend to be well differentiated in contrast to the poorly differentiated HCC seen in non-cirrhotic livers (Kanno et al, 1990).

Spread of HCC:

- ***Intra-hepatic spread:***

Metastases in the liver may be multiple or in one lobe. Spread is by the blood vessels, lymphatic permeation and direct infiltration (Anderson, 2002).

- ***Extra-hepatic spread:***

Involvement of small or large hepatic or portal veins or the vena cava may be seen. Metastases have also been found in oesophageal varices even if sclerosed. Regional lymph nodes at the porta hepatis are

frequently involved and the mediastinal and cervical chains can also be infiltrated. The tumour may involve the peritoneum with resulting haemorrhagic ascites; this may be terminal. Lung and bone metastasis were also reported (Levy, 2002).

Several clinical classification system describe HCC:

Table (5): TNM classification system of HCC (American Joint Committee on Cancer) (AJCC) (1998):

Primary tumour size	Denoting
PTx	Histologic examination of the primary tumour not possible.
PTO	Primary tumour not found.
PTl	Solitary tumour, <2 cm diameter, no vasoinvasion.
PT2'	Solitary tumour<2cm diameter, with vasoinvasion or solitary tumor>2cm diameter, no invasion.Or multiple tumors in one lobe ,<2cm diameter, no vasoinvasion.
PT3	Solitary tumour>2cm diameter, with vasoinvasion. Or multiple tumors in one lobe, <2cm diameter, with vasoinvasion.Or multiple tumors in one lobe>2cm diameter with or without vasoinvasion.
PT4	Multiple tumours in more than one lobe or Vasoinvasion in larger branches of portal or hepatic veins similarly regarding the regional lymph nodes:
NO	Denoting absence of nodal metastases.
N1	Denoting presence of nodal metastases.
N2	Denoting extensive nodal involvement and regarding the presence or absence of distant metastases.
MO	Denoting absence of distant metastases.
Ml	Denoting presence of distant metastases.

T: Tumour N: Lymph nodes M: Distant metastases

Several other staging systems have been developed as further aids in

assessing the prognosis for patients with HCC. The Okuda staging system *(Table 6)* evaluates HCC based on tumour size, presence of ascites and serum levels of albumin and bilirubin **(Okuda et aL, 1983 and Fleming, 2001)**.

Table **(6):** The Okuda staging system.

• Tumour size >50% extension	
• Presence of ascites	
• Albumin <3 g/dL	
• Bilirubin >3 mg/dL	
Stage I: None of the above present. Median survival =	11.5 months
Stage II: 1 or 2 of the above present. Median survival =	= 3.0 months
Stage III: 3 or 4 of the above present. Median survival	= 0.9 months

A new prognostic system for HCC, the CLIP scoring system (Table!), devised by the Cancer of the Liver Italian Program *(CLIP% 1998 and Henderson et al, 2003*.

Table **(7):** The CLIP scoring system.

Prognostic variable	0 point	1 point	2 points
Child-Pugh class	A	B	C
Tumour morphology	Uninodular and extension <50%	Multinodular and extension < 50%	Massive or extension >50%
Alpha fetoprotein level	< 400 ng/ml	> 400 ng/ml	
Portal vein thrombosis	Absent	Present	

The CLIP system should be the clinical staging system of choice,

because it is generally applicable to most patients and it includes easily collected variables (Sherman et al., 2004).

Barcelona Clinic Liver Cancer (BCLC) Group **(Liovet et al., 1999)**. This classification takes into consideration hepatic function, portal vein hypertension, bilirubin, symptoms related to the tumor, tumor morphology, presence of distant metastases, or vascular invasion. This is the only classification that correlates prognostic data with therapeutic possibilities **(Franca et al., 2004).**

French classification this classification includes as prognostic factors, serum bilirubin levels, AFP, alkaline phosphatase, and the presence of portal thrombosis detected by US. The score ranges from

0 to 11. The 1- and 2-year survival rate was 72 and 51% for low risk patients (score 0), 34 and 17% for patients of intermediate risk (score

1 to 5), and 7 and 3% for high risk patients (score >6), respectively. It should be pointed out that this classification was based on the analysis of patients (47%) submitted to some type of treatment. In addition, mean patient survival was only 4.3 months, suggesting selection of patients with an advanced stage of the disease **(Franca et al., 2004).**

Diagnosis of Hepatocellular Carcinoma: An early diagnosis of HCC is required for the institution of treatments considered to be curative. It has been suggested that monitoring should be performed by US and by measurements of serum AFP at 6-month interval **(Franca et al., 2004).**

Ultrasound:

US is considered to be the technique of choice for the diagnosis of focal hepatic lesions , permitting the detection of tumors of small size (1 cm) still in the early phase of development and thus being used for the screening of HCC in patients with cirrhosis of the liver **(Ebara et al., 1989 and Solmi et al., 1996).** The ultrasonographic characteristics of HCC depend on nodule size. Small nodules of less than 3 cm are frequently hypoechogenic. As they increase in size, they start to acquire isoechogenic characteristics with a peripheral halo or hyperechogenic or heterogeneous characteristics due to the neoformation of blood vessels and intratumoral necrosis. Other possible findings are lateral shadows, a posterior reinforcement and a

perilesional halo that corresponds to the presence of a peritumoral fibrous capsule consequent to the compression of the adjacent hepatic parenchyma **(Ebara et al., 1989).** US can also be used to assess the permeability of vascular structures and the existence of hilar adenopathies suggestive of tumoral extension **(Franca et al., 2004).**

The combination of Doppler with US can be useful for the identification of portal thrombosis in patients with HCC, with 89 to 92% sensitivity and 100% specificity in the identification of tumor thrombosis **(Tanaka et al., 1993).** The presence of a hepatofugal pulsatile flow inside the thrombus is suggestive of vascular invasion **(Vilana et al., 1993).** In addition to providing an imaging diagnosis, US can also be used to guide the needle for aspirative puncture or for a biopsy from the tumor nodule **(Ebara et al., 1989).**

Alpha-1 fetoprotein:

Patients with chronic liver disease, especially those with a high degree of hepatocyte regeneration, can express AFP in blood in the absence of malignant neoplasia.

Measurement of serum AFP levels is not useful for the early detection of HCC because, even though 80% of the patients with HCC have serum AFP concentrations exceeding normal levels (10-20 ng/ml), patients with a high liver regenerative activity may express higher than normal AFP values without having HCC **(Ebara et al., 1989 and Liovet and Beaugrand, 2003).**

AFP serum levels above 400-500 ng/ml are considered to be diagnostic of HCC in cirrhotic patients with focal hepatic lesions. However, only 1/3 of patients with HCC have AFP levels higher than 100 ng/ml, with levels above 400 ng/ml being quite infrequent in the presence of small tumors (<5 cm) **(Ebara et al., 1989).**

Progressively increasing AFP concentrations during screening are suggestive of a diagnosis of HCC. These patients should be submitted to helicoidal computed tomography in order to rule out the diagnosis of a tumor **(Franca et al., 2004).**

With the development of early detection programs, an increase in the number of cases with small tumors and normal AFP levels (29%) has been observed **(Nomura et al., 1989). Lescano et al., 2002** found that 42% of patients with HCC present serum AFP levels within normal limits.

At the time of tumor diagnosis, AFP seems to be of prognostic value **(Franca et al., 2004).**

Other tumor markers:

Des-gamma carboxyprothrombin (DCP) is another tumor marker used for the diagnosis of HCC. Other markers such as interleukin-2, urinary tumor growth factor-61 and MAGE-4 protein receptors have also been used in research to aid the diagnosis of HCC but their clinical applicability requires scientific confirmation **(Franca et al., 2004).**

The protein induced by vitamin K absence or antagonist II (PIVKA-II) increases in HCC. This is normal in chronic hepatitis and metastases. Specificity is superior to AFP. **(Sherlock and Dooley, 2002b).**

In the presence of a diagnostic suspicion by US and/or AFP, the patient should be further investigated by helicoidal computed tomography (CT) and/or magnetic resonance (MRI). In selected cases, hepatic arteriography (as a preoperative measure) was performed, in order to confirm the suspected diagnosis and determine the stage of the tumour **(Franca et al., 2004).**

Helicoidal computed tomography:

The main characteristic of HCC detected by CT is the early uptake of contrast in the arterial phase of the examination. Due to the hypovascularization of small-sized tumors, the diagnostic efficacy of CT is reduced in tumors measuring less than 2 cm (Ikeda et al, 1994). This technique increases by about 15% the diagnostic sensitivity for the detection of hepatic tumors smaller than 2 cm compared to standard CT. CT also reveals the presence of tumor involvement of lymph nodes, vascular invasion and extrahepatic involvement with

high sensitivity, specificity and diagnostic accuracy.

Magnetic resonance image:

One of the major useful properties of MRI is the differential diagnosis from hepatic **hemangioma** (Ebara et al., 1989). Because of the lack of anatomical limitation, MRI and CT are superior to US for the diagnosis of tumors close to the lung and of isoechogenic lesions (Liovet and Beaugrand, 2003).

Hepatic arteriography:

The diagnostic efficacy of hepatic arteriography (HA) depends on tumor size and on the extent of tumor vascularization (Ikeda et al., 1994). The diagnostic accuracy of imaging techniques for the detection of HCC depends on the characteristics of the tumor and on the experience of each study group. According to the experience of Franca et al., (2004) the ability to diagnose HCC is 84% for US, 79% for CT, 77% for MRI and 64% for HA. Thus, because of its low cost and availability, we believe that US continues to be the main choice for an early diagnosis of HCC, being useful for the monitoring of cirrhotic patients.

Cytology and / or histology:

The material to be used for cytology is obtained by fine needle aspiration biopsy (FNAB). This is a safe technique with minimal risks of complications due to the procedure, which provides adequate material when performed by trained personnel. Histopathological examination is the main method for a sure diagnosis of HCC (Franca et al., 2004).

Treatment of Hepatocellular Carcinoma:

Surgical resection

Surgical resection is considered to be the option of choice for the treatment of patients with HCC. However, due to the frequent

occurrence of postoperative hepatic decompensation, this treatment modality should be indicated only for patients with preserved hepatic function (Liovet et al., 1999 and Yamamoto et al., 2001). The presence of portal hypertension represented by the portal pressure gradient (difference between occluded and free hepatic venous pressure) >10 mmHg has been used as one of the main factors predictive of hepatic decompensation after surgical resection . This suggests that resection should be indicated for patients without portal **hypertension** (Liovet et al., 1999).

When possible, conservative surgery should be performed, such as segmentectomy or sub-segmentectomy which will preserve a functioning liver mass. Tumor recurrence is observed in about 12, 60 and 70% of patients after 1, 3 and 5 years, **respectively** (Liovet et al., 1999) and is related to the presence of satellite nodules and tumor **differentiation** (Michel et al., 1995).

Liver transplantation

Liver transplantation is the only curative treatment option for patients with cirrhosis and unresectable HCC without extrahepatic **dissemination** (Emiroglu et al., 2006). Liver transplantation not only eliminates the neoplasia, but can also cure the base liver disease. When strict selection criteria are used, such as a single, small tumor (<5 cm) without satellite nodules, without vascular invasion, without invasion of regional lymph nodes, without distant metastases, and without an indication for resection, a satisfactory survival can be obtained (Jonas et al., 2001). With single tumors smaller than 5 cm, the possibilities of survival reach 84, 74 and 74% in the 1st, 2nd and 5th years after liver transplantation, with a rate of tumor recurrence of only 3.5% (Michel et al., 1995). Thus, the ideal candidate for liver transplantation is a patient with a single HCC smaller than 5 cm or with up to 3 nodules, none of them larger than 3 cm, without signs of neoplastic invasion of the portal system or of distant metastases.

Immunosuppressive agents such as cyclosporine and tacrolimus are known to be stimulators of hepatic regeneration. However, their interference with tumor progression is still a matter of controversy (Franca et al., 2004).

Percutaneous treatment

Several types of percutaneous treatment are available for HCC, all of them aiming at destruction of the tumor with a safety margin of non-tumoral liver. The techniques most commonly used are alcoholization and radiofrequency. However, substances such as boiling saline solution and acetic acid can also be used. Coagulation by radiofrequency, microwaves, laser therapy, and electrocauterization are percutaneous techniques used for the treatment of HCC. Percutaneous ethanol injection (PEI) is the technique for which most experience has been obtained, with various studies showing its efficacy. El Kady et el., (2006) reported a new effective modality for treatment of HCC, Percutaneous ethanol lipiodol injection therapy (PELIT), with less number of sessions than ethanol injection.

A single tumor smaller than 3 cm or up to three nodules, none of them larger than 3 cm, without extrahepatic metastases and with only slightly deteriorated hepatic functional reserve (Child-Pugh A and B) and without surgical indication are the major indications for **PEI** (Aril et al., 2000 and Yamamoto et al., 2001).

Vilana et al. (1992), in an analysis of alcoholization of tumors measuring less than 5 cm, observed that tumors smaller than 3 cm gave a better response and concluded that the success of PEI is related to tumor size at the beginning of treatment, as also reported by others. PEI may have a therapeutic efficacy similar to resection or even to liver transplantation, especially when patients with poor hepatic function are selected for surgical treatment (Livraghi et al., 1995).

Local recurrence was observed in 17% of cases. The appearance of new lesions after treatment may reach 60% at 2 years and 80% at 5 years and is related to tumor size, inadequate necrosis, presence of pretreatment intrahepatic metastases (Vilana et al., 1992 and Livraghi et al., 1995).

Depending on the degree of hepatic dysfunction, the survival of patients submitted to PEI may reach 85% in the first year, 60% in the third, and 30% in the fifth. Rates similar to those obtained with surgical resection (Aril et al., 2000 and Yamamoto et al., 2001).

Radiofrequency has also been used successfully for patients with HCC. The indications are similar to those for PEI (Livraghi et al., 1999). No randomized studies comparing the two percutaneous techniques in terms of antitumoral effect and patient survival are available. The advantage of radiofrequency over PEI is the smaller number of sessions needed to obtain tumor necrosis also, radiofrequency is superior to PEI in ablation of HCC but PEI can be preferable for lesions abutting the GIT, portal vein or the biliary radic**les** (Mahran et al., 2006). PEI is less expensive and is easy to perform, requiring no hospitalization. Radiofrequency should be avoided in superficial lesions because of the risk of tumoral dissemination through the path of the needle. The 5-year survival rate for patients submitted to radiofrequency is about 33% (Buscarini et al., 2001). The option for PEI and radiofrequency depends on the experience in each group. Recently, an integrated system of CT and sonographic images helps radiofrequency ablation electrode placement in HCC, which cannot be adequately depicted on B-mode sonography (Minami and Kudo, 2006).

Transarterial embolization or chemoembolization

Since HCC is a tumor predominantly irrigated by the arterial system of the liver, blockade of the blood supply to the tumor is used as treatment. The surgical route has been abandoned due to its severe side effects and has been replaced with the use of arterial obstruction by a peripheral route using interventionist radiology. Tumors that cannot be submitted to radical treatment are considered for transarterial embolization (TAE)/ chemoembolization (TACE). Usually, patients with multiple diffuse tumors or single tumors larger than 5 cm, with deteriorated hepatic function are the main candidates for treatment by TAE (Bruix et al., 2001).

A higher frequency of post-embolization syndrome and of gastrointestinal toxicity tends to occur in cases in which lipiodol is used as the chemotherapeutic vehicle. Antibiotic prophylaxis should not be routinely applied to patients submitted to TAE (Castells et al., 1995).

Patients with a single tumor larger than 3 cm that cannot be treated

surgically may benefit from the combination of TAE and PEI. The combination of these two therapeutic techniques is based on the persistence of viable neoplastic cells after TAE, especially at the periphery of the lesion, where the predominant circulation seems to be the venous **one** (Franca et al., 2002). In addition, large tumors require larger amounts of alcohol to be necrotized. Thus, when TAE is performed first, necrosis of the central part of the HCC is obtained, and PEI is later performed at the periphery. This combination of techniques seems to be effective in reducing tumor size and increasing patient **survival** (Koda et al., 2001).

TACE has been used as co-adjuvant treatment when the patient is on the waiting list for liver **transplantation** (Vennok et al., 1995). The combination of TAE and PEI has shown a good anti tumoral effect in patients who are on the waiting list for liver transplantation (Franca et al., 2002). However, the possibility of tumor dissemination after treatment**,** detected by the presence of messenger RNA for AFP (Boix et al., 1996) and by a higher incidence of pulmonary metastases after TAE, should be considered at the time when a decision has to be made about co-adjuvant treatment in addition to radical therapy.

Evaluation of the therapeutic response (Franca et al., 2004).

Complete response: complete disappearance of known lesions and no occurrence of new lesions as evaluated during two observations separated by an interval of at least 4 weeks.

Partial response: reduction of the tumor mass >50% as evaluated during two observations separated by an interval of at least 4 weeks.

Stable disease: not classified as complete response, partial response or progressive disease.

Progressive disease: an increase >25% in the tumoral mass of one or more known lesions or appearance of new lesions.

Serum AFP levels can be used as a parameter of response to treatment only in cases in which their levels were elevated before the procedure. Elevation of their levels after treatment is suggestive of tumour recurrence.

Other therapies

In HCC, both estrogen and androgen receptors can be found on the membrane of neoplastic cells. The use of anti estrogens has been suggested for the treatment of HCC. However, tamoxifen (TAM), a non-steroid antiestrogen, did not prove to be useful in improving the quality of life of patients with HCC even when administered at high doses **(Chow et al., 2002).**

Gene therapy is approach to treat human diseases based on the transfer of genetic material to the cells. A cell is said to be transduced when it has incorporated and expresses a foreign gene **(Blau and Springer, 1995).** To facilitate cell transduction, the genetic material is packaged into molecular constructs named vectors, which can be of viral **(Giinzburg and Salmons, 1995)** and non-viral nature **(Ledley, 1995).** Viral vectors are frequently preferred because of their higher transduction efficiency and can be classified as long term and short-term expression vectors (retroviruses, adeno-associated viruses (AAV), gutless adenoviruses belong to the first category and first generation adenoviruses to the second) **(Giinzburg and Salmons, 1995).**

Because of the lack of curative treatment when the progression of the disease precludes surgical resection. Transfer of therapeutic genes to the tumour mass or to the peritumoral tissue provides a promising new approach to treat these processes. Different gene therapy based approaches have been tested to treat cancer including replacement of functional tumour suppressor genes, inhibition of oncogenes, transference to tumoral cells of genes conferring sensitisation to a specific prodrug ("suicide genes"), stimulation of antitumoral immunity, and inhibition of the formation of tumoral neovessels **(Prieto et al., 2003).**

Considerable hope is placed in the antitumoral effect of cytokines,

such as interleukin (IL) 12, endowed with potent antitumoral activity. IL12 acts by inducing a TH1 type of response, activating NK (Natural killer) cells and cytotoxic T lymphocytes, inhibiting tumoral neoangiogenesis, and increasing the expression of adhesion molecules on endothelial cells thus facilitating the traffic of lymphocytes to the tumour **(Shurin et al., 1997).** This cytokine, however, is toxic when administered systemically as a recombinant protein **(Lui et al., 2001).** The rational for IL12 gene therapy is to allow local production of the cytokine at the tumour site thus achieving high intratumour or peritumoral levels but low serum concentration, a scenario that might result in maximal antitumoral effect with minimal systemic toxicity.

It is known that dendritic cells are the most efficient antigen presenting cells. As activation of dendritic cells is critical for the induction of anti-tumour immunity, another possible way to take advantage of the therapeutic effect of IL12 is to infect dendritic cells with Ad.IL12 ex vivo and to inject these engineered dendritic cells into the tumour **(Melero et al., 1999).** Stimulation of dendritic cells is widely dependent on activation by costimulatory molecules like B7 and CD40 ligand.

The diversity of vectors, doses and routes of administration, and the variety of therapeutic genes used to treat different tumours make very premature the analysis of the potential of cancer gene therapy in humans. Moreover because gene therapy is still an investigational procedure many of the trials have been performed in patients with advanced tumours who have progressed despite chemotherapy **(Prieto et al., 2003).**

HBV and HCV co-infection

Epidemiology:

The exact number of patients infected with both HCV and HBV is unknown. An Eastern European study found a rate of dual infection in 0.68% of a randomly selected healthy population of over 2200 individuals **(Atanasova et al., 2004).** In patients with chronic hepatitis B, the rates of HCV co-infection vary from 9% to 30%, depending on

the geographic region **(Liaw, 1995).** In an Italian study the rate of dual infection increased with age, and was more common in patients over 50 years of age **(Gaeta et al., 2003).** These numbers may underestimate the true number of patients with both viral infections because no large-scale studies have been performed, and there is a well-described phenomenon of "serologically silent" occult HBV infection .In Egypt, 5% of population had markers for both hepatitis B and C **(El Gohary et al., 1995 and El-Sayed et al., 1997).**

Screening for Co-infection:

Persons with a first episode of acute hepatitis should be screened for all viral causes including HBV and HCV. Some patients may be inoculated with both viruses simultaneously and will present with acute hepatitis due to both viruses. In addition, HBV superinfection in patients with chronic hepatitis C, and HCV superinfection in patients with chronic hepatitis B have both been reported **(Liaw et al., 2000).** Therefore, episodes of acute hepatitis in patients with known chronic HBV or HCV infection, especially those with ongoing risk behavior for infection with the alternative virus such as injection drug users, should raise suspicion and prompt screening for superinfection. In addition, as will be described below, silent or occult HBV infection in patients with chronic hepatitis C may alter patients' clinical course and response to therapy **(Fukuda et al, 2001).**

Interaction of Hepatitis Viruses:

Several studies have shown that the HBV and HCV interact with each other and affect immune responses. HCV infection can suppress HBV replication, as demonstrated by studies showing that patients with chronic hepatitis B who are co-infected with HCV have lower HBV DNA levels, decreased activity of HBV DNA polymerase, and decreased expression of HBsAg and hepatitis B core antigen in the liver **(Chu et al., 1998).** Furthermore, patients with chronic HBV infection who become superinfected with HCV can undergo seroconversion of hepatitis B e antigen (HBeAg) and HBsAg to respective antibodies **(Dai et al., 2001). Sheen et al. (1994)** conducted a longitudinal follow-up study of a

large series of HBV infected patients and found that the annual incidence of HBsAg seroconversion was 2.08% in co-infected patients compared to 0.43% in patients with HBV monoinfection, and a subsequent study confirmed these results **(Utili et al., 1999).** Several mechanisms of replicative interference of HBV by HCV have been proposed and implication of the hepatitis C core protein in suppression of HBV was reported **(Shih et al., 1993).**

A subsequent study found that the hepatitis C core protein suppressed HBV enhancer activity, thereby affecting transcription **(Schuttler et al., 2002).** This inhibitory effect appears to be more pronounced with HCV genotype 1 both ***in vitro*** and ***in vivo*** **(Pontisso et al., 1996 and Schuttler et al., 2002).**

Several authors had reported that HBV can reciprocally inhibit HCV replication as well **(Pontisso et al., 1993).** Specifically, HBV DNA replication has been shown to correlate with decreased HCV RNA levels in co-infected patients **(Zarski et al., 1998).** In an Italian study, co-infected patients had a rate of HCV RNA clearance of 71% compared to 14% with HCV monoinfection **(Pontisso et al., 1996).** HBV replication in co-infected individuals may result in more liver inflammation, as demonstrated by studies in which HBV replication correlated with elevated ALT levels, while HCV replication did not **(Pontisso et al., 1993 and Ohkawa et al., 1995).**

Overall, the available evidence demonstrates that both viruses can inhibit each other simultaneously; either virus can play a dominant role; both viruses have the ability to induce seroconversion of the other; the chronicity of infection has a role in determining the dominant virus; and HBV and HCV can alternate their dominance **(Liaw, 1995).** However, the overall dominant effect appears to be HCV suppression of HBV **(Liaw, 2002).**

Clinical Picture:

Different patterns of infection have been described with dual infection with HBV and HCV Including:

- Acute dual viral hepatitis.
- Occult HBV co-infection of chronic hepatitis C**.**
- Super infection by either virus in patients with preexisting chronic hepatitis due to the alternative virus.

In addition, co-infected patients are often found to have evidence of both HBV and HCV infection without a clear chronology of infection. In areas with high endemic rates of HBV infection due to vertical transmission, co-infection can generally be assumed to be due to HCV superinfection. In other geographic areas, the sequence of infections is less clear. Acute co-infection or super infection with either virus can lead to fulminant hepatitis, chronic hepatitis, cirrhosis and HCC **(Crockett and Keeffe, 2005).**

Super infection with HBV in patients with chronic hepatitis C is less common **(Liaw et al., 2000).**

Fulminant Hepatitis in co-infection of HBV and HCV infection:

Several studies have addressed the role of co-infection with HBV and HCV in fulminant hepatitis. **Chu et al. (1994)** conducted a prospective study of patients admitted with acute hepatitis C in Taiwan. Eleven patients had fulminant hepatitis, and of these, 23% had underlying chronic HBV infection, compared to 2.9% for patients without fulminant hepatitis. A French study of 40 patients with fulminant and subfulminant hepatitis found that 5 of 40 patients (12.5%) had acute co-infection with HBV and HCV and 3 of 40 (7.5%) had superinfection of HCV **(Feray et al., 1993).** Another Taiwanese study of 25 subfulminant and fulminant hepatitis cases found similar rates of co-infection (9.4%) and HCV superinfection of chronic hepatitis B (3.1%) **(Wu et al., 1994).** These studies suggested an increased risk of fulminant hepatitis with HCV and HBV co- infection and superinfection.

Chronic Hepatitis of co-infection of HBV and HCV infection (Crockett and Keeffe, 2005):

There are various immune profiles of dually infected patients with chronic hepatitis, and their immune profiles have a bearing on the choice of treatment. One possibility is dually active HBV and HCV, in which patients have detectable serum HBV DNA and HCV RNA. It stands to reason that these patients are at highest risk of progression to cirrhosis and decompensated liver disease, and therefore, should be considered for treatment. Another possibility is active HCV infection (positive HCV RNA) in the setting of an inactive HBsAg carrier. Such patients behave similar to patients with HCV monoinfection, and likely exhibit HCV viral suppression of HBV activity. Another possibility is active HBV infection in patients with inactive or prior HCV infection (HBV DNA positive/HBeAg positive/HCV RNA negative/anti-HCV positive). It is less common, and indicates HBV suppression of HCV.

Inapparent co-infection (occult hepatitis B virus in co-infection).

Several studies have reported detectable HBV DNA in patients with chronic hepatitis C but negative HBsAg. This so-called "serologically silent" HBV infection or "inapparent co-infection" has been correlated with impaired response to IFN treatment **(Crockett and Keeffe, 2005). Zignego et al. (1997)** reported significantly worsened results in 14 chronically infected HCV patients with inapparent HBV co-infection (anti-HCV-positive, HBV DNA- positive, HBsAg-negative). Patients were treated with IFN alfa-2a twice/week for 12 months. Four out of 14 patients had normal ALT levels at the end of therapy (28%), but all had relapsed within 6 month post-treatment, and thus none had a SVR.

Fukuda et al. (1999) also found that silent HBV infection was associated with higher ALT levels, greater histological activity scores and poor efficacy of IFN treatment. Some have proposed that the impaired response to IFN in such patients may be due to HBV-mediated down-regulation of intrahepatic IFN receptor gene **(Crockett and Keeffe, 2005).**

A hepatitis C flare has been described in a co-infected patient who had HBeAg/HBV DNA clearance in response to IFN **(Liaw et al., 1997).**

Treatment of HBV and HCV co-infection;

Interferon plus Ribavirin:

Liu et al. (2003) ***recommended antiviral combination therapy with IFNa plus ribavirin: with IFN alfa-2a (6 MU TIW for 12 weeks***

followed by 3 MU TIW for 12 weeks), concurrently with ribavirin 1200 mg daily for 24 weeks. These results demonstrate the effectiveness of combined IFN and ribavirin in coinfected patients, with a rate of SVR and SBR comparable to HCV monoinfected patients. However **Yalcin et al. (2003)** *reported a severe hepatitis B flare in a patient with HBV/HCV co-infection (HBV DNA-negative) undergoing treatment with IFN and ribavirin. The infection of hepatitis improved after discontinuation of therapy, but a relapse of HCV infection with rapid progression to cirrhosis occurred thereafter. Clinicians must exercise caution when treating co-infected patients with combination IFN plus ribavirin given this risk of HBV reactivation.*

Interferon plus Lamivudine:

One study of lamivudine therapy in addition to IFN, (5 MU of IFN and lamivudine 100 mg/day for 12 months followed by lamivudine alone for 6 months), for co-infected patients has been published by **Marrone et al., 2004.** *This study suggested that the addition of lamivudine to IFN may be effective in coinfected patients with chronic hepatitis C and active HBV replication.*

Adefovir and Entecavir:

There have been no published studies regarding treatment of co-infected patients with the newer agents' adefovir and entecavir. However, these agents may be useful, particularly in patients with HBV-dominant disease. Studies need to be performed using these agents before they can be recommended for routine usage **(Marrone et al., 2004).**

Transplantation:

The United Network of Organ Sharing (UNOS) (2005) *reported that 14 patients were transplanted for combined hepatitis B and C in the United States in 2004, and 434 patients have been transplanted for this indication since 1988.*

***There is no currently established standard of care for patients who are co-infected with HBV and HCV. In general, the same treatment criteria should be applied to patients who are HBV/HCV dually infected as are applied to monoinfected patients. Initiation of treatment, as with both HBV and HCV, is recommended in patients with active chronic hepatitis or cirrhosis prior to decompensation. Given the complex interaction of HBV and HCV both with each other and with the immune system, care must be taken to select the most appropriate antiviral regimen based on serologic markers and levels of viremia* (Crockett *and* Keeffe, 2005).**

Triple hepatotropic viral infections with HBV, HCV and HDV can result in more severe hepatitis, and therefore compel the clinician to offer treatment* (Liaw,** *1995). Few studies have been published regarding treatment of patients with triple hepatitis virus infection.* **Weltman et al.** *(1995) studied 7 patients with triple infection who received IFN therapy. One patient had a reported SBR, and 2 patients were withdrawn from treatment due to side effects. Interferon treatment is a reasonable recommendation despite the paucity of data to support its use.

HBV and HCC

Mechanism of HBV induced carcinogen:

***From the viewpoint of carcinogenesis, the integration of HBV DNA into the cell genome and the production of the X protein (HBxAg) seem to be of significance. Integration of the provirus into the host genome is important in the replication cycle. It is not, however, a "necessary" part of the viral cycle in HBV replication* (Ferber et al., 2003).** ***The integration is random, usually multiple, does not preserve the viral genome sequence and is variable. The integrated viral DNA might therefore act as a mutagenic agent, causing secondary chromosomal rearrangement (duplications, translocations, deletions) and increasing genomic instability. The deletions might involve loss of tumor suppressor genes, or the amplification, over expression of growth factor genes which influence cell proliferation and cell cycle control* (Anthony, 2001).**

HBV x gene/protein

***The term "gene" is applied because its role during acute/chronic viral infection is not known, despite its essentiality for the viral cycle. The protein product (HBx) functions as a transcriptional transactivator of different host genes involved in cellular growth control* (Feitelson, 1999 and Moradpour and Wands, 2003).**

***HBx transactivates cellular genes involved in cell proliferation control (c-jun, c-fos, c-myc). This transactivation activity appears to involve stimulation of the protein kinase C (PKC) and nuclear factor kappa B (NF_kB) pathways. The hepatitis B virus X protein deregulates cell cycle control, interferes with cellular DNA repair and apoptosis. It is important that HBx may interact with p53 and retinoblastoma (RB)* (Wang-Shick Ryu, 2003).**

***Although the pleiotropic functions of HBx were illustrated, it is unclear which functions directly contribute to the viral carcinogenesis. It is relevant that HBx has been implicated in both the deregulation of the cell cycle control or cell proliferation* (Benn and Schneider, 1995), *as well as the apoptosis* (Kim et al., 1998).**

***Apoptosis normally eliminates the cells with damaged DNA; that is, the ones that are most likely to engender a neoplastic clone. The proapoptotic activity of HBx may contribute to viral oncogenesis by exerting a selective pressure that favors the emergence of mutated cells. Collectively, HBx has pleiotropic activities that might involve in viral carcinogenesis* (Wang-ShickRyu, 2003).**

Mechanism of HCV and HBV induced hepatocarcinogenesis

In contrast to HBV, the putative receptors for binding HCV are known, as CD81* (Pileri et al., 1998), *LDL-R* (Scarselli et al., 2002), *human scavenger receptor B1* (Agnello et al., 1999 and Saunier et al., 2003).** ***An interesting step in the "regular" viral cycle is the entrance of smaller viral core portions (pi9, p21) into the nucleus* (Scarselli et al., *2002). HCV core proteins can modulate various cellular signal transduction pathways, namely by mediating the transcription activity of NF_kB and* STAT*-3 proteins* (Waris and Siddiqui, *2003).

It is not clear whether evidence of previous, rather than chronic, infection with HBV has any impact on disease progression or the

***development of hepatocellular carcinoma (HCC) in patients with chronic HCV infection* (Marusawa., 2004)**

There is an increased risk of developing HCC, even in HBsAg negative, anti-HBs positive cases, most probably related to persistence of low levels of hepatic HBV DNA which can also be isolated from tumour tissue* (Brechot et al.,** *1998)* ***The HBV encoded X protein is known to regulate both cell proliferation and apoptosis, and the combination of chronic HCV infection with its attendant increase in hepatocyte turnover together with continuing production of HBV encoded proteins may be synergistic with regard to HCC development.

***The association between previous HBV infection and severity of HCV infection is less clear. In the UK, HBV infection is uncommon and evidence of previous HBV infection in patients with chronic HCV is most likely to have resulted from intravenous drug usage, with vertically acquired HBV being rare. HBV infection in adults behaves very differently to that in children and further studies are needed to clarify whether apparently resolved HBV infection in adults is truly a factor contributing to the progression of HCV related liver damage* (SZABO et al.,2004).**

***It is generally accepted that neither HBV nor HCV are directly cytopathic viruses* (Nakamoto and Kanekos, 2003).** ***An important effect of both viruses, however, is causing chronic infection, and repeating attacks of the host immune system against the viral infection. Continuous cell death, mainly by apoptosis, and reactive proliferation occur through the inflammation-necrosis-regeneration sequence, as the basis of cirrhosis* (Tornillo et al., 2000).**

***Summarizing the role of pathways playing a role in HBV and HCV induced HCC, several common and differing features can be observed. Chronic inflammation, cell death and proliferation, as a result of the oxidative stress, and up- and down-regulation of several growth factors and cytokines, play a central role. Viral integration is an essential part of cell transformation by HBV, which does not occur in HCV infection. However, viral proteins, especially HBx and to a certain extent (at least it is now believed) the core component of HCV, may directly participate in the hepatocarcinogenesis* (SZABO et al., 2004).**

Table 8. ***Common genetic alterations in HCC****

G → T transversion in codon 249 (AFB1) of the p53 gene
p53 mutation (15-50%)
Loss of heterozygosity (LOH) on 8p, 17p
p-catenin mutation (20-40%)
pi 6 INK4a promoter methylation (~ 70%)
Loss of pl6INK4a expression
E-cadherin promoter methylation (~ 70%)
Decreased p27 expression (50%)

*** based on** Edamoto et al. (2003)

Table 9. ***Common altered pathways in HCC****

• ***p53 pathway***
(p53 mutations, pl4ARF promoter methylation)
• ***Wnt pathway***
(mutation of P-catenin)
• ***RBI pathway***
(pl6INK4a methylation, loss of RBI expression cyclin D1 amplification)

***based on** Edamoto et al. (2003).

RB = Retinoblastoma.

Occult Hepatitis B Virus

Definition of occult HBV***, HBV infection in absence of detectable HBsAg* (Chan and Lok, 1999)*** ***and are usually characterised by very low serum HBV DNA levels usually less than*** 10^4 ***copies/ml (Allain, 2004).***

***Also, it was defined as the presence of HBV DNA without detectable HBsAg with or without anti-HBc or anti-HBs outside the pre-seroconversion window period* (Torbenson and Thomas, 2002).**

Long-term studies of HBV in chronic and recovered infections showed that after HBsAg is no longer detectable, HBV DNA persists for years in serum or in the liver* (Torbenson and Thomas, 2002 and Yuki et al.,** 2003). ***Sensitivity improvement of PCR methods led to the identification of an increasing number of individuals carrying HBV DNA as the only marker of active

infection.

Mechanisms of occult HBV, ***Several possibilities have been hypothesized as the mechanisms of occult HBV infection. These include:***

- ***Mutations of HBV-DNA sequence.***
- ***Integration of HBV-DNA into host's chromosomes.***
- ***Infection of peripheral blood mononuclear cells by HBV formation of HBV-containing immune complex, altered host immune response.***
- ***Interference of HBV by other viruses*** *(Rapicetta et al., 1989).*

***Occult HBV infection has been found in patients with hepatocellular carcinoma (HCC), cryptogenic cirrhosis, and individuals without HBV serological markers. The frequency of the diagnosis depends on the relative sensitivity of HBV DNA assays and the prevalence of HBV infection in the population* (Hou et al., 2005).**

Occult HBV and HCV in chronic liver disease:

Since the last decade of the past millennium many studies have pointed out to the strikingly high prevalence of hepatitis C virus (HCV) among Egyptian chronic liver disease patients **(Abdel-Wahab et al., 1994),** ***and the relative decline of HBV as the sole pathogen in this population*** **(Kabil et al., 1990).** ***It is noteworthy that multiple evidences have shown that combined chronic HBV infection (manifested as positive HBsAg) and HCV infection leads to more severe liver disease and an increased risk of hepatocellular carcinoma*** **(Raimondo et al., 2005).** ***Very low levels of HBV DNA in absence of any detectable HBsAg in the serum have also been identified*** **(Tanaka et al., 1990)** ***in the sera of patients with chronic HCV infection. But the prevalence of occult HBV among HCV related chronic liver disease has been quite variable among different populations*** **(Villa et al., 1995)** ***Some researchers detected that this occult HBV infection contributes to severer chronic liver damage and promotes HCC development but also, is associated with a lack of response to interferon treatment in patients with chronic hepatitis C*** **(Kao 2002).** ***Others mentioned that occult HBV infection may not have a significant impact on***

response to interferon therapy for chronic HCV and development of HCC after therapy **(Hassegawa et al., 2005).**

Prevalance of occult HBV:

Darwish et al., 2001 *reported the prevalence of HBV DNA in their group of healthy control group was 3.3% which matches an intermediate rate of HBsAg prevalence (5-8%) among the whole population. Hepatitis B virus infection, including occult infection has been associated with the high incidence of HBV integration and subsequent development of HCC. HBV DNA integration may cause rearrangement of the viral DNA sequence* **(Lai et al., 1990)**, *which combined with other factors, such as genetic instability, p53 gene mutation and telomerase activity, may play a contributory role in carcinogenesis* **(Fukuda et al., 1996& Cacciola et al., 2000 and Marusawa et al., 2000).**

The rate of detection of HBV DNA in liver tissue in HCC patients have always been higher than the rate of detection of serum HBV DNA in the same population **(Cacciola et al., 2000).**

The high occurrence of occult HBV infection in those with chronic HCV infection can be explained by the fact that both HBV and HCV are transmitted parenterally and share common routes of infection; thus infection with both viruses may occur. The highest prevalence of HCV is reported from Egypt, as a result of extensive schistosomiasis control programs that used intravenously administered tartar-emetic 20-50 years ago, and probably resulted in the establishment of a large reservoir of infection **(Frank et al., 2000).** *The campaigns were directed to school children, military recruits and villagers. Thus, they iatrogenically probably transmitted both viruses especially that HBV is more infectious than HCV* **(Van der et al., 2005).** *As the rate of chronicity in HCV infection is generally the rule and is in the order of 80%* **(McHutchison and Bacon, 2005).** *Unlike HBV, which as a DNA virus integrates into the hepatocyte and promotes carcinogenesis, the mechanism by which HCV induces the development of HCC is largely through promotion of cirrhosis but, recently HCV core protein was reported to have transactivating properties* **(Koike, 2005).**

Occult HBV and HCC

The potential mechanisms whereby an overt HBV might induce tumour formation are mostly maintained in cases of occult infection as both integrated viral DNA and covalently closed circular HBV genomes were detected in patients with occult HBV. The report by* Tamori et al. (2005) *has shown a contributing role of occult hepatitis B infection in the pathogenesis of HCC among reported HCV antiviral "sustained virologic responders", as they found demonstrable HBV in tumour and adjacent nonneopalstic liver. Studies on HBV and HCV co-infection revealed a mutual viral interference between HBV and HCV, with a more potent inhibitory effect of HCV on HBV replication. Human subjects and chimpanzee models with chronic HBV and HCV co-infection tend to have a low level of HBV-DNA and significantly enhanced HBsAg clearance* (Raimondo et al., 2005). *Cotransfection studies revealed that HCV core protein may be involved in the inhibition of HBV replication thus, HBV DNA is frequently found in the liver of patients with chronic hepatitis C (Shih* et al., 1993). *So the prevalence of serum HBV DNA may underestimate the true prevalence of occult HBV in our HCV series.

Patients and Methods

A) Patients:

This study was carried out on 21 patients with hepatocellular carcinoma and 18 patients with cirrhosis. The patients were selected from those admitted to Tropical Medicine Department, Tanta University Hospital during the period of the study between February and October 2006. Eighteen apparently healthy subjects were chosen as controls.

The involved subjects were divided into three groups:

- *Group I:* ***included 21 patients with hepatocellular carcinoma; 15 males & 6 females, their ages ranged from 40 to 71 years with a mean age 52.667 ± 8.278 years.***

- *Group II:* ***included 18 patients with liver cirrhosis; 12 males & 6 females.***

Their ages ranged from 30 to 55 years with a mean age of 42.000 ± 8.239 years

- *Control group:* ***included 18 apparently healthy subjects chosen from the patients relatives; 11 males & 7 females. Their ages ranged from 29 to 45 years with a mean age of 37.615 ± 5.052 years.***

Exclusion criteria:

- ***Patients with hypertension.***
- ***Patients with renal failure.***
- ***Patients with rheumatic diseases.***
- ***Patients with concurrent infections.***

B) Methods:

All patients were subjected to the following:

- ***History taking.***
- ***Clinical examination.***
- ***Laboratory investigations.***
- ***Abdominal ultrasonography.***
- ***Computed tomography (CT) scans for some patients.***
- ***Liver biopsy for some patients.***
- ***Special investigations including:-***
- ***Detection of hepatitis C virus (HCV) antibodies by (ELISA) followed by Polymerase Chain Reaction (PCR) for HCV RNA as a confirmatory test in positive patients.***
- ***Detection of hepatitis B surface antigen (HBsAg) by (ELISA). Confirmatory Polymerase Chain Reaction (PCR) for HBV DNA was performed in negative patients.***
- ***Alpha fetoprotein (AFP).***

I. History taking:

A careful history was taken considering history of operations, blood transfusion, history of intravenous drug abuse, history of anti-bilharzial treatment, using shared articles like razors, syringes, tooth brushes, needles, shaving machine, scissors, combs, forks and foments as risk factors of viral hepatitis infection and also asking about risk factors for hepatocellular carcinoma including residence , history of intake of oral contraceptive pills in females, special habits, exposure to chemicals e.g insecticides and pesticides and associated diseases specially diabetes mellitus.

II. Full Clinical Examination:

General examination was done to detect jaundice, lower limb oedema, signs of weight loss, cachexia or associated liver diseases. Abdominal examination was done to evaluate the liver size, consistency, tenderness, masses, spleen, ascites and other abdominal organs for complications or association of other diseases.

III. Laboratory Investigations:

- **Complete blood picture.**
- *Complete urine analysis.*
- *Complete stool analysis.*
- *Erythrocyte sedimentation rate (ESR).*
- *Blood urea and serum creatinine.*
- *Blood sugar (fasting and post prandial).*
- *Liver function tests:*
- *Serum albumin.*
- *Serum bilirubin (direct and indirect).*
- *Serum transaminases (AST and ALT).*
- *Prothrombin time and activity.*

IV- Abdominal ultrasonography:

Ultrasonography of the liver was done to assess its size and echo pattern in order to detect any pathology such as liver cirrhosis and to detect focal masses. The site, size and echogenicity of the detected masses were recorded. Ultrasonographic findings for HCC were searched for, such as focal lesion of hypoechoeic or mosaic pattern either with or without peripheral sonolucency (halo), produced by fibrotic pseudocapsule **(Ebara et al., 1986 and Franca et al., 2004).** *The portal and splenic veins were also evaluated as regards diameter and thrombosis if present.*

Other abdominal organs were scanned as well as the presence of ascites or retroperitoneal masses. Abdominal ultrasonography was performed in the Tropical Medicine Department for all studied patients and controls using Toshiba Capase Machine (770/25ANHz3-5) with a convex probe 3.5 MHz.

Technique:

Patients were examined after overnight fasting in the supine and lateral positions. Aquatic gel was spread as a film on the abdomen of the patients to prevent interposition of air between transducer and the skin. Survey scanning was done through several longitudinal, oblique and transverse cuts. Measurements were

taken in quiet respiration.

V. Triphasic Computed Tomography (CT) scans:

CT scanning of the abdomen was done for better evaluation, to some cases in group I which could not be proved properly by ultrasonography. Scanning was done using intravenous iodinated contrast e.g urographin. It has many advantages over ultrasonography: it is less operator dependant, capable of detecting small lesions (less than 1 cm in diameter), can calculate the percentage of hepatic replacement by the tumour. Triphasic CT can detect HCC as small as 0.5 cm with sensitivity up to 97% **(Gunver et al.,** *1985* **and Franca et al., 2004)**

VI. Liver Biopsy:

Some patients of group I were subjected to liver biopsy but some of the cases came to our department with already preformed liver biopsy.

A) Preparation of the patient:

Prothrombin time was not more than three seconds prolonged over control value. If prothrombin time is prolonged above three seconds, the patient should be given 10 mg vitamin K parenterally for 3 days after which prothrombin time is repeated. The platelet count should exceed 80.000/mm^3. Fresh blood was available during the procedure to be ready for any sign of bleeding. In cases where ascites may be present, measures should be taken to minimize it. The procedure was not be done with tense ascites or shrunken liver **(Huang et al., 1996).**

B) Procedure:

- ***The procedure was explained to the patient before hand to obtain his confidence as well as his cooperation, as he will have to hold his breath at intervals during the procedure. Sedation was not essential.***
- ***Sonographic scanning was done to determine the site and description of the lesion.***
- ***The skin entry site was marked.***
- ***Three to five ml of local infiltration anaesthesia e.g. lignocaine were injected into the puncture site.***
- ***A nick is made at the entry site using a scalpel blade.***
- ***The biopsy was taken using a 14-gauge tru-cut needle.***

- ***7- The hepatic tissue core was then fixed in 10% formalin and sent for histological examination. Routine stain with Haematoxyline and Eosin was done.***

VII. Special Investigations:

- ***Hepatitis C virus antibodies were tested using Hepatitis C Virus Test (Qualitest HCV- 3rd generation ELISA)*** **(Kuba et al., 1999).**

- ***Hepatitis B surface antigen (HBsAg) was tested using Hepatitis B Surface Antigen Test (Qualitest HBsAg - 3rd generation ELISA)*** **(Haverkos, 2004).**

- ***Detection of HCV-RNA by Polymerase chain reaction was performed for positive cases of HCV antibodies.***

- ***Detection of HBV-DNA by Polymerase chain reaction was performed for HBs Ag negative cases.***

- ***Serum alpha-fetoprotein was estimated using the one-step immunoenzymatic mediated assay (Sorin Biomedica- 3rd generation ELISA)*** **(Chieregatti, 1990).**

Polymerase Chain Reaction (PCR).

(QUIGEN &Roche)

Blood samples were collected aseptically. Serum was separated then stored frozen at -70°C till all samples were tested at the same time.
Samples were subjected to cell lysis, RNase treatment and DNA and RNA precipitation. A master mix was prepared for each of DNA and RNA. PCR was then performed using thermal cycler.
The following primers were used:
HCV primers sequence:
Primer h 5* TGC TCA TGG TGC ACG GTC TA 3'
Primer 2: 5' CCA TGG CGT TAG TAT GAG TG 3'
Primer 3: 5' AGA GCC ATA GTG GTC TGC GG 3'
Primer 4: 5' CTT TCG CGA CCC AAC ACT AC 3'
HBV Primers sequence:
Primer 1: 5' CCT GCT GGT GGC TCC AGT TC3'

Primer 2: 5' CCA CCA TTC ATT GAC ATA CTT TCC A3'
Primer 3: 5' GCA CAC GGA ATT CCG AGG ACT GGC GAC CCT G3'
Primer 4: 5' GCA CCA AGC TTG GTT AGG GTT TAA ATG TAA CC3'
DNA and RNA products were then detected by agarose gel electrophoresis. The gel was visualized on a UV transilluminator and photographed by a Polarotd camera. The results were interpreted. Positive control for HBV and HCV give sharp band at 250 bp and 186 bp respectively, compared to 100 bp ladder marker. Negative control lane did not give the expected band. The sample was considered positive when there was a band at the same level of the positive control band.

Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, Student t-test analysis of variance [ANOVA] test and chi-square test. By SPSS VI1

1. Mean **=**

Where Σ = sum & n = number of observations.

2. Standard Deviation ***[SD]:***

Student t-test [Unpaired]:

t =

Where:

X_x ***= Mean of the first group.***

X_2 ***— Mean of the second group.***

SE_1 ***= Standard error of the first group.***

SE_2***= Standard error of the second group.***

Unpaired Student T-test was used to compare between two groups in quantitative data.

P value is significant when it is below (0.05).

- Analysis of variance [ANOVA] tests. ***According to the computer program SPSS for Windows. ANOVA test was used for comparison among different times in the same group in quantitative data.***

Chi-square *the hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship.*

Mann-Whitney

A non parametric equivalent to the t test. Tests whether two independent samples are from the same population. It is more powerful than the median test since it uses the ranks of the cases. Requires an ordinal level of measurement.

Kruskal-Wallis

A non parametric equivalent to one-way ANOVA. Tests whether several independent samples are from the same population. Assumes that the underlying variable has a continuous distribution, and requires an ordinal level of measurement.

RESULTS

The present study was carried out on 39 patients with chronic liver diseases selected from the inpatient clinics of Tropical Medicine Department, Tanta University Hospital during the period of the study between February 2006 and October 2006. Eighteen apparently healthy subjects were involved as a control group (relatives of the patients). The involved subjects were divided into:

- *Group I: included 21 patients with hepatocellular carcinoma (HCC).*
- *Group II: included 18 patients with liver cirrhosis.*
- *Control group: included 18 apparently healthy subjects.*

The results were tabulated and statistically analyzed.

Age of the studied groups:

Table (10) and Figure (1) showed the age of the studied groups. In group I, the age ranged between 40-71 years with a mean value of (52.667±8.278) and between 30-55 years with a mean value of (42.000±8.239) in group II and between 29 - 45 years in control group with a mean value of (37.615±5.052).

There was a statistically significant increase in age in group I as compared to group II and control group ($p < 0.001$). But, there was no statistical difference in age of group II as compared to control group ($p > 0.05$).

Sex distribution among the studied groups:

Table (11) and Figure (2) showed the sex distribution among all studied groups. Group I included 15 males (71.43%) and 6 females (28.57%), group II included 12 males (66.67%) and 6 females (33.33%) and control group included 11 males (61.11%) and 7 females (38.89%).

There was no significant difference among all studied groups as regards sex distribution ($p>0.05$).

Some risk factors of viral hepatitis detected in all studied groups:

Table (12) and Figure (3) showed that: In group I, there were 15/21 patients

(71.43%) with history of blood transfusion and 11/21 patients (52.38%) with past history of operations; 2/ll patients (18.18%) with splenectomy, 5/1 l patients (45.45%) with cholecystectomy, 3/1 lpatients (27.27%)with appendectomy and 1/11 patients(9.09%) with tonsillectomy . Eight out of twenty one patients (38.10%) received intravenous antischistosomal drugs. In group II, there were 10/18 patients (55.56%) with history of blood transfusion, 10/18 patients (55.56%) with past history of operations; 3/10 patients (30%) with splenectomy, 3/10 patients (30%) with cholecystectomy, 2/10 patients (20%) with appendectomy and 2/10 patients (20%) with tonsillectomy. There were 8/18 patients (44.44%), who received intravenous antischistosomal drugs. In control group, there was neither history of blood transfusion nor past history of operations nor history of receiving intravenous antischistosomal treatment.

There was a statistically significant increase in number of patients with a past history of blood transfusion, past history of operations and history of intake of intravenous antischistosomal treatment in group I, II as compared to control group (P0.001).

All patients and subjects of all groups shared articles carrying risk of infection e.g. syringes, razors, tooth brushes, needles, shaving machin, scissors, combs, forks and foments.

Some non viral risk factors of malignancy detected in all studied groups;

Table (13) and figure (4) showed that:

In group I, 12/21 were smokers (57.14%) and 1/21 was ex-smoker (4.76%). There were 15/21 diabetic patients (71.43%). 7/21 patients (33.33%) with family history of malignancy and 5/6 females (83.33%) with past history of using oral contraceptive pills. In group II, 4/18 were smokers (22.22%) and 1/18 was ex-smoker (5.56%). There were 6/18 (33.33%) diabetic patients, 1/18 patient (5.56%) with positive family history of malignancy and 2/6 females (33.33%) with history of usage of oral contraceptive pills.

The control group included non smokers, non diabetics. They all had no family history of malignancy but only 1/7 female (14.29%) received oral contraceptive pills.

A statistically significant increase was found in the number of smokers, number of diabetic patients and those with positive family history of malignancy in group I compared to group II and control group ($p < 0.001$). And in group II compared to control group ($p<0.001$). Also a significant increase was found in number of females_who received oral contraceptive pills in group I as compared to group II and control group ($p<0.001$) but no significance difference was found between group II and control group ($P> 0.05$).

Clinical data of the studied groups:

Table (14) and Figure (5) showed; in group I, the following clinical data were observed:

- *Jaundice was found in 15/21 patients (71.43%).*
- *Lower limb oedema was noticed in 16/21 patients (76.19%).*
- *Ascites was noticed in 16/21 patients (76.19%).*
- *Splenomegaly was noticed in 16/21 patients (76.19%).*
- *Splenectomy was noticed in 3/21 patients (14.29%).*
- *Normal sized spleen was noticed in 2/21 patients (9.52%).*

- *Enlarged hard tender liver was observed in 7/21 patients (33.33%).*
- *Shrunken liver was observed in 14/21 patients (66.67%).*
- *Upper quadrant abdominal pain was found in 14/21 patients (66.67%).*
- *Flapping tremors was found in 14/21 patients (66.67%).*
- *Clubbing was found in 5/21 patients (23.81%).*
- *Skin echymosis was noticed in 10/21 patients (47.61%).*
- *Bleeding/gum was detected in 8/21 patients (38.10%).*
- *Bleeding varices was found in 7/21 patients (33.33%).*

While in group II, showed:

- *Jaundice was found in 9/18 patients (50%).*
- *Lower limb oedema was noticed in 11/18 patients (61.11%).*
- *Ascites was noticed in 15/18 patients (83.33%).*
- *Splenomegaly was noticed in 15/18 patients (83.33%).*
- *Splenectomy was reported in 3/18 patients (16.67%).*
- *Shrunken liver was observed in all patients.*
- *Upper quadrant abdominal pain was found in 2/18 patients* **(11.11%).**
- *Flapping tremors was found in 9/18 patients (50%).*
- *Clubbing was found in 4/18 patients (22.22%).*
- *Skin echymosis was noticed in 11 /18 patients (61.11%).*
- *Bleeding/gum was detected in 8/18 patients (44.44%).*
- *Bleeding varices was found in 9/18 patients (50%).*

Subjects comprising the control group were free from jaundice, lower limb oedema, ascites, organomegaly, pain, tremors, clubing, skin echymosis, bleeding per gum and bleeding varices as well.

Laboratory results:

Table (15) and Figure (6) showed the mean of haemoglobin concentration. It was (10.025±1.410) gm/dl in group I, (11.203±1.514) gm/dl in group II and (12.501±0.699) gm/dl in control group. There was statistically increase in group I as compared to group II (P < 0.001), group I and control group (P < 0.05) and between group II and control group (p <0.05).

Table (16) and Figure (7) showed total leucocytic count in all studied groups. The mean value of leucocytic count was (7.670 xlO3 ±1.03 8x103) /mm3 in group I, (5.228xl03±1.070xl03) /mm3 in group II and (5.909x 103± 1.263 x 103) /mm3 in control group. There was statistical significant increased values in group I than group II (p < 0.001), between group II and control group (p value < 0.001), also between group I and control group (P<0.05).

Table (17) and Figure (8) showed platelet count of all studied groups. The mean value of platelet count was (140.143 xl03±54.152xl03) / mm3 in group I, in group II it was (139.000 xl03±36.979xl03) / mm3 and (279.994x103±97.905xl03) / mm3 in control group. There was no statistical significant difference between group I and group II (p > 0.05), but there was statistical significant increased values in group I than control group (p < 0.001), also between group II and control group (P0.001).

Table (18) and Figures (9 & 10 & 11) showed the liver function tests in the studied groups.

Total serum bilirubin level ranged between 0.9 to 5.5 mg/dl with a mean value of 3.810 ± 1.681 mg /dl in group I, in group II it ranged between 0.6 to 4.00 mg /dl with a mean value of 2.538 ± 0.993 mg/dl and in control group ranged between 0.65 to 1.00 mg/dl with a mean value of .790±0.128 mg/dl. There was no statistically significant increase in total serum bilirubin level in group) I as compared to group II (p >0.05), but there was statistically significant increase in its level in group I and

group II as compared to control group (P value < 0.001).

Serum alanine transaminase (ALT) level had a mean value of 35.24±18.245 U/L in group I, in group II it was 34.650±9.113 U/L and in control group it was 23.520±2.633 U/L. Using Tukey's test, revealed no statistically significant difference in group I as compared to group II (P >0.05), but statistically significant increase in group I (P<0.001) and group II (P<0.05) as compared to control group was observed.

Serum aspartate transaminase (AST) had a mean value of 41.215±19.241 U/L ml in group I. In group II, it was 44.761±11.361 U/L and in control group it was 30.551±4.483 U/L. Using Tukey's test showed no statistically significant difference between group I and group II (P>0.05). But, it was significantly increased in group I and group II as compared to control group (p < 0.001).

Serum albumin level ranged between 2.1 to 4.00 gm/dL in group I with a mean value of 2.843±0.420 gm/dL, in group II its level ranged between 2.30 to 4.00 gm/dL with a mean value of 3.217±0.619 and in control group, its level ranged between 4.35 gm/dL to 5.00 gm/dL with a mean value 4.606±0.188 gm/dL. Using Tukey's test, revealed a significant decrease in group I and group II as compared to control group (p<0.001) , and in group I than group II (p<0.001).

Prothrombin activity ranged between 38% to 85% in group I, in group II it ranged between 48% to 82% and in control group it ranged between 95.7% to 100%. Using Tukey's test; prothrombin activity was

significantly decreased in group I and group II as compared to control group (PO.OOl) as well as in group I compared to group II (p<0.001).

Table (19) and Figure (12) showed erythrocyte sedimentation rate of all studied groups. After 1st hour (ESR1), it ranged between 15 to 130 mm/hour in group I with a mean of 76.571±36.060 mm/hour. In group II it ranged between 10 to 55 mm/hour with a mean of 20.444±11.638 mm/hour and in control group it ranged between 4.5 to 10 mm/hour with a mean of 7.072±1.668 mm/hour. Using Tukey's test it was significantly increased in group I as compared to group II and to control group (p<0.001), with no significant difference between group II and control group (P>0.05).

Table (20) and Figure (13) showed erythrocyte sedimentation rate of all studied groups after 2nd hour (ESR2). It ranged between 27 to 130 mm/hour with a mean of 99.143±33.346mm/hour in group L In group II, it ranged between 5 to 85 mm/ hour with a mean of 39.944 ±20.678mm/hour and in control group; it ranged between 10 to 30 mm/hour with a mean of 17.490±6.762 mm/hour. Using Tukey's test showed significant increase in group I as compared to group II and to control group (p<0.001), and also in group II as compared to control group (p <0.05).

Table (21) and Figure (14) showed blood urea of all studied groups, it ranged between 20 to 56 mg/dl in group I, in group II it ranged between 20 to 44 mg/ dl and in control group it ranged between 15 to 37 mg/dl. There was no significant difference between all studied groups (p>0.05).

Table (22) and Figure (15) showed serum creatinine of all studied groups. It ranged between 0.6 to 2.5 mg/ dl in group I, in group II it ranged between 0.5 to 1.8 mg/ dl and in control group it ranged between 0.5 to 1 mg/ dl. There was no significant difference between all studied groups (p>0.05).

Table (23) and Figure (16) showed levels of alpha fetoprotein (AFP) in all studied groups. It ranged between 9.70 to 95x 103 ng/ml in group I. In group II it ranged between 10.90 to 165.5 ng/ml and in control group it ranged between 0.80 to 9.00 ng/ml. Using Mann-Whitney test there was significant increase in group I as

compared to group II (P0.001) and to control group (p<0.001). Also, there was significant increase in group II as compared to control group (p<0.001).

Table (24) and Figure (17) showed the number and percentage of HCV antibodies positive and negative patients in all studied groups. All patients of group I were HCV antibodies positive, 16/18 patients (88.89%) in group II and also 1/18 subject (5.56%) of control group was positive. No significance difference between group I and group II (PI>0.05) but a significant increase was found between group I & group II and control group (P2<0.001).

Table (25) and Figures (18) showed the number and percentage of PCR positive and negative patients for HCV RNA in those with positive HCV antibodies in all studied groups. It was positive in 18/21 patients (85.71%) in group I, 14/16 patients (87.50%) in group II. In control group, PCR was done for the only positive subject who was HCV antibodies positive and was also positive for PCR. There was no significant difference in group I as compared to group II (Pl>0.05) but there was significant increase in group I &II as compared to control group (P2<0.001).

Table (26) and Figure (19) showed seroprevalence % of HBsAg among patients of all studied groups. It was positive in 4/21 patients (19.05%) in group I, 2/18 patients (11.11%) in group II while all the control group was HBsAg negative (0%). There was no significant difference between group I and group II (Pl>0.05). There was significant increase in group I& group II as compared to control group (P2<0.05).

Table (27) and Figures (20) showed the number and percentage of patients with positive PCR for HBV DNA in HBsAg negative patients (occult HBV) of all studied groups. Occult HBV infection was detected in 7/17 patients (41.18%) in group I and 3/16 (18.75%) in group DL No significant difference between group I and group II (PI>0.05) was found yet there was significant increase of occult HBV in group I & group II as compared to control group (P2<0.05).

Table (28) and Figures (21) showed the percentage of occult HBV in all studied groups. It was detected in 7/21 patients (33.33%) in group I and 3/18 patients (16.66%) in group II. No occult HBV was detected in control group. There was no significant difference in group I as compared to group II (Pl>0.05) but there was significant increase in group I& group II as compared to control group. (P2<0.05).

Table (29) and Figure (22) showed HBV and HCV co-infection prevalence in all studied groups. Co-infection was detected in 11/21 patients (52.38%) in group I, 5/18 patients (27.77%) in group II and no co-infection at all in control group, with no significant difference between group I and group II (PI>0.05). There was significant increase in group I& group II as compared to control group (P2<0.05).

Table (30) and Figure (23) showed percentage of co-infection % of HBV, either manifest or occult, with HCV in all studied groups:

In group I, co-infection of HCV was noticed in 4/21 patients (19.04%) with manifest HBV and 7/21 patients (33.33%) with occult HBV.

In group II, co-infection of HCV was noticed in 2/18 patients (11.11%) with manifest HBV and 3/18 patients (16.66%) with occult HBV. No co-infection was found in control group. There was no significant difference between group I and group II (Pl>0.05) but there was significant increase in group I& group II as compared to control group (P2<0.05).

Table (10): Age in years of all studied groups.

Groups	Age

	I *Range*	*Mean ± SD*	*ANOVA*	
			F	*P-value*
Group I	***40.00 - 71.00***	***52.667 ± 8.278***	***21.596***	***<0.001****
Group II	***30.00 - 55.00***	***42.000 ± 8.239***		
Control group	***29.00 - 45.00***	***37.615 ± 5.052***		
Tukey's test				
Group **I** &	Control group	***<0.001****	Control group & Group **II** ***>0.05***	
	Group **II**	***<0.001****		

P value is significant when it is below (0.05).
**** Significant.***

Figure (1): Age in years of all studied groups

Table (11): Sex distribution in all studied groups.

Groups		**Sex**		
	Group I	**Group II**	**Control group**	
Male	N	15	12	11
	%	71.43	66.67	61.11
Female	N	6	6	7
	%	28.57	33.33	38.89
Chi-square	**X^2**	0.464		
	P.value	>0.05		

P value is significant when it is below (0.05).

Figure (2): Sex distribution in all studied groups

Table (12): Some risk factors of viral hepatitis.

	Group I	**Group II**	**Control group**	**Chi-square**

				X^2	P-value
History of blood transfusion.	N	15	10	0	12.066	<0.001*
	%	71.43	55.56	0.00		
Past History of operations	N	11	10	0	15.388	<0.001*
	%	52.38	55.56	0.00		
History of intake of I.V. antibilharzial treatment.	N	8	8	0	17.489	<0.001*
	%	38.10	44.44	0.00		
Use of shared articles like razors.	N	21	18	18		
	%	100.00	100.00	100.00		

P value is significant when it is below (0.05).
*Significant
P value: between group I & group II and control group

Figure (3): Some risk factors of viral hepatitis

Table (13): Some non viral Risk factors of malignancy

Risk factors of malignancy	*Group I*	*Group II*	*Control*	*Chi-square*	
			group	*X*	*P-value*
Smokers	***N***	***12[a]***	***4[a]***	***0***		
	%	***57.14***	***22.22***	***0.00***	***16.120***	***<0.001****
Ex smoker	***N***	***1***	***1***	***0***		

	%	***4.76***	***5.56***	***0.00***		
Diabetes mellitus	***N***	***15[a]***	***6[a]***	***0***	***15.189***	***<0.001****
	%	***71.43***	***33.33***	***0.00***		
Family history of	***N***	***?[a]***	1[a]	***0***	***15.603***	***<0.001****
malignancy	%	***33.33***	5.56	***0.00***		
Use of oral contraceptive pills in females	***N***	***5[a]***	2[a]	***1***	***6.596***	***>0.05***
	%	"***83.33***	***33.33***	***14.29***		

P value is significant when it is below (0.05).
*Significant
Significant between group I and group II
P value: between group I & group II and control group

Figure (4): Some non viral risk factors of malignancy

Table (14): Clinical data detected in all studied groups.

Signs		**Group I**	**Group II**	**Control group**	**Chi-square**	
					X^2	*P-value*
Jaundice	N	15[a]	***9[a]***	***0***	***20.959***	***<0.001****
	%	71.43	***50.00***	***0.00***		
L.L edema	N	16	***11***	***0***	***24.561***	***<0.001****
	%	76.19	***61.11***	***0.00***		
Ascites	N	16	***15***	***0***	***26.615***	***<0.001****
	%	76.19	***83.33***	***0.00***		
Splen	**EnLarged**	N	16	***15***	***0***	***107.706***	***<0.001****
		%	76.19	***83.33***	***0.00***		
	Splenectomy	N	3	***3***	***0***		
		%	14.29	***16.67***	***0.00***		
	Normal size	N	2	***0***	***18***		

		%	9.52	***0.00***	***100.00***		
Liver	**Enlarged**	N	T	***0[a]***	***0***	***58.539***	***<0.001****
		%	33.33	***0.00***	***0.00***		
	Shrunken	N	14	***18***	***0***		
		%	66.67	***100.00***	***0.00***		
	Normal size	N	0	***0***	***18***		
		%	0.00	***0.00***	***100.00***		
Upper quadrant abdominal pain	N	14[a]	***2[a]***	***0***	***23.733***	***<0.001****	
	%	66.67	***11.11***	***0.00***			
Flapping tremors	N	14	9	0	***21.362***	***<0.001****	
	%	66.67	50.00	0.00			
Clubbing	N	5	4	0	***4.951***	***<0.001****	
	%	23.81	22.22	0.00			
Skin echymosis	N	10	11	0	***16.888***	***<0.001****	
	%	47.61	61.11	0.00			
Bleeding /gum	N	8	8	0	***10.460***	***<0.001****	
	%	38.10	44.44	0.00			
Bleeding varices	N	7	9	0	***11.600***	***<0.001****	
	%	***33.33***	***50.00***	***0.00***			

P value is significant when it is below (0.05). * Significant.

[a] Significant between group I and group II.

P value: between group I & group II and control group.

Figure (5): Clinical data detected in all studied groups.

Table (15): Haemoglobin gm/dl in all studied groups.

Groups		**Hb gm/dl**		
	Range	*Mean ± SD*	*ANOVA*	
			F	*P-value*
Group I	***7.80 - 13.10***	***10.025 ± 1.410***	***18.442***	***<0.001****
Group II	***8.60 - 13.50***	***11.203 ± 1.514***		

Control group	***12.14 - 15.74***	***12.501 ± 0.699***		
Tukey's test				
Group **I** &	Control group		***<0.05****	Control group & Group **II** ***<0.05****
	Group **II**		***<0.001****	

P value is significant when it is below (0.05).
**** Significant.***
Normal range of haemoglobin: male; 16-18 gm/dl & female; 12-16gm/dl

Figure (6): Haemoglobin gm/dl in all studied groups.

Table (16): Total white blood cell counts (WBCs) (xl0^3 /mm^3).

Groups		WBCs xlO^3/ mm^3	
	Range	***Mean + SD***	***ANOVA***
		F	***P-value***
Group I	***5.82 - 9.76***	***7.670 ± 1.038***	***24.865***	***<0.001****
Group II	***3.08 - 8.62***	***5.228 ± 1.070***		
Control group	***4.34 - 7.40***	***5.909 ± 1.263***		
Tukey's test				
Group I &	Control group		***<0.05****	Control group & Group II ***<0.001****
	Group II		***<0.001****	

P value is significant when it is below (0.05).
**** Significant.***
Normal range of total white blood cell counts :(4xl0^3-l lxl 0^3 /mm^3).

Figure (7): Total white blood cell counts (WBCs) (xl0^3 /mm^3).

Table (17): Platelet count of all studied groups (x 10^3/ mm^3,' in all studied groups.

Groups		**Platelet x 10^3 / mm^3**	
	Range	*Mean ± SD*	*ANOVA*

			F	*P-value*
Group I	***90.00 - 270.00***	***140.143 ± 54.152***	***26.764***	***<0.001****
Group II	***80.00 - 200.00***	***139.000 ± 36.979***		
Control group	***150.00 - 400.00***	***279.994 ± 97.905***		
Tukey's test				
Group I &	Control group	***<0.001****	Control group & Group II ***<0.001****	
	Group II	***>0.05***		

P value is significant when it is below (0.05).
**** Significant.***
(Normal range of Platelet count: 150 xl0^3-400 xl0^3 mm^3)

Figure (8): Platelet count of all studied groups (x 10^3/ mm^3,' in all studied groups.

Table (18): Liver functions of all studied groups.

Liver functions						
		Group I	**Group II**	**Control group**	**F**	**P-value**
Total serum bilurbin mg/dl	Range	0..90-5..5	0..60-4.00	0.65-1.00		
	Mean±SD	3.810±1.681	2..538±0..993	0.790±0.128	60.312	<0.001*
	Tukey's	G I & G II	C&GI	C&GII		
	test	>0.05	<0.001*	<0.001*		
ALT (U/L)	Range	18-60	19.92-55.00	218.88-30.06		
	Mean±SD	3 5.24± 18.245	34.650±9.113	23.520±2.633	159.123	<0.001*
	Tukeys	G I & GII	C&GI	C&GII		
	test	>0.05	<0.01*	<0.05*		
AST(U/L)	Range	20.24-63.24	21.08-67.35	20.10-38.37		
	MeaniSD	41.215±19.241	44.761±11.361	30.551±4.483	168.291	<0.001*
	Tukey's	G I & G II	C&GI	C&GII		
	test	>0.05	<0.001*	<0.001*		
Serum albumin gm/dl	Range	2.10-4.00	2.30-4.00	4.35-5.00		

	MeaniSD	2.843±0.420	3.217±0.619	4.606±0.188	82.529	<0.001*
	Tukey's	G I & GII	C&GI	C&GII		
	test	<0.001*	<0.001*	<0.05*		
Prothrombin activity %	Range	38.00-85.00	48.00-82.00	95.76-100.00		
	Mean±SD	55.857±14.766	71.167± 14.243	98.080±1.378	60.237	<0.001*
	Tukey's	G I & GII	C&GI	C&GII		
	test	<0.001*	<0.001*	<0.001*		

P value is significant when it is below (0.05). G I: group I.
* Significant G II: group II.
P value: between group I, group II and control group C: control group
Normal range:
Serum bilurbin 0.2-1 mg/dl
ALT up to 35 u/1
AST up to 41 u/1
Serum albumin 3.5-5.0 gm/dl
Prothrombin activity: 75-100%

Figure (9): Liver enzymes (ALT&AST) of all studied groups (u/1).

Figure (10): Total bilirbuin (mg/dl) and serum albumin (gm/dl) of all studied groups.

Figure (11): Prothrombin activity % of all studied groups.

Table (19): Erythrocyte sedimentation rate (ESR) after first hour of all studied groups (mm/h).

Groups			**ESR 1**		
		Range		**Mean +*SD***	*ANOVA*
				F	**P-value**
Group I	15.00 - 130.00	76.571 ±	36.060		
Group II	10.00 - 55.00	20.444 ±	11.638	S'1.358	<0.001*

Control group	4.50 - 10.00	7.072 ±	1.668		
			Tukey's test		
Group I &	Control group		<0.001*	Control group & Group II	
	Group II	<0.001*		>0.05	

P value is significant when it is below (0.05).
* Significant.
Normal range of erythrocyte sedimentation rate (ESR) after first hour by Westergren method: male 3-5mm /h and female7-12 mm/h.

Figure (12): Erythrocyte sedimentation rate (ESR) after first hour of all studied groups (mm/h).

Table (20): Erythrocyte sedimentation rate (ESR) after second hour of all studied groups (mm/h).

		ESR 2		
	Range	**Mean + SD**	**ANOVA**	
			F	**P-value**
Group I	27.00 - 130.00	99.143 ± 33.346	62.693	<0.001*
Group II	5.00 - 85.00	39.944 ± 20.678		
Control group	10.00 - 30.00	17.490 ± 6.762		
Tukey's test				
Group I &	Control group	<0.001*	Control group & Group II <0.05*	
	Group II	<0.001*		

P value is significant when it is below (0.05).
* Significant.
Normal range of erythrocyte sedimentation rate (ESR) after second hour by Westergren method: male 7-15mm /h and female 12-17 mm/h.

Figure (13): Erythrocyte sedimentation rate (ESR) after second hour of all studied groups (mm/h).

Table (21): Blood urea (mg/dl) of all studied groups.

Groups		**Blood urea**		
	Range	**Mean + SD**	**ANOVA**	
			F	**P-value**
Group I	20.00 - 56.00	37.381 ± 10.754		

Group II	20.00 - 44.00	33.944 ± 6.073	1.109	>0.05
Control group	15.00 - 37.00	35.037 ± 1.079		

P value is significant when it is below (0.05).
P. value: between group I & group II and control group. Normal value of blood urea 10-50 mg / dl.

Figure (14): Blood urea (mg/dl) of all studied groups.

Table (22): Serum creatinine (mg/dl) of all studied groups.

Groups		**Serum creatinine (mg/dl)**		
	Range	**Mean ± SD**	**ANOVA**	
			F	**P-value**
Group I	0.60 - 2.50	1.224 ± 0.453	2.711	>0.05
Group II	0.50 - 1.80	1.133 ± 0.340		
Control group	0.50 - 1.00	0.961 ± 0.210		

P value is significant when it is below (0.05).
P value: between group I & group II and control group.
Normal value of serum creatinine: 0.2-1.2 mg / dl

Figure (15): Serum creatinine (mg/dl) of all studied groups

Table (23): Alpha feto protein (ng/ml) of all studied groups.

Groups			**Alpha feto protein (ng/ml)**		
	Range	*Median*	*Mean rank*	*Kruskal Wallis Test*	
				X^2	*P-value*
Group I	***9.70 - 95.000***	***5275.000***	***43.778***	***37.73***	***<0.001****
Group II	***10.90 - 165.50***	***130.000***	***32.286***		
Control group	***0.80 - 9.00***	***8.45***	***8.352***		
Mann-Whitney test					

Group I &	Control group	***<0.001****	Control group & Group II ***<0.001****
	Group II	***<0.001****	

P value is significant when it is below (0.05).
**** Significant.***
Normal range up to 10 ng/ml.

Figure (16): Alpha feto protein (ng/ml) of all studied groups.

Table (24): Percentage of HCV antibodies seroprevalence in all studied groups.

		HCV antibodies		
	Group I	**Group II**	**Control group**	
Positive	N	21	16	1
	%	100.00	88.89	5.56
Negative	N	0	2	17
	%	0.00	11.11	94.44
Chi-square	X^2_1	2.459	X^2	46.756
	P-value 1	>0.05	P-value 2	<0.001*

P value is significant when it is below (0.05).
* Significant.
P-value 1 comparison between group I and group II.
P-value 2 comparison between group I, group II and control group

Figure (17): Percentage of HCV antibodies seroprevalence in all studied groups.

Table (25): PCR for HCV RNA in anti-HCV positive patients

		PCR for HCV RNA		
	Group I	**Group II**	**Control group**	
Positive	N	18/21	14/16	1/1
	%	85.71	87.50	100.00
Negative	N	3	2	0
	%	14.29	12.50	0.00
Chi-square	X^2_1	0.025	X^z_2	33.059
	P-value 1	>0.05	P-value2	<0.001*

P value is significant when it is below (0.05).
* Significant.
P-value 1 comparison between group I & group II.
P-value 2 comparison between group I, group II and control group.

Figure (18): PCR for HCV RNA in anti-HCV positive patients

Table (26): Percentage of hepatitis B surface antigen (HBsAg) seroprevalence in all studied groups.

		HBsAg antigen		
		Group I	**Group II**	**Control group**
Positive	N	4	2	0
	%	19.05	11.11	0.00
Negative	N	17	16	18
	%	80.95	88.89	100.00
Chi-square	X^2_1	0.469	X*2	3.743
	P-value 1	>0.05	P-value 2	* <0.05

P value is significant when it is below (0.05).
**** Significant.***
***P-value 1 comparison between group* I *& group* II.**
P-value 2 comparison between group* I*, group* II *and control group

Figure (19): Percentage of hepatitis B surface antigen (HBsAg) seroprevalence in all studied groups

Table (27): PCR for HBV DNA in HBsAg negative patients (occult HBV) in all studied groups.

		PCR for HBV DNA		
		Group I	**Group II**	**Control group**
Positive	N	7/17	3/16	0/18
	%	41.18	18.75	0.00
Negative	N	10/17	13/16	18/18
	%	58.82	81.25	100.00
Chi-square	X^2_1	1.045	X^2_2	9.415
	P-value 1	>0.05	P-value 2	<0.05*

P value is significant when it is below (0.05).
**** Significant.***
P-value 1 comparison between group I and group II.
P-value 2 comparison between group I, group II and control group

Figure (20): PCR for HBV DNA in HBsAg negative patients (occult HBV) in all studied groups

Table (28): Percentage of occult HBV in all studied groups..

Occult HBV		***N***		%
Group I		***7/21***		***33.33***
Group II		***3/18***		***16.66***
Control group		***0/18***		0.00
Chi-square	X^2_1	***1.412***	X^2_2	***1.045***
	P-value 1	***>0.05***	***P-value2***	***<0.05****

P value is significant when it is below (0.05).
* Significant.
P-value 1 comparison between group I & group II.
P-value 2 comparison between group I & group II and control group.

Figure (21): Percentage of occult HBV in all studied groups.

Table (29): Percentage of HBV and HCV co-infection in all studied groups.

Co-infection		N		%
Group I		11/21		52.38
Group II		5/18		27.77
Control group		0/18		0.00
Chi-square	X^2_1	***2.25***	X^2_2	***17.337***
	P-value 1	***>0.05***	***P-value 2***	***<0.05****

P value is significant when it is below (0.05).
**** Significant.***
P-value 1 comparison between group I & group II.
P-value 2 comparison between group I ***& group*** II ***and control group***

Figure (22): Percentage of HBV and HCV co-infection in all studied groups.

Table (30): Prevalence of co-infection of HBV (manifest and occult) with HCV in all studied groups.

Co-infection		***N***		%
Group I	**Manifest HBV**	***4/21***		***19.04***
	Occult HBV	***7/21***		***33.33***
Group II	**Manifest HBV**	***2/18***		***11.11***
	Occult HBV	***3/18***		***16.66***
Control group		***0/18***		0.00
Chi-square	X^2_1	***2.25***	X^2_2	***17.337***
	P-value 1	***>0.05***	***P-value 2***	***<0.05****

P value is significant when it is below (0.05).
* Significant.
P-value 1 comparison between group I & group II.

P-value 2 comparison between group I & group II and control group.

Figure (23): Prevalence of co-infection of HBV (manifest and occult) with HCV in all studied groups

Figure ***(24): A graose gel electrophoresis for PCR product stained with ethidium bromide, detecting HBV DNA:***
Upper gel:
M: Marker
Lane 1: positive control
Lane 2: negative control
Lane 3,4,5,6,7,8,9 positive samples
Lower gel:

M: marker.

Lane 1: positive control
Lane 2,3,5,7: positive samples
Lane 4,6,8 negative

Figure (25): Agrose gel electrophoresis for PCR products stained with ethidium bromide detecting HCV-RNA:
M: Marker
Lane 1: positive control
Lane 2: negative control
Lane 3,4,5,6,7,8,9 positive samples

Figure (26): Ultrasound finding of HCC patients

DISCUSSION

***Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are primarily hepatotropic. Chronic infection with these viruses causes progressive liver disease and hepatocellular carcinoma* (Janaki et al., 2006). *HBV/HCV co-infection further increases the risk of HCC* (Shi et al., 2005). *HCC is the fifth most common cancer worldwide* (Mboto et al., 2005). *Transformation of normal liver to cirrhosis and to cancer involves a number of successive and additional genetic and epigenetic events* (Paradis et al., 2003). *Carcinogenesis is believed to be a multistage process, occurring through a sequence of steps termed initiation, promotion and progression. This process may evolve over several or many years. Tumor initiation begins in cells through mutations induced by exposure to carcinogens. DNA changes, maintained during successive cell divisions, activation of oncogenes and inactivation of suppressor genes lead to dysregulation of the cell division* (Michielsen et al., 2005).

***Several studies have shown that HBV and HCV interact with each other and affect immune responses. HCV infection can suppress HBV replication however another study had reported that HBV can reciprocally inhibit HCV replication as well* (Crockett and Keeffe, *2005). However co-infection with HBV and HCV leads to aggravated course of disease and faster progression to HCC* (Yates et al., 1999).

Several studies found that patients co-infected with HBV and HCV have increased risks of HCC compared to those with mono-infection (Lok, 2004).

Recently it has been thought that occult HBV infection may result in cirrhosis and HCC (Lok, 2004). ***Occult HBV infection can be a co-factor in the development of HCC among patients with alcoholic cirrhosis or cirrhosis due to other etiologies*** (Tagger et al., 1999).

The aim of the present work is to study co infection of HBV and HCV in hepatocellular carcinoma with or without occult B.

In the present work, as regard to age in years of all studied groups, there was a statistically significant increase in age of HCC patients in group I with a mean value of (52.667±8.278) in comparison to group II with liver cirrhosis and control group , this without a significant difference between group II and control group. These data were in agreement with* El-Bolkainy *(1998) who reported a median age of 53 years for HCC in Egypt.

This could be explained by the possibility of exposure to risk factors and progressive

pathology with increasing age that is in agreement with **Mboto et al., (2005)** *who reported that, hepatocellular carcinoma is generally associated with increasing age and that significantly higher HBV and HCV prevalence that have been reported among persons in their 40th and 50th of age respectively.*

Abo-Al-Azm et al., (2005) *in a larger study reported that, the prevelance of HCC became more prominent in younger age than in the past and is related to increased frequency of exposure to environmental risks as pesticides than in the past that related to progressive increase of age.*

The present study showed an increase in number of male as compared to number of females. The male to female ratio was 2.5: 1 (Discussion *among HCC patients of group I while the male to female ratio was 2: 1 in cirrhotic patients of group II and was 1.6: 1 in control group.*

The increase in number of males as compared to females among HCC patients in this work was nearly similar to the results of **McGlynn and London (2005)** *who reported a marked male predominance in HCC Egyptian patients, with a 3.8:1 male to female ratio. Male predominance may be explained by the possibility of a higher frequency of exposure among males than females to risk factors which can provoke carcinogenesis like HBsAg carriage, liver cirrhosis, greater exposure to environmental risk factors, like chemicals and smoking* **Okuda** *et* **al., (1983).**

Our data disagree to some extent with **El-Bolkainy (1998)** who reported a male to female ratio of 5:1. This difference could be explained by the multiplicity of risk factors which could suggest that the females in our area are become more exposed to risks of HCC in their work and changing its nature and culture via viral hepatitis, common use of oral contraceptive pills, hormonal replacement therapy and exposure to chemicals.

Also, increased number of males to females in cirrhotic patients of group II was explained by **Okuda et al. (1983)** who reported that chronic liver diseases are more frequent in males than females which can yield higher incidence of HCC.

In the present work, we noticed that blood transfusion, previous operations and intravenous antibilharzial treatment were prominent risk factors for HBV and HCV which could have a role in carcinogenesis **(Yates et al., 1999).**

In the present study, we detected an increase of family history of malignancy in group I than other studied groups. Presence of family history could be explained by the possibility of genetic predisposition by multiple mechanisms. Patterns of gene expression in cirrhosis and

hepatocellular carcinoma have recently been shown to be of value in predicting prognosis. Two hundred seventy three gene signatures were present in samples from patients with proven HCC. A subset of 30 genes was most significantly altered in both the high risk types of cirrhosis and the HCC patients. The TACSTD1, a gene associated with HCC development in other studies, is a leader gene in this gene signature **(Kim et al., 2004).**

Lee et al., (2004) could identify a limited number of genes that accurately predicted survival in a series of 91 HCC patients. The genes involved are not only implicated in cell proliferation and apoptosis, but also in ubiquitination and histone modification. **Delpuech et al., (2002)** identified distinct patterns of gene expression according to the viral aetiology, and **Hann et al., (2004)** could demonstrate the presence of antibodies to differentially expressed genes in hepatitis B and C, and this appeared to be linked with decreased survival.

In the current study, cigarette smoking was significantly increased in HCC patients of group I than group II cirrhotic patients while neither smokers nor exsmoker were detected among control group. These data were in agreement with the results of **Sun et al. (2001), Donato et al. (2002), Yu et al. (2002), and Abdel Aziz et al., (2006)** who mentioned that there is a direct relation between smoking and HCC development. Also, **Ogimoto et al. (2004)** suggested cigarette smoking to be an important risk factor for death from HCC regardless of whether the smoking habits were in the past or were continuous at present. **Munaka et al. (2003)** explained the carcinogenic effect of tobacco by the possibility of induction of genetic polymorphism for glutathione S-transferase through a study on a group of Japanese patients with HCC, while **Goa et al. (2003)** explained this carcinogenic effect by slowing acetylation genotype of N-acetyl transferase. Also our results could agree with the results of **Jee et al. (2004)** who mentioned that smoking is independently associated with increased risk of HCC taking in consideration the difference in culture of patients, severity of smoking and / or absence of other risk factors.

In the present work, we noticed a significant increase in incidence of diabetes mellitus among HCC patients of group I compared to group II cirrhotic patients and control group. The association of diabetes mellitus and HCC in the present work were in accordance with **Moor et al. (1998)**

and Vineis et al. (2004) who reported a strong association between DM and HCC development which may be due to increased levels of insulin like growth factors that are potential carcinogenic factors. Diabetes may modulate the immune response to carcinogens and may have a role in progress of HBV and HCV towards cirrhosis and malignancy **(Qureshi et al., (2002)**. Our results also were in agreement with **Yu and Yuan (2004)** who noticed that diabetic patients were at a high risk for HCC probably as a result of hepatic injury, steatosis, fibrosis and eventual cirrhosis.

The association of diabetes mellitus and cirrhosis may be attributed to the association of diabetes mellitus and HCV and / or HBV that could be explained by pancreatic B cell injury by direct virus replication, immune complexes, cryoglobulins and the possibility of abnormal insulin and blockage of insulin receptors as described by **Qureshi et al., (2002)** who also reported that diabetes mellitus was more frequent in patients having liver cirrhosis than in those having chronic hepatitis.

Yuan et al. (2004) concluded that DM, heavy alcohol consumption, and viral hepatitis were found to exert an independent and synergistic risk on the development of HCC, This could suggest that HCC pathogenesis is due to multiplicity of risks which could react synergistically with the hepatocyte and not a single factor.

We noticed that clinical manifestations were more manifest in patients of HCC rather than cirrhotic patients. That was in agreement with **Anthony (1987)** who reported that primary carcinoma of the liver should be suspected if a patient with cirrhosis deteriorates or if there is no improvement when ascites is adequately treated and local signs as hard nodular tender liver with progressive loss of weight and deterioration of liver functions tests are present.

The decrease of platelet count or leucocytes in group I and group II may reflect portal hypertension and the possibility of associated hypersplenism **(Okuda, 1996)** or bone marrow suppression due to HCV infection **(Abo Al-Azm et al, 2002).**

In the current study, it was noticed that there was a significant elevation of erythrocyte sedimentation rate (ESR) in group I as compared to group II and control group. Elevation of ESR after first hour above one hundred in cirrhotic patients could suggest malignant transformation **(Okuda, 1994).**

In the present study, there was a significant increase in the level of AFP in group I (with great variation in patients in group I) as compared to group II and control group. These data were in agreement with **Cotton et al. (1994)** who mentioned that the rises of AFP are important in the diagnosis and screening of HCC while **Frazer (1999)** mentioned that AFP may or may not be increased in patients with HCC and also, they noticed that AFP titres rose with flares of active hepatitis and they concluded from their study that the sensitivity of AFP was 39-64%, the specificity was 76-91% and the positive predictive value was 9-32%.

Di Bisceglie and Hoofnagle, (1989) who found that slight increases in AFP are usual in acute hepatitis, chronic hepatitis and cirrhosis, and suggested that overlaps can cause diagnostic difficulties.

As regards the prevalence of HCV in our patients: HCV antibodies were detected in 100% in group I, 88.89% in group II and in one accidentally discovered asymptomatic subject in control group . This could reflect high prevalence of HCV in our area due to many risks as past use of common syringes, dental manipulations, previous operations, blood transfusion, shistosomiasis and its parentral therapy **(Abo-Al-Azm and El -Sheikh, 1996).** PCR for HCV RNA was performed in all patients positive for HCV antibodies. We noticed that 85.71% were positive in group I, 87.50% were positive in group II and 1/18 subject was positive in control group. There was no statistical significant difference between group I and group II. These data were In agreement with **Badran et al. (2006)** who found that the prevalence of HCV-Ab and HCV RNA seropositive patients was 90% in patients with HCC and 82% in cirrhotic patients. They also reported that, HBV DNA was positive in 34% of patients with HCC and 28% in cirrhotic patients. They detected combined HCV infection and occult hepatitis B in 31.1% in patients with HCC and 29.3% in cirrhotic patients.

The HCV single positive patient in our control group could be explained by the endemicity of HCV in Nile Delta region **(Abdel Whab et al., 1994)** and the main bulk of HCV patients usually presented asymptomatic till complications occurred. There are some explanations for increased prevalence of both HBV and HCV in Egypt. Firstly, Egyptians in rural areas often chose traditional healers and drug vendors who give injections, and in small and poorly equipped health clinics a shortage of syringes and needles may lead to their reuse. Secondly, the

reuse of needles when schistosomiasis was treated parenterally may have transmitted hepatitis viruses if syringes were contaminated. Thirdly, screening for anti-HCV started as a routine procedure in blood banks in Egypt since (1994), and since (1987) for HBV. Transfusion of contaminated blood prior these dates may thus have trasmitted HBV and HCV **(El-Sayed et al., 1997).** Furthermore, other studies in Egypt had shown very high prevalence of HCV among blood donors especially paid donors. **(Bassily et al., 1995b and El Gohary et al., 1995 & Abdo Al-Azm and El-Sheikh, 1996).**

HCV RNA was detected in 85.7% of the studied HCC patients, in this work, the observation that HCV infection rather than the level of replication was associated with HCC suggests that, HCV viral load is not the detrimental of disease progression, but rather the immune response raised against HCV. **Khakoo et al. (1997)** reported that the hepatic injury found in patients with chronic hepatitis is mainly induced by cytotoxic T cell responses against infected liver cells. In this view, this continuing process of hepatic damage might eventually lead to the development of HCC **(Yates et al., 1999).**

HCV core protein was reported to have transactivating properties **(Koike, 2005).** HCV core proteins can modulate various cellular signal transduction pathways, namely by mediating the transcription activity of NFkB and STAT-3 proteins **(Waris and Siddiqui, 2003).**

Yates et al. (1999) reported that infection with HCV was present in 76% of HCC patients which is higher than the 65% found by **Waked et al (1995).** Yates et al. stated that HCC was strongly related to HCV infection irrespective of HBV. Also, they noticed a higher association of HCC with HCV and HCV-HBV double infection than with HBV infection alone. **El-Zayadi et al. (2001)** reported that HCV seroprevalence in HCC patients was 71.1% and HBsAg was 22.4%. On the other hand, **Kirk et al. (2004)** found that the aetiologic fraction for HCC related to HBV was 57.2%, related to HCV was 19.9% and to dual infection was 3.5%. **Shimada et al. (2003)** and Abo **Al-Azm et al. (2005),** mentioned that the prevalence of DNA fragments of HBV irrespective of the status of antibodies (anti HBV antibodies e.g. Anti- HBe) is a pivotal factor associated with development of HCC .

The present results showed that HBsAg was positive in 19.05% of group I and 11.11% in group II and negative in control group. The increased

prevalence in group I was insignificant .When PCR test was carried out in negative HBs Ag patients, HBV-DNA could be detected in 41.81% in group I and 18.75% in group II. This ratio could reflect the prevalence of occult HBV in both groups with insignificant increase in group I as compared to group II.

We also noticed that all HBV positive patients in group I (11/21; 52.38%) and group II (5/18;27.77%) were co-infected with HCV either presented in a manifest state with positive HBsAg (4/11;36.36% in group I & 2/5; 40% in group II) or in an occult state with negative HBsAg and positive HBV-DNA (7/ll;63.63% in group I & 3/5;60% in group II). The total incidence of occult HBV in HCC group was 33.33% (7/21) and in cirrhotic group was 16.66% (3/18).

Occult HBV could reflect underestimation of HBV seroprevalences when screening depends on HBs Ag only and add another risk factor for hepatic carcinogenesis. The incidence of occult HBV could be attributed to co-infection with HCV as all cases with occult HBV, in this work, were noticed to be co-infected with HCV, with higher incidence in HCC group compared to cirrhotic patients that could represent an apparent risk factor for hepatic carcinogenesis **(Lok, 2004).**

The mechanism of occult HBV in HBV/HCV co-infection could be explained by **Rodriguez et al., (2005)** who reported that super infection of HBV with HCV may inhibit HBV replication This inhibition may be mediated by the host immune response (via the induction of cytokines such as interferons) or by a direct effect of HCV proteins. In this regard, it has been shown that HCV core and NS2 proteins inhibit HBV replication and gene expression in vitro. HCV also can substantially suppress the expression of HBV surface proteins **(Chu et al., 1998).**

Lok (2004) concluded that occult HBV could increase the incidence of HCV induced liver cirrhosis and HCC. However, Kao et al., 2002 reported that where HBV infection is hyper endemic, with higher incidence of infection since childhood with immune modulation, HBV co-infection has no effect on the clinicopathologic status of patients with chronic hepatitis C or the therapeutic response to combination therapy (interferon & ribavirin).

The insignificant difference in occult HBV incidence between HCC and cirrhotic groups put those cirrhotic patients, in our study, at high risk for development of HCC. **Lok (2004)** stated that many patients have developed cirrhosis or HCC after decades of chronic HBV infection with spontaneously cleared HBsAg at the time of presentation. Most studies explained occult HBV infection was related to low level HBV infection with subdetectable levels of HBsAg or infection with HBV variants that cannot express S proteins or variants that express S proteins with aberrant epitopes that cannot be detected by con-ventional serology assays. While some HBV S variants such as the glycine-arginine substitution at codon 145(G145R) may be missed by monoclonal antibody based HBsAg assays, most HBV S variants can be detected by polyclonal antibody based serology assays. Thus, many patients with occult HBV infection could have overt chronic HBV infection for many years with subsequent spontaneous clearance of HBsAg **(Brechot., et al, 2001).**

Spontaneous clearance of HBsAg in patients with chronic HBV infection has been reported to occur at the rate of 0.5-1% per year. Although the outcome of these patients is improved, HCC has been reported **(Huo et al., 1998).**

Other patients might have transient acute HBV infection in the past with serological recovery. Numerous studies showed that low level HBV DNA can be detected in the^ liver of these patients more than 10 years later. Recent study from Japan found that inflammation and fibrosis can be detected up to 10 years after serological recovery from acute hepatitis B. These data could suggest that transient acute HBV infection can result in chronic liver injury **(Lok, 2004).**

Our data were in agreement with the results of **Badran et al. (2006)** who found that overall prevalence of occult HBV among HCC patients was 34% and 28% in cirrhotic patients. Our data were in agreement also with **Brechot et al. (1998)** who reported that prevalence of serum HBV DNA in HCC patients ranged from 29% to 40%. HBV including occult infection has been associated with the high incidence of HBV integration and subsequent development of HCC. HBV DNA integration may cause rearrangement of the host DNA sequence **(Lai et al., 1990).** Also, the high occurrence of occult HBV infection in those with chronic HCV infection can be explained by the fact that both HBV and HCV are transmitted parenterally and share common routes of infection.

These results could indicate different patterns of risk factors, morphogenesis and incidence of HCC development in HBV and HCV associated cirrhosis, suggesting different mechanisms of carcinogenesis **(Benvegnu and Alberti, 2001).**

The results of the present work disagree with the results of **Saker et al., (2006)** who noticed that occult HBV is not frequent in Egyptian patients. However, **Zeinab et al. (2005),** reported occult hepatitis B virus, in patients with chronic HCV in various frequencies (50% to 87%).

In Egypt, unlike most of the world in which HBV and heavy alcohol consumption are major causes for HCC, chronic HCV infection is exceedingly common **(Abdel-Aziz et al., 2000 and Nafeh et al., 2000).**

HCC pathogenesis depends on multifactorial risks **(Abo Al-Azm et al., 2005)**. The insignificant increase in HCV and HBV incidence in group I than group II may also indicate that HCV and/or HBV are not the only factors in hepatic carcinogenesis but need other environmental risk factors as fungal toxins, chemicals and also other factors related to these viruses as duration of infection, viral load and severity of hepatic pathology could be involved.

However, co-infection with HBV and HCV with higher incidence of occult HBV could be considered as a potential HCC risk, higher than the risk attributed to infection with either type of virus alone **(Lok, 2004).** The growing higher incidence of HCV in Egypt is thought to have agreat role in the increasing incidence of occult HBV with false HBV underestimation depending on HbsAg evaluation. The two viruses may interact by causing more induction of inflammation and accelerated progression to cirrhosis and HCC **(Lok, 2004).** Alternatively, direct oncogenic effects of these two viruses may be additive or synergistic **(Lok, 2004).** A multivariate analysis done by **(Benvegnu and Alberti, 2001)** showed that the risk of HCC is significantly higher in HBV and HCV co-infected patients compared with those with single infection **(Drucker et al., 2001).**

Summary

Hepatocellular carcinoma (HCC) is ranked the eighth in frequency among cancers world wide. It is the fifth most common malignancy in men and ninth most common in women. It is a rapidly fatal tumor, making its incidence rate equal to its mortality rate.

Several studies have shown that HBV and HCV interact with each other and affect immune response. HCV infection can suppress HBV replication however; other studies had reported that HBV can reciprocally inhibit HCV replication as well. HBV and HCV co-infection lead to aggravated course of the disease and faster progression to HCC. Occult HBV infection can be a co factor in the development of HCC.

The aim of this work was to study co-infection of HBV and HCV in HCC with or without occult HBV.

The study was conducted during the period between February 2006 and October 2006. It included three groups of patients and subjects:

> ***Group I:*** composed of 21 inpatients with HCC admitted to (Tropical Medicine Department Tanta University). They were 15 males and 6 females and their ages ranged between 40-71 years.
>
> ***Group II:*** composed of 18 inpatients with liver cirrhosis admitted to (Tropical Medicine Department Tanta University), 12 were males and 6 were females. Their ages ranged between 30- 55 years.

Control group: composed of 18 apparently healthy subjects (relatives of the patients).They were 11 males and 7 females, with age range between 29-45 years.

All patients and control group were subjected to the following:

Thorough history taking, full clinical examination, laboratory investigations which included: complete blood picture, complete urine analysis, complete stool analysis , erythrocyte sedimentation

rate (ESR), blood urea and serum creatinine, blood sugar (fasting and post prandial), liver function tests:- serum albumin, serum bilirubin, serum transaminases (AST and ALT), prothrombin time and activity, abdominal US , triphasic CT and liver biopsy for some patients.

- Estimation of Hepatitis C virus antibody by 3 rd generation ELISA

- Estimation of Hepatitis B surface antigen (HBsAg) by 3rd generation ELISA

- Polymerase chain reaction for HCV RNA in anti HCV positive cases was performed.

- Polymerase chain reaction for HBV DNA was performed in HBs Ag negative cases.

- Estimation of Serum alpha-fetoprotein using the one-step immunoenzymatic mediated assay

The results of the present study revealed that:

Males were noticed to be more risky than females in all groups and this was explained as to be due to more exposure to risk factors.

In this study we detected that right hypochondrial pain, weight loss, anorexia, encephalopathy, hematemesis, ascites, jaundice, splenomegaly and lower limb edema were the most frequent symptoms of the associated liver disease in both disease groups. The local factors as hard nodular liver with tenderness and general cachexia and deterioration of liver function tests could suggest malignant liver.

It was also noticed that alpha-fetoprotein level in spite of its great variation in HCC patients was a good suggestive in high rise in HCC patients as compared to cirrhotic patients.

In the present study, a considerable number of HCC patients were diabetics which suggest a strong relation between HCC and diabetes mellitus. The mechanism of carcinogenesis is not clear but may be due to steatosis, presence of insulin like growth factors which leads to cirrhosis that may be a co-factor in hepatic carcinogenesis.

In the present work, most of HCC patients in group I were from rural areas. Most of them had received intravenous anti bilharzial drugs and most of them were hepatitis C virus positive (hyper endemic area).

The seroprevalence of both HBV&HCV and co-infection were insignificantly higher in group I as compared to group II putting cirrhotic patients of group II at high risk for HCC and may also denoting the role of other factors.

Occult HBV is increased among HCC patients than cirrhotic patients, which could suggest having a role in the pathogenesis added to exposure to carcinogens like chemicals, smoking, DM, oral contraceptive pills and hormonal replacement therapy in females.

Occult HBV is a potential risk for hepatic carcinogenesis and denotes HBV underestimation.

Conclusions:

From the present study we can concluded that:

- Hepatocellular carcinoma (HCC) is more frequent in old age due to more exposure to risk factors.
- The prevalence of HCC is more common in males as compared to females, however, it is more evident in females of the present study who received oral contraceptive pills in large percent and hormonal replacement therapy in presence of viral hepatitis, so contraceptive pills must be replaced by other methods.
- There is underestimation of hepatitis B in most surveys when they depend on HBsAg screening only. Usage of polymerase chain reaction (PCR) for HBV- DNA is essential for better evaluation.

- Occult HBV emerge as an important risk factor of HCC, in Egypt suspected to be mainly induced by HCV co-infection.
- HCV induced occult HBV could be considered as an additional factor in the pathogenesis of HCV induced HCC.
- HCC is the sequele of multi risks inspite of the importance of HCV and HBV co-infection and occult HBV(potential risks) in the process of pathogenesis.
- Diabetes in liver patients must be controlled because they are more prone to HCC.
- E.S.R, alpha fetoprotein, U.S and CT are non invasive investigations and are of great help in diagnosis of HCC after clinical suspicion.

Recommendations:

We can recommend the following:

- A wider study for the relation between HCC and HBV&HCV for the role of occult HBV in development of HCC.
- PCR is essential in diagnosis of HBV estimating HBV-DNA especially in HCV patients detecting cases with occult HBV with high risk for HCC.
- A wider study for the relation between HCC and chemicals and the role of aflatoxin.
- There are major needs for further researches to evaluate the relation between HCC, insulin-resistance syndromes and diabetes mellitus.
- Surveillance for HCC should be considered in cirrhotic patients especially those with deterioration of liver function.

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الملخص العربى

يحتل سرطان الكبد المركز الثامن من حيث الانتشار على مستوى العالم بين السرطانات المختلفة ويحتل ايضا المركز الخامس بين الأورام التى تصيب الذكور والمركز التاسع بين الأورام التى تصيب الإناث كما يسبب الوفاة سريعاً مما جعل معدل حدوثه مساوياً تقريباً لمعدل الوفيات الناتجة عنه. وقد أوضحت العديد من الدراسات تفاعل الفيروس الكبدى سى مع الفيروس الكبدى بى مما يؤثر على المناعة كذلك اظهرت الدراسات ان الفيروس الكبدى سى يحبط تكاثر الفيروس الكبدى بى ولكن فى بعض الدراسات الأخرى اظهرت العكس.

هذا وقد وجد ان العدوى المشتركة للفيروس الكبدى بى وسى يمكن ان تؤدى الى تدهور اكثر فى المرض وتحول اسرع إلى حدوث سرطان الكبد.

لذلك فقد استهدفت هذه الدراسة تقييم العدوى المشتركة لفيروس الالتهاب الكبدى بى وسى فى مرضى سرطانات الكبد مصحوبة أو غير مصحوبة بالعدوى المتخفية للفيروس الكبدى بى.

وقد اجرى البحث (أثناء الفترة ما بين فبراير 2006 وأكتوبر 2006) على ثلاث مجموعات من الاشخاص:

المجموعة الأولى: اشتملت على 21 مريضا مصابين بسرطان الكبد تم احتجازهم بقسم طب المناطق الحارة والحميات – جامعة طنطا وقد اشتملت المجموعة على 15 رجلا و6 اناث تراوحت اعمارهم بين 40 – 71 سنة.

المجموعة الثانية: اشتملت على 18 مريضا مصابين بالتشمع الكبدى تم احتجازهم بقسم طب المناطق الحارة والحميات – جامعة طنطا وكان من بينهم 12 رجلا و6 اناث تراوحت اعمارهم

بين 30- 55 سنة.

المجموعة الثالثة: اشتملت هذه المجموعة على 18 فردا صحيحا من أقارب مرضى المجموعتين الأولى والثانية منهم 11 رجلا و7 اناث تراوحت اعمارهم بين 29 – 40 سنة.

وقد خضعت مجموعات المرضى الى:

- اخذ التاريخ المرضى
- الفحص الاكلينيكى الظاهرى
- التحاليل المعملية (صورة دم كاملة – تحليل بول كامل – تحليل براز كامل – سرعة ترسيب والألفا فيتو بروتين)
- دلائل فيروسات لفيروس الكبدى بى – وسى ثم عمل تفاعل البلمرة المتسلسلة لبعض الحالات.
- أشعة تليفزيونية على البطن والحوض.
- عينة كبدية فى بعض الحالات.

وقد تنوعت شكوى المرضى بين آلام فى الجانب الأيمن العلوى من البطن، نقص الوزن، غيبوبة كبدية، قيىء دموى وفقدان للشهية أما الأعراض التى اكتشفت عند فحص المرضى فقد تراوحت بين استسقاء البطن، الصفراء، تضخم بالكبد ، تورم بالساقين وتضخم بالطحال.

وقد لوحظ زيادة نسبة الفا بروتين فى مرضى سرطان الكبد عنه فى مرضى التشمع الكبدى مما يعكس أهميتة فى تشخيص ومتابعة السرطان فى حالة الزيادة.

أوضحت الدراسة أن سرطان الكبد أكثر انتشاراً فى الرجال عن الإناث وأن عدداً ملحوظاً من الاناث المصابات بسرطان الكبد كن قد تعاطين أما حبوب منع ا لحمل أو الهرمونات الأنثوية التعويضية لفترات طويلة وأنها من الاسباب المحتملة لحدوث سرطان الكبد فيهن.

وقد لاحظنا أن عددا غير قليل من المرضى فى المجموعتين كانوا من المدخنين أو قد تعرضوا لمواد كيمياوية فى صورة مبيدات حشرية ومبيدات زراعية وقد لوحظ أيضا أن عدد غير قليل من مرضى أورام الكبد كانوا يعانون من مرض البول السكرى وهذا يرجح وجود علاقة قوية بين مرض البول السكر وسرطان الكبد وأنه يجب السيطرة عليه فى مرضى الكبد.

وقد وجد كذلك أن معظم المصابين بالورم الكبدى كانوا من سكان القرى وأن معظهم هؤلاء المرضى قد أخذوا علاج البلهارسيا الوريدى وأن معظمهم ايجابى لاختبار تفاعل البلمرة المتسلل (PCR) للفيروس الكبدى سى.

كذلك أظهرت الدراسة ان الفيروسات الكبدية بى وسى كانت أكثر انتشاراً فى مرضى سرطانات الكبد مقارنة بمرض التشمع الكبدى.

كما أتضح من خلال الدراسة أن العدوى المشتركة لفيروس الالتهاب الكبدى بى وسى أكثر انتشاراً فى مرضى سرطان الكبد بالمقارنة عن مرضى التشمع الكبدى والذى يساعد على حدوث سرطانات الكبد مع وجود فارق غير ذو دلالة إحصائية بين المجموعتين مما يجعل مرضى التشمع الكبدى أكثر عرضه لإحتمال حدوث سرطان الكبد. وقد كان جميع المرضى المصابين بالفيروس الكبدى بى يعانون من عدوى مشتركة بالفيروس الكبدى سى.

ومن اللافت للنظر ان هذه الدراسة اثبت زيادة عدد المرضى المصابين بالعدوىالمتخفية للفيروس الكبدى بى خاصة فى مرضى سرطان الكبد وذلك بعد إجراء تفاعل البلمرة المتسلل (PCR) للحالات السلبية للانتيجين السطحى للفروس الكبدى بى (HBsAg) وبالتالى فإن انتشار الفيروس الكبدى بى يقدر بأقل من الواقع وأنه يعد خطر محتمل للتحول إلى سرطان الكبد.

أظهرت نتائج البحث أن زيادة إنتشار الفيروس سى قد يكون من أهم أسباب حدوث العدوى المتخفية للفروس الكبدى بى مما يعد خطراً لسرعة التحول إلى سرطان الكبد.

الاستنتاجات

- زيادة معدل حدوث سرطانات الكبد فى السن الكبير نتيجة للتعرض للعديد من المخاطر
- يعد انتشار سرطانات الكبد فى الذكور أكثر منه مقارنة لمعدل حدوثه فى الإناث. وقد أصبح أكثر حدوثاُ فى الاناث اللاتى يتناولن حبوب منع الحمل بنسبة كبيرة والهرمونات التعويضية ويزداد حدوثه فى وجود إصابة بالالتهاب الكبدى الفيروسى وبالتالى فإن هذه الوسائل (حبوب منع الحمل) يجب استبدالها بوسائل اخرى.
- أن الفيروس تالكبدى بى لم يقدر حق قدره فى معظم الدراسات السابقة والتى كانت تعتمد على المعامل السطحى للفيروس بى (HbsAg) فى عمل مسح للفيروس وبالتالى فإنه يحتاج الى وسائل اكثر دقة مثل تفاعل البلمرة المتسلسل (PCR).
- تعد العدوى المتخفية للفيروس الكبدى بى من أهم العوامل التى تشارك فى حدوث سرطانات الكبد فى مصر والتى ترجع الى العدوى المشتركة مع الفيروس الكبدى بى وسى.
- يرجع حدوث سرطانات الكبد الى العديد من العوامل وبالرغم من اهمية

الالتهاب الكبدى الفيروسى بى وسى والعدوى المشتركة لكليهما والعدوى المتخفية للفيروس الكبدى بى كعوامل تساعد على حدوث المرض إلا إنه يوجد عوامل اخرى فى إحداث المرض.

- ولابد من معالجة مرضى الكبد المصابين بمرض البول السكرى بدقة لانهم أكثر عرضة لحدوث سرطانات الكبد.
- يوجد العديد من الوسائل والتى تساعد فى تشخيص المرض بعد الكشف المبدئى مثل سرعة الترسيب والفافيتوبروتين والأشعة التليفزيونية.

دراسة العدوى المشتركة لفيروس الالتهاب الكبدى سى وبى فى مرضى سرطان الكبد مصحوبة أو غير مصحوبة بالعدوى المختفية لفيروس بى

رسالة مقدمة من الطبيبة

رحاب بدوى محمد الششتاوى

بكالوريوس الطب والجراحة – جامعة طنطا

طيب مقيم بقسم طب المناطق الحارة والحميات

بمستشفيات جامعة طنطا

إيفاءً جزئياً للحصول على درجة الماجستير فى

طب المناطق الحارة والحميات

المشــرفــون

الدكتور منى أحمد حلمى شحاتة أستاذ طب المناطق الحارة والحميات كلية الطب – جامعة طنطا	الأستاذ الدكتور هالة أحمد فؤاد إسماعيل أستاذ الميركوبيولوجيا والمناعة كلية الطب – جامعة طنطا

الأستاذ الدكتور

عبد الرؤوف عبد البارى أبو العزم

أستاذ طب المناطق الحارة والحميات

كلية الطب – جامعة طنطا

كلية الطب
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