Retrospective study of thrombocytopenia in patients with chronic hepatitis C after interferon therapy

A thesis submitted for partial fulfillment of master degree of internal medicine

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بسم الله الرحمن الرحيم

وعلَّمك ما لم تكن تَعْلَمُ وَكَانَ فضَّلُ الله عَلَيْكَ عَظِيمًا
صدق الله العظيم

(النسخة: من الآية 113)
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**Abbreviation**

**ADCC**: antibody-dependent cellular cytotoxicity

**ADP**: Adenosine diphosphate

**ALT**: Alanine transaminase

**ANA**: antinuclear antibody

**Anti-LKM**: liver-kidney membrane

**Ash Test**: diagnostic for Alcoholic liver disease inflammation.

**AST**: Aspartate transaminase

**ATP**: Adenosine triphosphate

**CD**: cluster differentiation

**CDC**: Center of disease control

**CFU-MK**: colony-forming units of megakaryocytes

**CIFN**: consensus interferon

**CPT classification**: current procedural terminology classification

**CREs**: Cis-acting replication elements

**CTL**: cytotoxic T-lymphocyte

**EBV**: Epstein-Barr virus
EDTA : ethylenediaminetetraacetic acid

EGF : epidermal growth factor

EIA : enzymes immunoassay

E1, E2: the HCV envelope proteins

EVR : early virological response

GB: gall bladder

GP : antiglycoprotein

HBV: hepatitis B virus

HCC :Hepatocellular carcinoma

HCV RNA: HCV ribonucleic acid

HCV: hepatitis C virus

HGF : hepatocyte growth factor

HIT: heparin-induced thrombocytopenia

HIV: human immunodeficiency virus

HRS :hepatorenal syndrome

HTLV: human T-cell lymphoma virus

IFN : interferon

IL: interleukin
INOS2: IFN also induces a form of nitric oxide synthase

IRES: an internal ribosome entry site

ISDR: the interferon-sensitivity determining region.

ITP: autoimmune thrombocytopenic purpura

LSM: liver stiffness measurement

MELD: Model of End-Stage Liver Disease

MHC: Major histocompatibility complex

NASH: Non-alcoholic fatty liver disease

NHANES: National Health and Nutrition Examination Survey

NHL: non-Hodgkins lymphoma

NIH: the National Institutes of Health

NK cell: natural killer

NTRs: nontranslated regions

PCT: Porphyria cutanea tarda

PDGF: platelet-derived growth factor

Peg IFN: pegilated interferon

PF: platelet factor

PMPs: platelet microbial proteins
PT: prothrombin time
rh: recombinant human
RN: regenerative nodules
SBP: spontaneous bacterial peritonitis
STAT: The signal transducer and activator of transcription
SVR: sustained virological response
TGF: tissue growth factor
TMA-based assay HCV RNA: transcription mediated amplification
HCV RNA
TNF-α: tumor necrosis factor alpha
TPO: thrombopoietin
UDCA: The University District Community Association of University District, Detroit
VEGF: vascular endothelial growth factor
vWF: von Willebrand factor
WHO: The world health organization
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Introduction

Platelets are produced in the bone marrow; the progenitor cell for platelets is the megakaryocyte. This large, multinucleated cell sheds platelets into the circulation. Thrombopoietin (c-mpl ligand) is a hormone, mainly produced by the liver, that stimulates platelet production. It is bound to circulating platelets; if platelet levels are adequate, serum levels remain low. If the platelet count is decreased, more thrombopoietin circulates freely and increases marrow production (Rob et al. 2005).

The circulating life of a platelet is 9-10 days. After this it is sequestered in the spleen. Decreased function (or absence) of the spleen may increase platelet counts, while hypersplenism (overactivity of the spleen, e.g. in Gaucher's disease or leukemia) may lead to increased elimination and hence low platelet counts (Robert et al. 2005).

Blood platelets have several important functions. They adhere to sites of vascular injury, generate biological mediators, secrete their granule contents, form multicellular aggregates and serve as a nidus for plasma coagulation reactions. In order to carry out these tasks, the platelet undergoes dramatic structural rearrangements, utilizes multiple membrane receptors, which bind small molecule mediators, adhesive glycoproteins and constituents of the vascular subendothelium, and activates a network of complex signaling pathways. All of these events
occur within seconds of vascular injury. Collectively, they help to maintain the integrity of the vascular system. It should not be surprising that mutations occasionally arise that perturb these complex reactions and lead, in some cases, to disordered hemostasis. While inherited disorders of platelet function are relatively rare, they have provided important information about normal platelet physiology (Robert et al. 2005).

Thrombocytopenia is a frequent complication of chronic liver disease and is considered an indicator of advanced disease. The low platelet count is due partly to the effects of portal hypertension and hypersplenism, decreased thrombopoietin production, and virus-induced bone marrow suppression (Giannini 2006). (G, Bordin G, Ballare and M, Zigrossip et al. 1995).

Patients with chronic liver disease due to infection with the hepatitis C virus (HCV) who have thrombocytopenia (<75,000 platelets per cubic millimeter) have been routinely excluded from clinical trials of interferon and ribavirin, and few published reports have described the treatment of chronic HCV infection in patients with platelet counts of less than 50,000 per cubic millimeter. Although a reduced platelet count is not an absolute contraindication to treatment with pegylated interferon (peginterferon) and ribavirin, product labels advise that caution be used in treating patients with clinically significant thrombocytopenia. Furthermore, if thrombocytopenia develops during antiviral therapy, peginterferon may need to be delivered at a reduced dose or discontinued. Currently, there is no
approved treatment for thrombocytopenia in patients with HCV infection (Shiffman ML, Ghany MG and Morgant Retal 2007).

Persistent hepatitis C virus (HCV) infection evokes autoimmune response including production of autoantibodies and concomitant autoimmune disorders. Numerous types of autoantibodies such as non-organ-specific autoantibodies and liver-specific autoantibodies have been identified in sera of patients with HCV-related chronic liver disease (CLD). The production of these autoantibodies in HCV-related CLD reflects "virus-induced autoimmunity." Molecular mimicry between the HCV polyprotein and self-proteins, and polyclonal B cell activation by chronic HCV infection have been proposed as possible mechanisms for the occurrence of autoantibodies in HCV-related CLD. Some autoantibodies are tightly associated with concurrent autoimmune diseases, and others closely associated with peculiar human leukocyte antigen (HLA) haplotypes. Changes in the titers of autoantibodies during the antiviral treatment may predict the sustained virological response in individuals. In this article, we mainly focus on the interpretations of autoantibodies in HCV-related CLD (Takashihimoto and Mikionishioka etal 2008)

Human interferon (IFN) is the standard therapy for chronic hepatitis C to prevent its progression to liver cirrhosis and hepatocellular carcinoma. Thrombocytopenia is one of the major adverse effects of IFN- and often leads to dose reduction or treatment discontinuation. However, there is little information on how IFN inhibits human megakaryopoiesis (Akikio Yamane, Takanori Nokamera HIdenori Suzuki etal 2008).
Aim of the work

The aim of this work is to evaluate the occurrence of thrombocytopenia after treatment with interferon therapy used in patients with chronic hepatitis C.
Epidemiology of HCV

HCV was first identified in 1989 as the primary agent responsible for post transfusion non-A, non-B hepatitis. Choo and his colleagues, utilized molecular biology techniques to isolate, clone, and characterize a viral agent from the blood of chimpanzees who developed chronic hepatitis after they underwent transfusion with plasma obtained from patients with chronic post transfusion non-A, non-B hepatitis (Choo et al., 1989). No vaccine against hepatitis C is available. The existence of hepatitis C (originally "non-A non-B hepatitis") was postulated in the 1970s and proven conclusively in 1989. It is one of five known hepatitis viruses: A, B, C, D, and E. (Karmochkine et al., 2006)

Prevalence of HCV

I- Geographical Distribution:

It is estimated that about 170 million people worldwide are infected with HCV. The sero-prevalence rates is about 1% in Asian countries and up to 10-20% in parts of central Africa and Egypt (Wasley and Alter, 2000 & WHO 2000)

Prevalence of HCV infection among blood donors and general population:

Studies of anti-HCV sero-prevalence has distinguished 3 geographical areas with different prevalences:

1-Area with mild prevalence (<0.5%) North of Europe, Canada and Australia.
2- Area with intermediate prevalence (0.5-1%) France, United Kingdom, Germany & United States.

3- Area with high prevalence (>1%): Southern & Eastern Europe, Japan

In the USA, the National Health and Nutrition Examination Survey (NHANES III) revealed that an estimated 3.9 million US citizens (1.8%) have been infected with HCV, of those approximately 2.7 million persons are chronically infected with HCV (Williams et al., 1999).

![HCV Has Broad Global Prevalence](image)

**Figure NO.(1):** Global Prevalence of Hepatitis C Virus (WHO 1999).

The world health organization (WHO) has declared hepatitis C a global health problem, with approximately 3% of the world's population (roughly 170-200 million people) infected with HCV (Mohamed, 2004). In the US approximately 3 million people are chronically infected, many of whom are still undiagnosed (Mohamed, 2004). Region-specific estimates range from <1% in northern Europe to >2.9% in northern Africa. The lowest prevalence (0.01%-0.1%) has been reported from countries in the United Kingdom and Scandinavia (Shepard et al., 2005).
**HCV status in Egypt:**

Egypt has possibly the highest HCV prevalence in the world; 10-20% of the general population is infected and HCV is the leading cause of HCC and chronic liver disease in the country (*Arthur et al., 1997*, *El-Zayadi et al., 2001* and *Hassan et al., 2001*). Approximately 90% of Egyptian HCV isolates belong to a single subtype 4a which responds less successfully to interferon therapy than other subtypes (*El-Zayadi et al., 1996*, *Angelico et al., 1997* and *Ray et al., 2004*).

The prevalence of HCV infection in Egypt increases steadily with age and high rates of infection are observed among persons in all age groups (*Abdel-Aziz et al., 2000*). This pattern indicates an increased risk in the distant past followed by an ongoing high risk for acquiring HCV infection although there are regional differences in average overall prevalence (*Perz et al., 2006*).

Cohort studies in Upper Egypt showed incidence rates of 0.8/1000 persons per year with background prevalence was 9% while 6.8/1000 in the Nile delta with a background prevalence of 24% (*Mohammed et al., 2005*).

**II- Age and Race:**

In the United States, the highest rate of hepatitis C is in persons aged 30-49 years, and is more prevalent in blacks than other racial groups (*Centers for Disease Control and Prevention 1998*).

**Figure NO.(2):** Prevalence of HCV Infection by Age and Race in the United States. (*Centers for Disease Control and Prevention, 1998*)
### III- Routes of transmission:

**Table No.(1) Outline of Hepatitis C Viral Transmission Routes (Lauer and Walker 2001).**

<table>
<thead>
<tr>
<th>Category</th>
<th>Routes of Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percutaneous</strong></td>
<td></td>
</tr>
<tr>
<td>Transfusion of blood products</td>
<td>Red blood cell transfusion</td>
</tr>
<tr>
<td></td>
<td>Intravenous immunoglobulin</td>
</tr>
<tr>
<td>Intravenous drug abusers</td>
<td>Sharing needles and equipment</td>
</tr>
<tr>
<td><strong>Nosocomial</strong></td>
<td></td>
</tr>
<tr>
<td>Patient-to-patient</td>
<td>Hemodialysis</td>
</tr>
<tr>
<td></td>
<td>Organ donation</td>
</tr>
<tr>
<td>Patient-to-healthcare worker</td>
<td>Needle-stick</td>
</tr>
<tr>
<td>Healthcare worker-to-patient</td>
<td>Surgery</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tattoo, intranasal cocaine,</td>
</tr>
<tr>
<td><strong>Sexual</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple sexual partners</td>
</tr>
<tr>
<td><strong>Perinatal</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infants born to HCV-positive mothers</td>
</tr>
</tbody>
</table>

The transmission of HCV is primarily through exposure to infected
blood (Karmochkine et al., 2006).

• Parenteral transmission:

1- Transfusion of blood and blood products

HCV is a blood-born virus; hence the transfusion of blood and blood products, as well as the transplant of organs that have not undergone viral inactivation, all are potential sources of HCV transmission (Lavanchy, 1999).

2- Iatrogenic infections

These are infections that occur unintentionally from medical or dental procedures. For example, injection with contaminated needles or syringes. Iatrogenic transmission of HCV is possible when disinfection and sterilization techniques are inadequate and contaminated equipments are shared among patients. Infection can occur among patients on dialysis due to poor infection control and sharing contaminated medical vials and supplies (WHO, 2000). Researches have attributed the high prevalence of HCV infection in Egypt to the use of unsterile syringes during the mass treatment of the general population with parenteral antischistosomal therapy till 80ies (Frank et al., 2000).

3- Occupational exposure to blood

Medical and dental personnel, first responders (e.g., paramedics, emergency medical technicians) can be exposed to HCV through accidental exposure to blood through accidental needle sticks or blood spatter to the eye or open wound. Universal precautions to protect against such accidental exposures significantly reduce the risk of exposure to HCV (Karmochkine et al., 2006).

• Non parenteral transmission:

1- Drugs used by nasal inhalation (drugs that are snorted)
The transmission of HCV may be possible through the nasal inhalation of illegal drugs such as cocaine and crystal methamphetamine when straws are shared among users (Thompson et al., 2006).

2- Sexual transmission

Sexual transmission of HCV is considered to be rare. Center of disease control (CDC) does not recommend the use of condoms between couples (where one partner is positive and the other is negative) (Ray et al., 2004 and Hahn, 2007).

3- Vertical transmission and breast feeding

In 2003, Ferrero and colleagues reported that the rate of perinatal transmission of HCV ranges from 2-8% in women not coinfected with HIV (Ferrero et al., 2003).

There is no evidence that breast feeding transmits HCV, however an infected mother should avoid breast feeding if her nipples are cracked or bleeding (Voyer et al., 2001).

4- Non identifiable source of infection:

Approximately 10% of patients in most epidemiological studies have no identifiable source of infection (Flamm et al., 1998).

Virology of Hepatitis C Virus

**Molecular genetic structure:**

The first molecular clones of hepatitis C virus (HCV) were reported in 1989 (Choo et al., 1989). Comparative sequence analysis revealed that HCV is related to flavi- and pestiviruses (Choo et al., 1991), and HCV was subsequently placed in the family flaviviridae (Murphy et al., 1995).
The HCV genome consist of a single open reading frame flanked by 5' and 3' nontranslated regions (NTRs). The 5' NTR contain a series of stem-loop structures that interact with host factors to initiate synthesis of the polyprotein through an internal ribosome entry site (IRES) (Rijnbrond and Lemon 2000). The 3' NTR consist of a short variable sequence, a poly (U)- poly (UC) tract, and a highly conserved X region, and it is critical for HCV RNA replication and HCV infection (Yi and Lemon 2003). The 5' and 3' NTR of HCV has Cis-acting replication elements (CREs) which are essential for viral life cycle (Friebe and Bartenschlager 2002).

HCV encodes a single polyprotein of 3011 amino acids, which is then processed into 10 viral proteins: three structural proteins (core, E1 and E2), and seven nonstructural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Bartenschlager and Lohmann 2000). Two regions of the envelope E2 protein, designated hypervariable region 1 and 2, have an extremely high rate of mutation, believed to be the result of selective pressure by virus specific antibodies. E2 also contain the binding site for CD81 (Pileri et al., 1998).

HCV also encodes a virus specific helicase, protease, and polymerase, and because of the critical function of these proteins in the viral life cycle, they represent attractive targets for antiviral therapy (Kolykhalov et al., 2000). A region in NS5A has been linked to the response to interferon alpha therapy and is therefore called the interferon- sensitivity determining region (ISDR).

A recent meta-analysis has confirmed a close correlation between the number of mutations in ISDR of the HCV protein NS5A and the outcome of interferon monotherapy for hepatitis C virus type 1b infection worldwide (Schinkel et al., 2004).
Genetic heterogenicity of HCV: Genotypes and Quasispecies:

Genetic heterogenicity is one of the most relevant biological characteristics of the HCV. Comparative analysis of the genomic sequences of the virus isolated in different parts of the world has led to identification of up to six different HCV genotypes, 1-6. In each genotype there are subtypes; genotypes 5 and 6 have a particularly large number of subtypes (Mondelli and Sillini 2000).

The geographical distribution of different types of HCV varies significantly. HCV genotype 1, 2, and 3 are distributed worldwide, whereas genotype 4, 5, and 6 are found mainly in specific areas (Zein 2000). For example, genotype 4 is relatively frequent in many Central Africans Countries (Ndomou et al., 2002), and highly prevalent in Egypt (Ray et al., 2002). In Europe, there have been dynamic changes over time in the prevalence of the different HCV subtypes. For example, the prevalence of HCV 1b has decreased, while conversely, that of HCV 1a and 3a has increased (Dal Molin et al., 2002-b).
In recent study, Pybus et al., (2001) used a mathematical model to analyze the epidemic behavior of HCV infection. Strikingly, it seems that HCV genotype 1a and 1b originated about 100 years ago and are evolving at a faster rate than genotype 4 and 6.

**Figure NO.(4):** Worldwide distribution of Various Genotypes of Hepatitis C Virus (Forns and Bukh, 1999)

HCV genotyping is important in the clinical setting, since genotype 1 and 4 isolates are less likely to respond to interferon (IFN) therapy than genotype 2 and 3 (Koshy et al., 2002). Recent studies in the United States have suggested that blacks are more likely to be infected with genotype 1, which has lower response to INF treatment than other genotypes (De Maria et al., 2002), which might be partially overcome by IFN-ribavarin combination therapy (McHutchison et al., 2000).

**Immunopathogenesis of hepatitis C:**

Most studies on the HCV specific immune response have been performed in chronically rather than acutely infected or recovered patients. Collectively these data imply that the immune response can mediate chronic inflammatory liver cell injury if it is not able to clear the virus early after infection (Alter et al., 1992).
In most patients with chronic HCV infection, both the humoral and cellular arms of the immune response appear to be active. Antibodies to HCV structural and nonstructural proteins develop during infection and form the basis for the detection assay of the host’s exposure to the virus. The cellular immune response results in the emergence of CD4+ and CD8+ cells that recognize and respond to processed HCV antigens (Ludewig et al., 1998).

**Humoral immune response:**

The role of the humoral arm of the immune response in HCV infection is suggested by the presence of hepatic lymphoid aggregates containing activated B cells, elevated levels of the B-cell-activating interleukin-4 (IL-4), and a B-cell-mediated response with production of antibodies to several structural and nonstructural polypeptides (Reiser et al., 1997).

Antibodies have two effects that might play a role in the immunopathogenesis of hepatitis C. These are viral neutralization and antibody-dependent cellular cytotoxicity (ADCC). Antibodies against envelope proteins often have neutralizing ability. Antibodies against conserved epitopes of the HCV envelope proteins (E1, E2) are found in more than 90% of patients with chronic HCV infection (Ray et al., 1994).

Antibodies may also direct destruction of its bound target through activation of other mechanisms, specifically the complement-mediated ADCC. However, for these antibodies to contribute to cell injury, they must recognize HCV antigens on the hepatocyte cell membrane.

Although HCV antigens (Core, E1, E2, NS3, and NS4) have been detected in the cytoplasm of infected hepatocytes, membranous antigens have not been observed; even when intracellular expression of HCV viral proteins is driven to very high levels by a recombinant vaccinia system, neither HCV antigens nor immunoglobulins could be detected on the cell membrane.

These data suggest that ADCC is unlikely to play an important role in mediating hepatocellular damage (Choo et al., 1994).
Finally, the antibody response to HCV may provide clues to the clinical course of infection. Anti-NS4 may decline or even disappear in patients who recover from acute hepatitis or respond to IFN therapy.

Core-specific IgM may also decline with successful IFN therapy, and some have suggested that the antibody titer may correlate with disease activity, although this is controversial (Nagayoma et al., 1994)

The production of IgM antibodies has been extensively investigated as a possible marker for recent infection and reactivation.

The proportion of acutely infected patients exhibiting IgM antibodies has ranged between 50 and 90% in different studies, but IgM antibodies specific for structural and nonstructural HCV protein have also been detected in 50-70% of patients with chronic hepatitis, these are generally believed to represent an indication of active virus replication (Hellstrom et al., 1993).

IgM level have been seen to correlate directly with the level of Alanine transaminase (ALT) and viraemia (Chen et al., 1995) and have been reported to be highly prevalent in individuals infected with genotype 1b, which appear to be associated with severe liver damage (Pawlotsky et al., 1995)

IgG antibodies to the envelop, core and NS3 proteins usually become detectable after serum ALT and viremia have peaked and remain demonstrable for protracted periods or indefinitely (Lesniewsk et al., 1995).

**Cellular Immune Response:**

The cellular immune response to viral infection involves nonspecific mechanisms, such as IFN release and NK cell activity, and antigen-specific mechanisms including cytotoxic T lymphocytes and inflammatory cytokine release.

Although nonspecific cellular responses may act very early to limit some infections, eradication of infection most likely depends on specific and classical CD4+ and CD8+ cytotoxic T lymphocyte (CTL) responses. (Kita et al., 1995).
In contrast to the humoral response, “which is triggered” by the binding of unprocessed extracellular antigens to B-cell immunoglobulin receptors, the cellular (T-cell) immune response is triggered by peptides that have been processed within the cell cytoplasm and expressed on cell membranes in conjunction with a major histocompatibility complex (MHC) molecule. Processed peptides are generally presented to CD8+ T cells by MHC class I molecules, which are expressed on virtually all cells, or to CD4+ T cells by the MHC class II molecules, which are found on specialized antigen presenting cells. Although the subsequent events that lead to death of the infected presenting cells are not entirely clear, both direct cytolysis and secreted antiviral factors (tumor necrosis factor alpha [TNF-α] and IFN-γ) can be implicated (Kita et al., 1995).

**CD4+ T-Lymphocyte response:**

The CD4+ T-cell response to viral proteins is critical for host protection because it occurs relatively early, augments antibody production by B cells, and stimulates CD8+ T cells, including those that are specific for virus-infected cells. Thus, its role has typically been viewed as a protective one. CD4+ T-cell responses to viral infection have traditionally been determined by measuring the ability of these peripheral lymphocytes to proliferate or produce IFN-γ when exposed to viral proteins. No one viral antigen is responsible for this CD4+ response, although peptides derived from core and NS4 result in the greatest proliferative responses. Interestingly, proliferative responses are most healthy in infected individuals who resolve acute infection, have persistent infection without histologic evidence of liver damage, or have chronic hepatitis that responds to IFN. These observations suggest that a vigorous CD4+ response to HCV infection provides early control of infection and protects against subsequent hepatocellular damage (Missale et al., 1996).

**CD8+ T-Lymphocyte response:**

The CD8+ CTL arm of the cellular immune system has been shown to be important in the control of viral infections and pathogenesis of cell injury in vivo. Several lines of evidence suggest that these cells also play an important
role in HCV infection.

**First**, immunophenotyping studies have demonstrated that a significant proportion of the activated cells in the liver of patients with chronic hepatitis C are CD8+ T lymphocytes.

**Second**, expression of adhesion molecules, one pathway for recruitment and priming of T cells, is up regulated in the inflamed hepatic portal tracts.

**Third**, most important, HCV-specific cytotoxic CD8+ T lymphocytes have been isolated from both liver and peripheral blood in a significant proportion of patients with chronic HCV infection. *(Koziel et al., 1993).*

**Cytokine response:**

The rapid replication rate of HCV and the large number of infected hepatocytes present a formidable challenge to the cellular immune system. The fact that this infectious burden exceeds the capacity of the CTL response is apparent from the persistent nature of the infection. However, other mechanisms may assist in control of the infection. Cytokine responses are referred to as Th1-like and Th2-like after the original description of the cytokine profiles produced by subsets of the CD4+ Th cells. Th1-like responses include IL-2, TNF-α, and IFN-γ secretion and are required for CTL generation and NK cell activation during the host’s antiviral immune response. Th2-like responses produce IL-4 and IL-10, which help augment antibody production and inhibit the development of the Th 1 response *(Fiorentino et al., 1991).*

**Direct Viral Cytopathicity:**

It has been difficult to determine whether HCV is directly cytopathic because an efficient cell culture system has not yet been developed. However, several lines of evidence support a cytopathic role for HCV.

However, there is also considerable evidence to suggest that HCV is not directly cytopathic. In the overwhelming majority of patients, particularly immunocompetent patients, biochemical or histologic markers of disease
activity do not correlate with serum viral levels or the amount of HCV RNA or antigen in the liver. In fact, many patients with HCV infection have persistently normal serum ALT levels and minimal liver injury despite the presence of detectable HCV RNA in serum (*Shindo et al., 1995*).

**Mechanism of HCV persistence:**

Several mechanisms suspected to contribute to failure to contain HCV have been suggested (*Recanelli and Rehermann 2003*), including impairment of cellular effector function (proliferation, secretion, and cytolytic activity) (*Wedemeyer et al., 2002*) or T cell exhaustion (*Kantzanou et al., 2003*).

As a second hypothetical explanation for HCV persistence, sequence variation of the quasispecies and the high mutation rate of HCV. Circulating HCV has a half-life of only about 3 hours indicating relatively efficient virus replication and rebase, at least in patients with high levels of viral load. This dynamic process, capable of generating viral variants continuously, suggests that viral variation may be important for the establishment and maintenance of persistent infection (*Neumann et al., 1998*).

Viral variants that may mediate escape from the cellular immune response have been demonstrated in many viral infection such as HBV, human T-cell lymphoma virus (HTLV) and HIV infection (*Borrow et al., 1997*) and recently in patients with chronic hepatitis (*Chang et al., 1997 and Kaneko et al., 1997*).

HCV may diminish its visibility to the immune response sufficiently to avoid clearance but not enough to prevent inflammatory liver injury (*Large et al., 1999*).

**Clinical Feature**

**Acute hepatitis C**
The course of acute hepatitis is highly variable and ranges in severity from a transient, asymptomatic infection to severe or fulminant disease. The disease may be self-limited and resolve, run a relapsing course, or lead to chronic infection. In a typical, clinically apparent course of acute resolving viral hepatitis (Fig. 1), the *incubation period* varies from 15 to 120 days (mean of 50), largely on the basis of the viral etiology and exposure dose. During this phase, virus becomes detectable in blood, but serum aminotransferase and bilirubin levels are normal, and antibody is not detected (*Hoofnagle JH*, 2007).

The *preicteric phase* of illness is marked by the onset of nonspecific symptoms such as fatigue, nausea, poor appetite, and vague right upper quadrant pain. Viral-specific antibody first appears during this phase. The preicteric phase typically lasts 3 to 10 days, but this phase may last longer and even constitute the entire course of illness in patients with subclinical or anicteric forms of acute hepatitis. Viral titers are generally highest at this point, and serum aminotransferase levels start to increase. The onset of dark urine marks the *icteric phase* of illness, during which jaundice appears and symptoms of fatigue and nausea worsen. Typically, acute viral hepatitis is rarely diagnosed correctly before the onset of jaundice. If jaundice is severe, stool color lightens, and pruritus may appear. Anorexia, dysgeusia, and weight loss may also occur. Physical examination usually shows jaundice and hepatic tenderness. In more severe cases, hepatomegaly and splenomegaly may be present. Serum bilirubin levels (total and direct) rise, and aminotransferase levels are generally greater than 10 times the upper limit of normal, at least at the onset. During the icteric, symptomatic phase, levels of hepatitis virus begin to decrease in serum and liver. The duration of clinical illness is variable; it typically lasts 1 to 3 weeks. Recovery is first manifested by return of appetite and is accompanied by resolution of the serum bilirubin and aminotransferase elevations and clearance of virus. Convalescence can be prolonged, however, before full energy and stamina return. Neutralizing antibodies usually appear during the icteric phase and rise to high levels during convalescence (*Hoofnagle JH*, 2007).
Figure No.(5): **Typical course of acute viral hepatitis** (*Hoofnagle JH*, 2007).

**Chronic hepatitis C**

**Symptoms:**

Most patients with chronic infection are asymptomatic or have only mild nonspecific symptoms the most frequent complaint is fatigue; other less common manifestations include nausea, anorexia, myalgia, arthralgia, weakness, and weight loss (*Merican I et al, 1993*) The symptoms of chronic HCV infection do not reliably reflect disease activity. Abdominal pain, itching, and dark urine were the only complaints that were significantly more common among the HCV patients, although they were present in only a small number of patients (*Shakil AO et al, 1995*).

**Complication of chronic hepatitis C:**

**A-Cirrhosis:**

Patients with cirrhosis are susceptible to a variety of complications and their life expectancy is markedly reduced. Cirrhosis and chronic liver disease accounted for more than 25,000 deaths and 373,000 hospital discharges in the United States in 1998 according to a report from The National Center for Health Statistics (*Gordon, E et al, 1998*).

**Clinical Manifestation:**

Patients with cirrhosis may present in a variety of ways.

1-They may have stigmata of chronic liver disease discovered on routine physical examination such as:
• **Spider angiomata** (also referred to as spider telangiectasias) are vascular lesions consisting of a central arteriole surrounded by many smaller vessels. They are most frequently found on the trunk, face, and upper limbs *(Pirovino M et al, 1988)*.

• **Palmar erythema** is an exaggeration of the normal speckled mottling of the palm, and is also believed to be caused by altered sex hormone metabolism *(Erlinger et al, 1991)*.

• **Clubbing and hypertrophic osteoarthropathy** Clubbing is more common in biliary causes of cirrhosis (particularly primary biliary cirrhosis) while hypertrophic osteoarthropathy can be seen with various causes of liver disease *(Mills PR et al, 1981)*.

• **Hepatomegaly** : The cirrhotic liver may be enlarged, normal sized, or small.

• **Splenomegaly** It is believed to be caused primarily by congestion of the red pulp as the result of portal hypertension. However, splenic size does not correlate well with portal pressures, suggesting that other factors may be contributing *(Erlinger et al, 1991)*.

• **Ascites** : probability of ascites being present was less than 10 percent in such patients *(Cattau EL Jr et al, 1982)*.

• **Caput medusa**

• **Jaundice**

2- They may have undergone laboratory or radiologic testing or an unrelated surgical procedure that incidentally uncovered the presence of cirrhosis.

3- They may present with decompensated cirrhosis, which is characterized by the presence of dramatic and life-threatening complications, such as variceal hemorrhage, ascites, spontaneous bacterial peritonitis (SBP), or hepatic encephalopathy.
- **Figure No. (6):** Complications of cirrhosis result from portal hypertension or liver insufficiency. Varices and variceal hemorrhage are a direct consequence of portal hypertension. Ascites results from sinusoidal portal hypertension and can be complicated by infection (spontaneous bacterial peritonitis [SBP]) or renal dysfunction (hepatorenal syndrome [HRS]). Hepatic encephalopathy results from portosystemic shunting (i.e., portal hypertension) and liver insufficiency. Jaundice results solely from liver insufficiency *(Garcia-Tsao G, 2007).*
Table 2: The Two Most Commonly Used Scoring Systems In Cirrhosis: *(Garcia-Tsao G, 2007)*

1. **Child-Pugh-Turcotte (CPT) score (range, 5–15)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Points Ascribed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites</td>
<td>None</td>
</tr>
<tr>
<td>Hepatic encephalopathy</td>
<td>None</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>&gt;3.5</td>
</tr>
<tr>
<td>Prothrombin time (seconds &gt; control) or</td>
<td>&lt;4</td>
</tr>
<tr>
<td>INR</td>
<td>&lt;1.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites Grade 1–2 (or easy to treat)</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Ascites Grade 3–4 (or refractory)</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hepatic encephalopathy Grade 1–2 (or induced by a precipitant)</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Hepatic encephalopathy Grade 3–4 (or spontaneous)</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Prothrombin time (seconds &gt; control) or</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>INR</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

CPT classification: Child A: score of 5–6; Child B: score of 7–9; Child C: score of 10–15

2. **Model of End-Stage Liver Disease (MELD) score (range, 6–40):**

\[
[0.957 \times \text{LN (creatinine in mg/dL)} + 0.378 \times \text{LN (bilirubin in mg/dL)} + 1.12 \times \text{LN (INR)} + 0.643] \times 10
\]

In interpreting the MELD Score in hospitalized patients, the 3 month mortality is:

- 40 or more - 100% mortality
- 30-39 - 83% mortality
- 20-29 - 76% mortality
- 10-19 - 27% mortality
- <10 - 4% mortality

INR = international normalized ratio; LN = natural logarithm.

*B-Hepatic decompensation* The most common form of decompensation was ascites, followed by variceal bleeding, encephalopathy, and jaundice (which is almost always a sign of advanced liver disease in patients with chronic hepatitis C).

*C-Hepatocellular carcinoma (HCC):*
HCV accounts for approximately one-third of HCC cases in the United States. Estimates of the risk of developing HCC once cirrhosis has developed have varied from 0 to 3 percent per year in various reports (Hu KQ et al, 1999).

**Extrahepatic manifestations of hepatitis C virus infection**

**A-HEMATOLOGIC DISORDERS**

1) **Essential mixed cryoglobulinemia:**
   
   Treatment of patients with cryoglobulinemia due to HCV should be based upon the presence of cryoglobulinemia symptoms rather than the usual criteria used in patients with chronic hepatitis alone (Saadoun D et al, 2006). The response should be assessed by symptomatic improvement of cryoglobulinemia, a reduction in cryocrit, and an increase in serum complement levels. Complete responses may be more common in patients with low pretreatment levels of viremia and with high dose interferon regimens (Casato M et al, 1997).

2) **Monoclonal gammopathies:** Hepatitis C may be a risk factor for the development of monoclonal gammopathies (Andreone P et al, 1998).

3) **Lymphoma:** Multiple reports have described an association between HCV infection and B-cell non-Hodgkins lymphoma (NHL) (Monti G et al, 2005).

**B-DIABETES MELLITUS**

HCV infection has been linked to diabetes mellitus in several epidemiologic studies (Zein CO et al, 2005) HCV genotype 2a was overrepresented among the diabetic patients.

Risk factors for the development of diabetes mellitus in HCV infected patients included older age, obesity, severe liver fibrosis, and a family history of diabetes mellitus (Petit JM et al, 2001).

HCV has also been linked to insulin resistance without overt diabetes (Moucari R et al, 2008). The insulin resistance may contribute to fibrosis progression, particularly with HCV genotypes 1 and 4 and high serum RNA levels (Moucari R et al, 2008). Insulin resistance may also impair the response to antiviral therapy with interferon and ribavirin.

**C-AUTOIMMUNE DISORDERS**
1. **Autoantibodies** are common in patients with chronic HCV infection; antinuclear antibodies, antibodies directed against the Fc portion of IgG (rheumatoid factor1997), anticardiolipin antibodies, smooth muscle antibodies, or antithyroid antibodies are detected in 40 to 65 percent of patients*(Cacoub P et al,)* Antibodies to actin and to liver/kidney microsomes (anti-LKM-1) are characteristic of types 1 and 2 autoimmune hepatitis*(Zauli D et al, 1997).*

2. **Thyroid disease** Thyroid disorders are common in patients with chronic HCV, particularly women *(Antonelli A et al, 2004)*

3. **Sialadenitis** A lymphocytic sialadenitis suggestive of Sjögren's syndrome has been described in patients with chronic HCV infection *(Ramos-Casals M et al, 2005).*

4. Anti-HCV antibodies occur in 10 to 19 percent of patients with autoimmune thrombocytopenic purpura (ITP). Some of these patients acquired anti-HCV antibodies passively following use of IVIG with a high titer of anti-HCV antibodies, while others developed actual infection following transfusion of HCV-infected blood products employed for the treatment of ITP (eg, contaminated IVIG). In other cases, ITP developed following the acquisition of HCV infection, or during its treatment *(Pawlotsky JM et al, 1995).*

5. **Myasthenia gravis**

**D-OCULAR DISEASE**

HCV infection has been associated with a variety ophthalmologic disorders including dry eyes, corneal ulcers (Mooren's ulcer), uveitis, and scleritis *(Jacobi C et al,2007),* and sicca syndrome in patients with HCV-related Sjogren's syndrome *(Ramos-Casals M et al,2005).* In addition, ophthalmologic disorders (retinal hemorrhages, cotton wool spots, and rarely retinal artery or vein obstruction) can occur during interferon therapy.

**E-RENAL DISEASE:**

Glomerular disease may occur in patients with chronic HCV infection. The most common patterns are membranoproliferative glomerulonephritis (usually associated with essential mixed cryoglobulinemia) and, less frequently, membranous nephropathy *(McGuire BM et al, 2006).*

**F-DERMATOLOGIC DISEASE**
lichen planus, vitiligo, Porphyria cutanea tarda (PCT), Sjogren’s syndrome, palpable purpura and necrolytic acral erythema.

G-MUSCULOSKELETAL

• Myalgia (muscle pains), fatigue, arthralgias (joint pains), arthritis

**Diagnosis of chronic HCV**

After full history taking and general, local abdominal examination, the investigations are:

*A-Biochemical assessment of liver condition:*

Diagnosis of hepatitis is made by biochemical assessment of liver function. Initial laboratory evaluation is shown in table (3) (*Sulkowski and Thomas, 2003*).

Patients with chronic HCV infection should be monitored at least twice yearly with laboratory studies, including measurement of serum ALT, bilirubin, albumin, and prothrombin time (*Schiff, 1997*).

**Table (3): Biochemical indicators of hepatitis C virus infection**

- In chronic hepatitis C, increase in the alanine and aspartate aminotransferase (ALT and AST respectively) range from zero to 20 times (but usually less than five times) the upper limit of normal.
- ALT levels are usually higher than AST levels, but that finding may be reversed in patients who have cirrhosis.
- Alkaline phosphatase and gamma glutamyl transpeptidase are usually normal. If elevated, they may indicate cirrhosis.
- Low platelet and white blood cell counts and raised levels of serum globulins (including immunoglobulins and rheumatoid factor) are frequent in patients with severe fibrosis or cirrhosis, providing clue to presence of advanced liver disease.
• The enzymes lactate dehydrogenase and creatinine kinase are usually normal.
• Albumin level, bilirubin, and prothrombin time are normal till late stage.
• Iron and ferritin levels may be slightly elevated.

(Schiff, 1997).

B- Virological assays for HCV:

• Enzymes immunoassay:

The third generation anti HCV enzymes immunoassay (EIA) in use since the mid 90ies has a sensitivity of 95-99% and can detect HCV antibody 6-8 weeks after exposure (Alter et al., 2002).

• Recombinant immunoblot assay (RIBA):

For confirmation the presence of HCV- specific serum antibodies after detection by EIA (Zignego et al., 2006).

• Direct assay for HCV-RNA:

Qualitative detection assays are based on the principle of target amplification using either "classic" polymerase chain reaction (PCR), "real-time" PCR or TMA. Qualitative detection assays must detect 50 HCV RNA IU/ml or less, and have equal sensitivity for the detection of all HCV genotypes. The lower limit of detection of the qualitative, non quantitative reverse-transcriptase PCR-based assay is 50 IU/ml, whereas that of the TMA-based assay HCV RNA Qualitative Assay is 10 IU/ml (Table 1). Real-time PCR assays, which are also able to quantify HCV RNA, have lower limits of detection of the order of 5-30 IU/ml when they are used as purely qualitative, non-quantitative assays. (Ge D, et al, 2009)

Polymerase chain reaction (PCR) can detect low levels of HCV-
RNA in patient serum, testing for HCV-RNA is a reliable way of demonstrating that hepatitis C infection is present and the most specific test for infection. Testing for HCV-RNA is particularly useful to confirm active infection, detect acute infection and in monitoring patients undergoing therapy (Armstrong et al., 2002).

**C- Liver biopsy:**

It is traditionally the 'gold standard' for the evaluation of liver diseases (Campbell and Reddy 2004).

In a recent study, Bain and colleagues (2004) supports the use of liver biopsy for the accurate assessment of the hepatic inflammation (grade) and fibrosis (stage) of patients with chronic hepatitis C. The use of liver biopsy has grown to serve multiple purposes:

2. Assessment of severity of necroinflammation and fibrosis.
3. Evaluation of possible concomitant disease processes.
4. Assessment of therapeutic intervention.

(Brunt, 2000).

**Routes of liver biopsy:**

- Percutaneous approach which is a quick and safe procedure commonly performed in out-patient settings.
- Transjugular approach to liver biopsy is often used in patients with a contraindication to percutaneous biopsy (e.g ascites).
- Laparoscopic liver biopsy allows visualization of the peritoneal cavity and liver surface.

(Campbell and Reddy 2004).

**Histologic Stages of HCV Infection:**

(In Panel A), a core-biopsy specimen from a patient with chronic HCV infection shows dense portal lymphocytic infiltrates (arrow) (fig.7) and
architectural changes (arrowhead) (fig.7). The lymphocytes are not limited to the portal tract but also extend into the lobules (arrowheads) (in Panel B). (Panel C) shows normal liver architecture with scant fibrous tissue (arrows) limited to the portal tracts. During the progressive course of infection, the fibrotic areas expand and bridging fibrosis develops (arrows) (in Panel D).

The final stage of cirrhosis (Panel E) is characterized by marked fibrosis and regenerative nodules (RN). Once cirrhosis has become established, hepatocellular carcinoma (Panel F) is a feared complication (Lauer and Walker 2001).

Figure NO.(7): Histologic Stages of HCV Infection. (Lauer and Walker 2001).
Table No.(4) Biopsy Evaluation Score:

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation (grade)</strong></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>Mild or absent interface necrosis and lobular activity</td>
</tr>
<tr>
<td>Moderate</td>
<td>Moderate interface necrosis and lobular activity</td>
</tr>
<tr>
<td>Severe</td>
<td>Marked interface necrosis and lobular activity with or without bridging necrosis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibrosis (stage)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None/minimal</td>
<td>Absence of fibrosis or fibrosis confined to the portal tracts.</td>
</tr>
<tr>
<td>Moderate</td>
<td>Fibrous septa or bridging fibrosis</td>
</tr>
<tr>
<td>Advanced bridging/cirrhosis</td>
<td>Advanced bridging (&gt;3 bridges) or cirrhosis</td>
</tr>
</tbody>
</table>

(Hytiroglou et al., 1995)

Histological grading and staging of chronic hepatitis

*Grading of chronic hepatitis:*

Table No.(5) Necroinflammatory score (Ishak et al., 1995).

<table>
<thead>
<tr>
<th>Change</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Periportal or periseptal interface hepatitis</strong></td>
<td></td>
</tr>
<tr>
<td>(piecemeal necrosis)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Mild (focal, few portal areas)</td>
<td>1</td>
</tr>
<tr>
<td>Mild/ moderate (focal, most portal areas)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate (continuous around &lt; 50% of tracts or septa)</td>
<td>3</td>
</tr>
<tr>
<td>Severe (continuous around &gt; 50% of tracts or septa)</td>
<td>4</td>
</tr>
</tbody>
</table>

| **Confluent necrosis**                         |       |
| Absent                                         | 0     |
| Focal confluent necrosis                       | 1     |
| Zone 3 necrosis in some areas                  | 2     |
| Zone 3 necrosis in most areas                  | 3     |
Zone necrosis + occasional portal-central (P-C) bridging
Zone 3 necrosis + multiple (P-C) bridging
Panacinar or multiacinar necrosis

**Focal (spotty) lytic necrosis, apoptosis and focal inflammation**
- Absent
- One focus or less per 10 x objective
- Two to four foci per 10 x objective
- Five to ten foci per 10 x objective
- More than ten foci per 10 x objective

**Portal inflammation**
- None
- Mild, some or all portal areas
- Moderate, some or all portal areas
- Moderate/ marked, all portal areas
- Marked, all portal areas

**Maximum possible score** 18

**Staging of chronic hepatitis:**

**Table No.(6)** Architectural changes, fibrosis and cirrhosis (Ishak et al., 1995).

<table>
<thead>
<tr>
<th>Change</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Fibrosis</td>
<td>0</td>
</tr>
<tr>
<td>Fibrous expansion of some portal areas, with or without short fibrous septa</td>
<td>1</td>
</tr>
<tr>
<td>Fibrous expansion of most portal areas, with or without short fibrous septa</td>
<td>2</td>
</tr>
<tr>
<td>Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging</td>
<td>3</td>
</tr>
<tr>
<td>Fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)]</td>
<td>4</td>
</tr>
<tr>
<td>Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)</td>
<td>5</td>
</tr>
<tr>
<td>Cirrhosis, probable or definite</td>
<td>6</td>
</tr>
</tbody>
</table>

**Maximum possible score** 6

**Complications:**

Bleeding, infections, pneumothorax, pain, hypotension and injury of other organs (e.g kidney, GB and vessels) (Friedman and Keefe, 2004).
**D- Other methods for assessment:**

Fibro Test, known as FibroSure in the US, is a patented biomarker test that uses the results of six blood serum tests to generate a score that is correlated with the degree of liver damage in people with a variety of liver diseases. FibroTest has the same prognostic value as a liver biopsy. *(Ngo Y, et al, 2006)*

Recently, Fibroscan evaluation of liver stiffness measurement (LSM) was proved to be useful for predicting hepatic fibrosis and assessing cirrhosis *(Wang et al., 2009).*

Fibro Test has been evaluated in relation to liver biopsy (the current gold standard in liver disease assessment) in a large number of patients with hepatitis C, hepatitis B, alcoholic liver disease, Non-alcoholic fatty liver disease and in the general population. By 2008 it had been used in over 350,000 patients. FibroTest has been validated for the initial diagnosis of fibrosis, but also for the monitoring of patients. In 2006, the French National Authority for Health recommended the use of FibroTest as a first-line assessment tool for fibrosis with untreated chronic hepatitis C. *(Ngo Y, et al, 2006)*

- The Fibro Test score (in this case 0.88) may indicate the presence of cirrhosis.
- The conversion of Fibro Test score into stages according to the three most used histological classifications (METAVIR, Knodell and Ishak) for liver biopsies,
Table (7):-the conversion of fibro Test into score,

<table>
<thead>
<tr>
<th>FibroTest</th>
<th>METAVIR</th>
<th>Knodell</th>
<th>Ishak</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75-1.00</td>
<td>F4</td>
<td>F4</td>
<td>F6</td>
</tr>
<tr>
<td>0.73-0.74</td>
<td>F3-F4</td>
<td>F3-F4</td>
<td>F5</td>
</tr>
<tr>
<td>0.59-0.72</td>
<td>F3</td>
<td>F3</td>
<td>F4</td>
</tr>
<tr>
<td>0.49-0.58</td>
<td>F2</td>
<td>F1-F3</td>
<td>F3</td>
</tr>
<tr>
<td>0.32-0.48</td>
<td>F1-F2</td>
<td>F1-F3</td>
<td>F2-F3</td>
</tr>
<tr>
<td>0.28-0.31</td>
<td>F1</td>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>0.22-0.27</td>
<td>FO-F1</td>
<td>FO-F1</td>
<td>F1</td>
</tr>
<tr>
<td>0.00-0.21</td>
<td>FO</td>
<td>FO</td>
<td>FO</td>
</tr>
</tbody>
</table>

*(Cacoub, et al, 2008)*

**FibroTest derivatives**

Four other tests derive from Fibro Test, and are part of the Fibro Max package of tests:

- ActiTest: have been used in several countries, since 2002, as an alternative to liver biopsy in order to estimate liver fibrosis and necroinflammatory activity in chronic viral hepatitis C *(Hofmann et al., 2008).*
- SteaoTest: diagnostic for liver steatosis;
- NashTest: diagnostic for NASH (Non-alcoholic fatty liver disease) inflammation;
- AshTest: diagnostic for Alcoholic liver disease inflammation.

**TREATMENT OF CHRONIC HEPATITIS**

HCV virus is a major cause of chronic hepatitis that may progress in some patients to cirrhosis and hepatocellular carcinoma. The natural history of HCV-related liver disease is variable among individuals, but without effective treatment strategies, HCV-related morbidity and mentality is
expected to increase 3-fold by the year 2015 (Wong et al., 2000). Treatment of chronic HCV infection with interferon based therapies has been part of clinical practice for more than 10 years with incremental steps in improving sustained virological response (Rossi and Wright, 2003).

The current standard treatment for chronic hepatitis C is the combination of pegylated interferon (IFN) alfa and ribavirin. The efficacy endpoint of hepatitis C treatment is the "sustained virological response" (SVR), defined by the absence of detectable HCV RNA in serum as assessed by an HCV RNA assay with a lower limit of detection of 50 IU/ml or less 24 weeks after the end of treatment. (Enomoto N, et al, 2010).

1- Interferons:

Interferons are a family of proteins discovered as an antiviral agents during studies on virus interference (Isaacs and Lindenmann, 1957). They are produced spontaneously in minute amounts in the body without any specific inducers (Tovey, 1988).

Interferons are commonly grouped into two types. Type I IFNs (also known as viral IFNs) include INF-α, INF- β and INF-γ. Type II IFN is also known as immune IFN (IFN- γ). The viral IFNs are induced by virus infection, where as type II INF is induced by mitogenic or antigenic stimuli (Biron and Sen, 2001).

The INF-a genes can be divided into two groups; an immediate early response gene (IFN-a4), which is induced rapidly and without the need for ongoing protein synthesis, and a set of IFN-a genes, consisting of IFN-α2, IFN-α5, IFN~α6 and IFN-α8 that display delayed induction and are synthesized more slowly and require protein synthesis (Marie et al, 1998).

INF- β is produced by fibroblasts (Taniguichi, 1988). Whereas INF-γ is synthesized only by certain cells of the immune system including natural killer (NK) cells, CD4 Th1 cells and CD8 cytotoxic suppressor cells (Bachet et al., 1997).
Mechanism of action of interferons:

Interferon receptors:

IFNs exert their actions through cognate cell surface receptors that are largely species specific (Helm, 1999). The alpha, beta and γ-IFNs appear to have a common receptor consisting to two subunits, IFNAR-1 and IFNAR-2. Both IFNAR-1 and IFNAR-2 map of chromosome 21 in human- IFN-y receptor complex. The IFN-y ligand binding IFNGR-1 subunit and the accessory IFNGR-2 subunit map to the chromosome 6 and 21 in human (Bach et al, 1997).

IFN-mediated signaling and transcriptional activation of cellular gene expression are the best understood in the context of JAK-STAT pathway proteins (Darnell et al., 1994).

The signal transducer and activator of transcription (STAT) family of proteins are latent cytoplasmic transcription factors that become tyrosine phosphorylated by the janus family of tyrosine kinase(JAk)enzyme in response to cytokine stimulation. There are seven known members of the STAT protein family and four members of the JAK family. Different members of the JAK and STAT families have distinct functions in cytokine signaling. Receptors associated JAKs are activated following binding of IFNs to their cognate multi-subunit transmembrane I receptor of the known JAKs and STATs. The JAK-1, JAK-2, and TYK-2 kinases and the stat-1 and stat-2 transcription factors play central role in mediating IFN-dependent biological responses, including induction of the antiviral state (Darnell, 1997).

Actions of interferon

A- Antiviral effect of interferon:

The replication of wide range of viruses is inhibited by IFN-The primary step of the virus multiplication cycle inhibited typically is the synthesis of viral polypeptides (Biron and Sen, 2001). Among the IFN- I induced proteins implicated in the antiviral actions of IFNs in virus- infected
cells are PKR (RNA dependent protein kinase), 2, 5T- oligoadenylate synthetase (OAS) and RNase L, the RNA-specific adenosine deaminase (ADAR) and the MX protein GTPases (Jacobs and Langtaluul, 1996).

IFN also induces a form of nitric oxide synthase (INOS2) and the IMHC class I and II molecule all of which play important roles in immune response to infections (Samuel, 20001).

The PKR inhibits translation initiation through the phosphorylation of protein synthesis initiation factor IF-2a, several studies establish the changes in protein phosphorylation mediated by the IFN-inducible PKR play an important role in the antiviral actions of IFNs as well as the control of cell growth mediated by IFNs (Lengyel, 1993).

2-5 oligoadenylate synthetase and RNase L:

Binding of interferon to specific receptors on the cell surface induces transcription of the messenger RNA for 2-5 OAS. The level of this enzymatic activity in interferon treated cells correlates with antiviral effects of interferons (Ballonl et al, 1979). When 2-5 OAS is activated it in turn activates RNase, which cleaves both host and viral RNA. It appears however, that viral RNA is preferentially cleaved by the RNase (Vilcek and DeMaeyer, 1984).

RNA-specific Adenosine Deaminase ADAR1:

Several studies implicate the IFN-inducible ADAR1 deaminase in the process of eating of viral RNA transcripts and cellular RNAs. The biological importance of RNA editing in animal cells is significant and far ranging (Bass et al., 1997). Accumulation of ADAR1 transcripts is increased about five fold by IFN treatment (Patlerson et al., 1995). Both IFN-α and IFN-γ induce ADAR1 transcript accumulation (Patterson and Samuel, 1995).

Protein MX GTPasc:

MX proteins are GTPases that belong 10 the superfamily of dynamin-
like GTPases *(Staeheli et al., 1993).* The intrinsic GTPase activity of MX proteins is required for their antiviral activity *(Ptossi et al, 1993).*

**Inducible nitric oxide synthase:**

INOS is implicated in the function of activated macrophages as well as the pathogenesis of inflammatory and autoimmune disease *(Nathan, 1997).*

**B- Immunomodulatory effect of interferon:**

Elevation of MHC class I and II antigen levels mediated by IFN believed to increase the efficiency of cellular immune responses to infections MHC antigen levels are increased in human cells treated with IFN *(Biron and Sen, 2001).*

Treatment with both IFN-α/β and IFN-y lead to increased levels of class 1 (HLA-A, HLA-B and HLA-C) MHC molecules, whereas IFN-y is only an efficient inducer of class II (HLA-D) MHC molecules *(Boehm et al, 1997).* So, it plays a major immunomodulatory role and is a key mediator of virus-specific cellular immunity *(Karupiah et al., 1993).*

**C- Anti-proliferative effect of interferon:**

Interferons exert also a powerful effect on the growth of tumours through both direct and indirect mechanisms. They exert their direct antiproliferative effect primarily by a cytostatic mechanism that slow the growth of tumour cells by increasing the length of their multiplication cycle. This lies behind the wide spread use of interferons in the trials for treatment of malignant tumours *(Wells et al, 1985).*

**Side effects of alpha interferon therapy**

**1- Acute adverse reactions:**

The most common side effects seen with interferon alfa treatment are fever, myalgia, chills, anorexia, fatigue and sleep disturbances which are called collectively (influenza like syndrome) *(Jones and Itri, 1986).*
2- Bone marrow suppression:-

Interferon-α is a mildly myelosuppressive and decrease in peripheral blood counts occur in almost all patients (Liang and Hoffnagle, 2000). Haemoglobin decreases minimally (less than 1g/dl) and usually only after several months of treatment. In contrast, white blood cells decrease within 1 to 2 weeks of starting treatment and is a common reason for dose adjustment or early discontinuation, neutrophil counts decrease by an average of 34% (Soza et al., 2002). The platelet count decreases more slowly than the white count, reaching a nadir of 25- 40% of baseline values after 3 to 4 weeks of treatment and these decreases are usually not clinically significant and rarely require dose adjustment (Liang and Hoofnagle, 2000). No difference was observed between standard and pegylated IFN, except for haemoglobin, which fall more during pegylated IFN therapy (Sow et al., 2002).

3- Auto-immune disease:

The most common severe adverse events are thyroid disease and induction of autoimmune conditions. Between 10 and 25% of patients treated for 6 months with interferon-a develop autoantibodies, but only a small proportion of them develop a clinically apparent autoimmune disease (Lisker-Melman et al., 1992). The most common autoimmune condition induced by interferon therapy is thyroiditis. Careful monitoring shows that thyroid disturbances occur commonly before and during treatment (7 to 15%). These abnormalities are usually short-lived but they can lead to chronic thyroid hormone replacement therapy. Frank thyrotoxicosis occurs less commonly during interferon therapy but is more likely to require discontinuation of treatment (Deufsch et al, 1997), Other autoimmune conditions that can be induced by interferon therapy include haemolytic anaemia, thromboyctopenic purpura, seronegative arthritis, a systemic-lupus-like syndrome, psoriasis, diabetes and lichen planus (Dusheiko, 1997).

4- Exacerbation of liver disease:

A rare but clearly defined complication of interferon-a therapy of
hepatitis C is a paradoxical worsening of liver disease with therapy (Pnpo et al., 1992). In most instances, this has been attributed to an induction of autoimmune hepatitis in a susceptible patient. Thus, most patients who have developed this complication had autoantibodies before therapy or developed high levels of antinuclear (ANA) or liver-kidney membrane (anti-LKM) antibodies during treatment (Linng and Hoofnagle, 2000). The liver disease improves with stopping therapy in most patients, but some have required corticosteroid therapy for variable periods. Patients with chronic hepatitis C who develop worsening of liver disease on interferon (increase in ALT levels above twice the baseline values, particularly if accompanied by jaundice) should have therapy stopped and should be evaluated for the possibility of an autoimmune form of hepatitis (Liang and Hoofnagle, 2000).

5- Psychiatric effects:

The most common psychiatric side effects of interferon therapy fall into three distinct patterns, characterized by irritability, emotionality, or depression (Renault et al., 1987). These side effects usually respond to dose reduction, but are also the major reason for early discontinuation of therapy (Renault et al, 1987 and Janssen et al., 1994).

6- Uncommon severe adverse reactions:

Other severe side effects of interferon-a include seizures, acute psychosis, confusion, coma, bacterial infections (independent of its effects on peripheral neutrophil counts), pneumonitis, acute renal failure, acute congestive heart failure and visual or hearing disturbances (Liang am HFFnagle,2000)

2-Ribavirin

Ribavirin has a broad spectrum antiviral effect in vitro against both DNA and RNA viruses. The mechanism of action is not entirely clear but it may act through depletion of intracellular phosphate pools, inhibition of viral polymerase, or shifting of the cytokine profile (Ning el al., 1996). It also has immunomodulatory effects, used alone in patients with chronic HCV, it
decreases transaminase levels and hepatic histology may improve, but HCV RNA levels do not fall. Relapse occurs after withdrawal of therapy (Sherlock and Dooley, 2002) and when combined with interferon-a the antiviral effect is enhanced (McHutchinson et al., 1998).

**Side effects of ribavirin:**

1-Haemolytic anaemia:

The average decrease in haemoglobin during a 24-week course of combination therapy with interferon is 2.5 to 3 g/dl. The fall in haemoglobin starts between weeks 2 and 3 and reaches a nadir at 6 to 8 weeks of treatment (McHutchison et al., 1998). About 10% to 20% of patients shows decrease more than 4 g/dl in haemoglobin (Davis et al., 1998). If haemoglobin falls to lower than 10g/dl, the ribavirin dose should be reduced to 600 mg/d. However, further decrease of haemoglobin to lower than 8g/dl is an indication to stop ribavirin (Poynard et al., 1998). Recently, the use of recombinant human erythropoietin has been tried in the treatment of ribavirin/interferon induced anaemia in patients with hepatitis C. None of the patients on recombinant human erythropoietin stopped treatment because of anaemia. (Gargely et al., 2002).

2- Teratogenic effects:

Ribavirin has been reported to be teratogenic in rats (Johnson, 1990). Outcomes in humans who have accidental pregnancy during combination therapy indicates that total mortality is high (Maddrey, 1999)

3- Itching and Rash:

Itching with or without skin rash occurs in 15% to 20% of patients receiving ribavirin. The cause may be due to a histamine-like effect of this nucleoside analogue. Accordingly, antihistaminics can be helpful in managing this side effect (Liang and Hoofnagle, 2000).

4- Nasal congestion:

Nasal stuffiness, cough and shortness of breath occur in approximately 20%
of patients receiving interferon and ribavirin (DiBisceglie el al., 1995). Antihistaminics may be helpful in these situations, rarely dose reduction

**3-Pegylated interferons**

The use of pegylated interferons have further improved response rates, so that more than half of treated patients can expect to have a sustained response with combination therapy using peg interferon and ribavirin(DiBisceglie and Hoofnagle,2002)

Polyethylene glycols(PEG)are amplile polymers of ethylene glycol of varying average molecular weights that can be covalently attach to proteins.Modifications of proteins with PEG has resulted in increased serum half- life and reduced immunogenicity for a number of proteins(Nucci et al.,1991)

PEG-IFNa-2a is synthesized by the covalent attachment of a branched methoxy PEG molecule to IFN-a2a(Bailon et al.,1996) The size and position of the PEG molecule used in the two forms of Peg-IFN, Peg-IFN alpha-2a and Peg-IFN alpha-2b,differ significantly in their respective physical- chemical characteristics (Grace MJ, Cutler D.,2004).

. Peg-IFN-alpha-2a (40 kD) is a conjugate of recombinant interferon-alpha-2a and a 40 kD branched Peg-IFN moiety while Peg-IFN alpha-2b (12 kD) is a conjugate of recombinant interferon alpha-2b and a 12 KD branched Peg -IFN moiety . With regard to time-concentration profiles following subcutaneous administration of Peg-IFN alpha-2a 180 µg or Peg-IFN alpha-2b 1.5 µg/kg, there was a -16-fold greater exposure in the serum of patients treated with Peg- IFN alpha-2a than in those treated with Peg-IFN alpha-2b and the biological half-life of interferon activity was longer in patients receiving Peg-IFN alpha-2a than in those receiving Peg-IFN alpha-2b. Reflecting these facts, a significantly greater reduction in white blood cell and neutrophil counts is associated with Peg-IFN alpha-2a treatment. Interferon therapy can induce multiple autoantibodies and approximately 2% of patients
receiving treatment with IFN develop an autoimmune condition, such as thyroiditis, Sjogren’s syndrome, lupus-like syndrome, hemolytic anemia, and thrombocytopenic purpura. Virological responses were higher in the peginterferon (69%) than the standard interferon arm (28%). However, relapse was common when therapy was stopped, so that the SVR with peginterferon was 39%, compared with 19% with standard interferon which was still a highly statistically significant difference (Silva M, et al. 2006). Discontinuation of ribavirin is required.

**Indications for therapy of hepatitis C**

Patients with anti-HCV, HCV RNA, elevated serum aminotransferase levels, and evidence of chronic hepatitis on liver biopsy, and with no contraindications, should be offered therapy with the combination of peginterferon and ribavirin. The National Institutes of Health Consensus Development Conference Panel recommended that therapy for hepatitis C be limited to those patients who have histological evidence of progressive disease. Thus, the panel recommended that all patients with fibrosis or moderate to severe degrees of inflammation and necrosis on liver biopsy should be treated and that patients with less severe histological disease be managed on an individual basis. Patient selection should not be based on the presence or absence of symptoms, the mode of acquisition, the genotype of HCV RNA, or serum HCV RNA levels. *(Thomas, et al, 2009)*

**Selection of patients**

**Age**

A more favourable outcome is seen in young patients *(Causse et al., 1991)*. However, the physiological age (age from infection) is more important than the chronological *(Sherlock and Dooley, 2002)*.

**Sex:**

*Causse et al. (1991)* came to conclusion that females are more likely to respond to treatment where as oilier studies have failed to link female sex to
better response.

**Race.**

In analysis of the United States trials of combination therapy of previously untreated patients with chronic hepatitis C, end of treatment and sustained response rates among African-Americans were three fold lower than in caucasins. The reason for the lower response rate among African-Americans is not clear, but it may be due in part to a higher prevalence of genotype I and possibly other host factors such as duration of disease and degree of fibrosis (*McHutchison et al., 1999*).

**Clinical status:**

Symptoms in hepatitis C are often non specific and do not correlate with the severity or histological stage of liver disease (*Hoofnagle, 1997*). However, symptoms do lessen in patients who achieve a sustained loss of HCV RNA after treatment (*SheHock and Dooley, 2002*).

**HIV co-infection:**

Patients who are coinfected with HCV and HIV-1 are at increased risk for disease progression (*Sanche-Quljano et al., 1995*)

**Genotype:**

The HCV genotype should be systematically determined before treatment, as it determines the indication, the duration of treatment, the dose of ribavirin and the virological monitoring procedure. (*Enomoto N, et al, 2010*)

Improved responses are found in those patients infected with HCV genotype 2 (*Kanai et al., 1992*), or HCV genotype 2 and 3 compared with patients infected with HCV genotype 1 (*Booth et al, 1995*). In a review of 15 IFN trials sustained response was seen in 18.1% of HCV genotype 1 infected patients compared with 54.9% of patients infected with other genotypes (*Davis and Lau, 1997*). The viral genotype is of clinical importance in determining the duration of treatment as well as the difference in response
rates. Poynard et al. (1998) found that patients with genotype 2 and 3 should be treated for 24 weeks only and that continuation of therapy for 48 weeks does not increase the sustained response rate. In contrast, among patients with genotype 1, prolonging therapy to 48 weeks did appear to increase the sustained response rate.

Viral level:

The likelihood of response was higher among patients who had low initial levels of HCV RNA than among those with high levels (McHutchison, et al., 1998 and Poynard et al., 1998). Lau et al. (1993)

Viral heterogeneity:

Sequence analysis of the E2/NS1 region of HCV by analyzing multiple clones from different patients has shown that the degree of variability in this region correlates with response to IFN (Okacia et al., 1992).

Treatment of Special Patients with hepatitis C

I- Treatment of patients with hepatitis C and liver cirrhosis:

Compensated cirrhosis is defined by the absence of clinical complications of liver disease (ascites, variceal haemorrhage and encephalopathy) and presence of preserved hepatic synthetic function. Therapeutic trials in patients with cirrhosis have also required the presence of blood cell counts that are adequate to tolerate treatment (haemoglobin >11 to 12 gm/m², absolute neutrophil count >1.500/mm³, and platelets > 75.000 to 100.000 cells/mm³) (Wright, 2002).

In a large multicenter study of peginterferon alfa-2a combination therapy (Fried et al, 2002) treated 1121 patient with chronic hepatitis C using both peginterferon combination therapy and standard interferon combination therapy. In this trial, only 144 patients had stage 3 or 4 fibrosis Among these patients, 43% had an SVR with peginterferon combination therapy compared with only 33% with standard interferon combination therapy.

On the other hand (Manns et al,2001) found no clear superiority of
peginterferon over standard interferon in combination therapy with ribavirin in treating patients with advanced liver disease. Hepatitis C patients with advanced liver disease have a higher rate of adverse side effects to interferon and ribavirin combination therapy than those with mild disease. Adverse effects such as neutropenia, thrombocytopenia and anaemia lead to greater dose reductions in patients receiving peginterferon than standard interferon preparations (Heathcote et al., 2002 and Fried et al., 2002).

II- Treatment of patients with hepatitis C and normal serum aminotransferase levels:

In all studies of patients with chronic hepatitis C and normal or near normal ALT levels, SVR rates with the combination of alpha interferone and ribavirin have been similar to rates reported in patients with abnormal ALT levels (Bacon, 2002).

Although most of patients with normal ALT levels have mild disease histologically, between 1% and 29% of patients have stage 3 or 4 fibrosis on liver biopsy (Bacon, 2002).

Thus, patients with normal ALT levels cannot be completely assured that they have mild liver disease. The recent NIH consensus conference on the management of hepatitis C recommended treatment of patients with normal ALT levels in special situations like favourable genotype, presence of hepatic fibrosis, patient motivation, presence of symptoms, severity of comorbid illness and the patient’s age.

III-Treatment of acute hepatitis C:

The diagnosis of acute hepatitis C may be delayed or inaccurate because hepatitis C is often clinically mild or completely asymptomatic (Orland et al., 2001).

Pooling data from 17 studies enrolled a total of 369 treated and 201 untreated patients. Sustained loss of HCV RNA was found in 62% (37% to 100%) of interferon-treated patients compared with only 12% (0 to 20%).
Despite the multiplicity, small size and heterogeneity of these 17% studies, the pooled results strongly support the benefit of interferon therapy in reducing chronicity of acute hepatitis C (Alberti et al., 2002).

According to high SVR rates reported by these small uncontrolled trials with interferon monotherapy, the treatment of persons with acute hepatitis C is warranted, but the timing of therapy and the type of regimen to use remains to be determined from future trials (National Institutes of Health Consensus Development Conference, 2002).

IV-Treatment of hepatitis C after liver transplantation:

Recurrent hepatitis C after transplantation can be mild and may be associated with no or minimal elevations in serum aminotransferases and minimal, non-specific changes on liver biopsy. However, a proportion of patients develop recurrent disease that is progressive and severe and leads to recurrent cirrhosis and graft failure (Wright, 2002).

Patients received combination therapy with standard interferon (3Mu 3 times a week) and ribavirin (800 mg/d) for 24 weeks. Biochemical responses were observed in 27% and virological responses in 25% of patients on therapy. However, several severe adverse events occurred. Additional recent studies using combination therapy have yielded similar results with SVR rates ranging from 9% to 33% (Wright, 2002). Thus, preliminary findings indicate that responses to combination therapy with standard interferon and ribavirin are generally lower in patients after liver transplantation than in immune complete patients. The last NIH consensus conference recommended that treatment of HCV recurrence after liver transplantation should be considered experimental and carried out in the context of clinical trials.

Criteria of good respond to therapy

Responses to therapy in chronic hepatitis C can be categorized as biochemical as shown by 1- normal alanine aminotransferase (ALT) levels2- virological as shown by absence of detectable HCV RNA 3- histological as shown by improvements in liver biopsy results (Lindsay, 1997). Responses
can also be categorized by timing of the measurements of success as early during treatment (initial response), at the end of therapy (end-of-treatment response), or 6 months after therapy (sustained response). Persons with a sustained virologic response have a high probability of having a durable biochemical, virologic, and histologic response (Reichard et al., 1999).

**Contraindications to therapy**

Contraindications to peginterferon therapy include severe depression or other neuropsychiatric syndromes, active substance or alcohol abuse, autoimmune disease (such as rheumatoid arthritis, lupus erythematosus, or psoriasis) that is not well controlled, bone marrow compromise, and inability to practice birth control. Contraindications to ribavirin and thus combination therapy include marked anemia, renal dysfunction, and coronary artery or cerebrovascular disease, and inability to practice birth control. (Shiftman, et al, 2007).

**Initiation of therapy in chronic hepatitis C**

**Interferon monotherapy:**

Interferon-a has multiple biological effects, including antiviral, antiproliferative and immunomodulatory actions. These characteristics lead to the initial studies of interferon-a as treatment for chronic hepatitis C (Hoofnagle et al., 1986). Most of the treatment trials have used dose of 3 million units (Mu) of IFN three times a week for 6 months (Causse et al., 1991). Albert, et al. (1993) have shown that 6mu three times a week leads to a higher proportion of patients with normal ALT at the end of treatment compared with those treated with 3mu three times a week. Another study using 10mu three times a week suggested that sustained response rates could be as high as 50% although there is greater risk of treatment failures due to side effects (lioo et al., 1993).

Longer treatment regimen of 12 or 18 months also resulted in greater numbers of sustained responders. In one study with a three year follow up period, treatment for 48 weeks lead to a sustained biochemical response in
51.7% of patients compared with 15.4% in patients treated with the same dose for 24 weeks (Surncco et al., 1993).

**Type of interferon:**

Four forms of alfa-IFN have been evaluated in adequate numbers of HCV infected patients: alfa-2b, alfa-2a, alfa-nl, and consensus IFN (CIFN) (Booth et al., 2001). Both alfa-2b and alfa-2a are produced by recombinant DNA techniques using a strain of Escherichia coli, genetically engineered to possess plasmid containing an IFN gene from a human leucocyte. alfa-2b differs from alfa-2a by a single amino acid. IFN alfa-nl is a mixture of nine IFN subtypes produced from a human B lymphoblastoid cell line while CIFN was produced by scanning subtypes of IFN and assigning the most frequently observed amino acid at each position to form a consensus molecule (Booth et al., 2001).

**Combination therapy:**

In the American Hepatitis Interventional Therapy Group Study, 912 IFN naive patients were randomly assigned to combination therapy for 24 or 48 weeks or IFN monotherapy for 24 or 48 weeks (McHufcllnson et al., 1998). The virological response rates were 31% and 38% for the combination groups (24 and 48 weeks therapy) and 6% I and 13% for IFN monotherapy improvements in histology were seen in 57% and 61% of cases treated with combination therapy versus 44% and 41% for the monotherapy groups. The results of these randomized studies I suggest that combination therapy leads to a sustained virological response approximately 30-40% of IFN naive patients (a 2-3 fold better response when compared with IFN monotherapy) (Booth et al., 2001).

High does of interferon in combination with ribavirin were proved to be more effective than the standard regimen. In a study done on 298 previously untreated patients (Mangia et al., 2002)

**Peginterferon in combination with ribavirin:**
Due to the increased response rates to therapy observed with peginterferon compared with standard interferon, it was natural to assess the combination of the pegylated form of interferon alfa with ribavirin. Manns et al. (2001), from Europe, North America, and Australia, randomized 1530 patients with chronic hepatitis C to 1 of 3 treatment groups:

1- Standard interferon 3mu thrice weekly with ribavirin 1000 to 1200 mg daily based on body weight.

2- Peginterferon alfa-2b 1.5 ug/kg/wk with ribavirin 800mg daily for 48 weeks.

3- Peginterferon alfa-2b 1.5 ug/kg/wk for 4 weeks and then 0.5 ug/kg/wk for 44 weeks with ribavirin 1000 to 1200 mg daily based on body weight.

The rates of SVR were greatest in the group receiving higher dose of peginterferon alfa-2b with ribavirin (54%), compared with both the lower dose peginterferon (47%) and standard interferon (47%) combined with ribavirin. The pretreatment variable that correlate with SVR included HCV genotypes 2 and 3, low viral levels, lower body weight, younger age, and lesser degree of fibrosis. Patients infected with genotype 1 and having high viral levels before treatment had the lowest rate of response, with only 30% achieving SVR, even with optimal therapy. On the basis of this study, the combination of peginterferon alfa-2b/ ribavirin has been approved as therapy for hepatitis C in the United States.
Fried et al. (2002), from North America, Europe, and Asia have completed a large international trial using peginterferon alfa-2a in combination with ribavirin. A total of 1121 patients with chronic hepatitis C were randomized into 3 groups to receive:

1 - Standard interferon alfa-2b 3mu thrice weekly with ribavirin.
2 - Peginterferon alfa-2a 180 ug weekly with ribavirin.
3 - Peginterferon alfa-2a 180 ug weekly with placebo.

The dose of ribavirin was the standard regimen of 1000 to 1200 mg based on body weight, and all patients were treated for 48 weeks. End of treatment responses were 52% with standard interferon and ribavirin, 59% with peginterferon monotherapy, and 69% with peginterferon and ribavirin. Sustained virological response rates were 45% with standard interferon and ribavirin, 30% with peginterferon alone, and 58% with peginterferon in combination with ribavirin. Thus, relapse rates in the groups receiving ribavirin were 15% and 19% compared with 51% in patients receiving peginterferon alone. These results showed that combination therapy with peginterferon and ribavirin was more effective than peginterferon monotherapy, and that combination therapy using peginterferon was more effective than combination therapy using standard interferon.

Another study of peginterferon alfa-2a and ribavirin therapy reported by Hadziyannis et al. (2002) from 91 centers in Europe, North and South America, Australia, New Zealand and Asia. They used a sophisticated randomization scheme that stratified patients based on genotype and initial viral level. The investigators treated 1284 patients with 1 of 4 regimens using a constant dose of peginterferon alfa-2a but varying duration of therapy and dose of ribavirin. Patients were treated with
peginterferon alfa-2a (180 ug weekly) and either:

1- Ribavirin 800 mg daily for 24 weeks.
2- Ribavirin 1000 to 1200 mg daily for 24 weeks.
3- Ribavirin 800 mg daily for 48 weeks.
4- Ribavirin 1000 to 1200 mg daily for 48 weeks.

In patients infected with HCV genotype 1, the highest rate of response were achieved in patients treated for 48 weeks with the higher dose of ribavirin (51%). The superiority of the standard dose of ribavirin and longer course of therapy was shown both in patients with high and low initial HCV RNA levels. In patients with genotypes 2 or 3, SVR were excellent, ranging from 73% to 78% regardless of duration of therapy or ribavirin dosage.

These findings indicate that patients with genotype 2 or 3 are adequately treated with a 24-week course of peginterferon therapy and that the dose of ribavirin can be reduced to 800 mg in this group. On the other hand, patients with genotype 1 need to receive the full, standard dose of ribavirin and 48 weeks of therapy to achieve optimal response rates.

**Method of monitoring of patients Before, during and after therapy:**

If possible liver biopsy is performed. Genotype is recorded and quantitative tests for HCV RNA are done before treatment, after 3 months treatment, at the end of treatment and 6 months later (*EAS Consensus Conference on Hepatitis C, 1999*).

A full blood count is performed before treatment and at weekly intervals for 4 weeks, then every 3 months during therapy and 6 months later. All patients should be tested for thyroid function before treatment
and at regular intervals. Because of the risk of tertalogenicity with ribavirin, women of reproductive potential should have a negative pregnancy test. Also, men and women should practice safe sex or use contraceptive method during therapy and for 6 months afterwards (Sherlock and Dooley, 2002).

**Monitoring of viral levels during therapy**

Alpha interferon therapy of chronic hepatitis C is typically accompanied by a biphasic decrease in HCV RNA levels. The initial rapid decline occurs during the first 24 to 48 hours reflecting direct inhibition of intracellular HCV production and release (Neumann et al., 1998) and a second more gradual decline during the following weeks which reflects continued inhibition of replication and the gradual elimination of virus infected cells (Layden and Layden, 2001).

The rate of the second-phase decline correlates with ultimate response to interferon treatment, thus assessment of early virological response (EVR) may predict outcome (Davis, 2002).

**Retreatment of patients with chronic hepatitis C:**

Patients being considered for retreatment can be categorized into 2 groups: relapsers and non-responders (Shiffman, 2002). Relapsers are patients who become HCV RNA negative during a course of therapy, but then relapse and redevelop HCV RNA after stopping treatment. Non-responders are patients who do not become HCV RNA negative during therapy. A third pattern of response is referred to as break through, in which patients become HCV RNA negative initially and then redevelop HCV RNA while still receiving therapy. Patients with this pattern are identified only by repeat HCV RNA testing during therapy and should be considered as non-responders (Shiffman, 2002).

**Retreatment of non-responders:**

The retreatment of interferon non-responders with interferon and
ribavirin has been the subject of several publications Cheng et al. (2001) and Cummings et al. (2001). These reports represent the treatment of over 1,000 interferon non-responders, the majority of these studies utilized biochemical criteria to define non-response. The patients were retreated for either 24 or 48 weeks and followed for an additional 24 weeks after discontinuation of therapy and the dose of ribavirin varied from 600 to 1,200 mg/d. Despite variations in the inclusion criteria, study design, and the dose and duration of interferon and/or ribavirin among these studies, the results were generally consistent on average, 26% to 32% of interferon non-responders become HCV RNA negative when retreated with interferon and ribavirin. SVR ranged widely, from 0% to 21%, but in most studies, rates were 12% to 15%.

Higher response rates to retreatment were associated with HCV genotype non-1, treatment duration of 48 rather than 24 weeks, low baseline HCV RNA levels, non-black race, absence of cirrhosis, use of standard doses of ribavirin (1,000 to 1,200 mg/d), and previous partial virological responses to interferon monotherapy (Cheng et al., 2001 and Shiffman, 2001).

In another multicenter study, 212 non-responders to either interferon monotherapy or interferon and ribavirin were retreated with peginterferon alfa-2a (180 ug/wk) and the standard dose of ribavirin (1000 to 1200 mg/d). All patients included in this trial had advanced fibrosis or cirrhosis, and 88% had HCV genotype 1. Of these patients who had previously received interferon monotherapy, 34% achieved an SVR. In contrast only 11% of non-responders to interferon with ribavirin achieved an SVR. These data suggest that IFN and ribavirin non-responders are urgently in need for new strategies for treatment (Shiffman, 2002).

**Retreatment of relapsers:**

Data regarding the effectiveness of peginterferon and ribavirin for retreatment of patients who relapsed after initial treatment with standard
interferon and ribavirin are still lacking. Fifteens patients retreated with peginterferon alfa-2b at a dose of 1.5 ug/kg/wk and ribavirin 800 mg/d, 87% of them achieved an end of treatment response and 60% an SVR. Unfortunately, the actual increase in SVR with peginterferon combination over rates with standard interferon combination therapy is only 10% to 11%. For these reasons combination retreatment using peginterferon should probably be limited to the patients in greatest need for therapy like those with advanced fibrosis or cirrhosis, patients with symptoms or extrahepatic manifestations of hepatitis C those with the greatest likelihood of responding like relapses and those who had a significant decline (at least 2 log_{10} units) in serum HCV RNA levels during previous therapy (Shiffman, 2002).

It has been suggested that non responders to therapy could benefit from long-term administration of interferon, with the aim of reducing disease activity, fibrosis progression and abnormal hepatocyte proliferation, thus reducing or delaying progression to cirrhosis and development of hepatocellular carcinoma (Shiffman, 2002).

However, this is a quite controversial issue and although several retrospective studies would suggest that virological non-responders might have marginal benefit from long-term administration of interferon, this has not yet been proved by properly designed prospective studies. The use of IFN therapy as a suppressive treatment for hepatitis C should be considered experimental and cannot be recommended in clinical practice (Alberti and Benvegnu, 2003).

**Other lines for HCV treatment**

**Amantadine**

selectively inhibit replication of type A influenza viruses in vivo and vitro. Early studies suggested that site of action of amantadine was either penetration or uncoating of virus (Oxford and Galbraith,1980).
Tested the result of amantadine as a single antiviral agent and found that amantadine decreased HCV RNA values in conjunction with diminution of ALT levels. (Smith, 1997)

**Ursodeoxycholic acid**

It may have a particularly beneficial effect on the biliary component with reduction in transaminases, improve liver functions in patients with chronic hepatitis C. (Attilli et al., 1994)

**Oral enzymes**

Are fibrinolytic and anti-inflammatory agents. Also induce release of beneficial mediators required for proper control of inflammation including tumor necrosis factor (TNF), interleukin (IL-1) and (IL-6). (Dresser and Rheberger, 1990)

**Silymarin**

Several trials using it to treat chronic liver disease have been reported with decrease in symptoms, fall in aminotransferase levels and improvement in survival. (Liango and Hoofnagle, 2000)

**Future therapy of HCV**

New approaches under evaluation of HCV include small molecule inhibitors of HCV enzymes most relevant for virus replication activities (protease, helicase and polymerase), new immune modulators, new ribavirin analogues deprived of haematological toxicity, ribozymes and antisense molecules (Alberti and Benvegnu, 2003). Large molecule inhibitors of replication such as ribozymes and antisense oligonucleotides have been tested but their usefulness appears to be limited by their size and toxicities (Rossi and Wright, 2003).

Recently reported on antiviral efficacy of an oral HCV serine protease inhibitor in patients with HCV, including non-responders to IFN and/or IFN plus ribavirin standard therapies (Hinrichsen, 2002).
Platelets

Human platelets are the second most numerous corpuscles in the blood. Platelets are a nucleated cells derived from bone marrow megakaryocytes (Harrison, 2005). They are smooth, biconvex disks, 1 to 4 μm in diameter, with a role in primary hemostasis. Adequate numbers are therefore required for normal hemostasis. Once released from the marrow; platelets are sequestered in the spleen for 24 to 48 h (Robetorye and Rodgers, 2001).

The spleen can contain up to 30% of the normal circulating mass of platelets. This proportion can be significantly increased in splenomegaly, leading to thrombocytopenia (Lange and Hillis, 2005).

Normal platelet life span is 8 to 14 days, dependent upon the method by which platelet survival time is measured. Platelets are removed from the circulation by the reticulo-endothelial system on the basis of senescence rather than by random utilization. However, there is a small Fixed component to platelet turnover due to random utilization of platelets that maintain vascular integrity (George and Veseley, 2003).

Platelet formation

Platelets are formed from megakaryocytes in the bone marrow. Megakaryocytes are one of the largest cells in the body, reaching up to 50 μm in diameter, and are characterized by a large nuclear content. Each megakaryocyte produces about 1000 to 5000 platelets (Italiano and Shivdasani, 2003). In normal individuals, platelet production is
approximately 35,000 to 50,000/μl of whole blood per day; this value can be increased up to eight fold during times of increased demand. There is no reserve of platelets in the bone marrow: 80% are in circulation and 20% are in the red pulp of the spleen (Plaut, 2003).

The differentiation of bone marrow progenitor cells along the megakaryocytes lineage is regulated by the cytokine thrombopoietin (TPO), which was first identified in 1995 (Prevost et al., 2003). Thrombopoietin (TPO) is the most important growth factor in the regulation of megakaryocytes development and platelet production (Wolber and Jelkmann, 2002).

The liver is an important source of TPO production in the body. Other sites of TPO production include the kidneys and the bone marrow stromal cells. The rate of TPO production in the liver and kidneys is constant and not influenced by the circulating platelet concentration. There are mechanisms have been postulated for the regulation of blood TPO levels. One of them is a passive mechanism in which the circulating TPO level is regulated, by its binding to platelets and megakaryocytes and its subsequent degradation, inside the cells. When circulating platelets increase, more TPO molecules are taken up by the cells and degraded. The plasma TPO level falls. The reverse occurs when the platelet level decreases. The increase in TPO level is greater in thrombocytopenic states in which the bone marrow megakaryocytes are markedly decreased, as in a plastic anemia (Hou et al., 1998).

As TPO is synthesized mainly by hepatocytes so inadequate production in patients with chronic liver disease may contribute to thrombocytopenia that is rapidly reversed by the living donor allograft
Thus, if the platelet count decreases, the free level of TPO rises and there is an increase in megakaryocytopenia. TPO offers great hope as a form of treatment for patients with thrombocytopenia and is the focus of a number of ongoing clinical trials (Bhatt and Topol, 2003).

**Platelet structure and organelles**

Platelets have several unique features that enable them to efficiently perform their primary function, namely the rapid formation of a vascular plug following vessel injury. The discoid shape of the platelet is formed by the platelet cytoskeleton which consists of a spectrin-based membrane skeleton, circumferential bands of single microtubule that lies beneath the plasma membrane, and a rigid actin filament network that fills the cytoplasm of the cell (Hartwig and Italiano, 2003).

This shape and small size enables the platelets to be pushed to the edge of the vessel, placing them next to the endothelial cells and in the right place to respond to vascular damage (Watson and Harrison, 2005).

Platelets contain three main types of storage granules: α-granules, dense grannies and lysosomes, each of which rapidly releases their contents upon activation. α-Granules are the storage site of β-thromboglobulin, platelet factor (PF) 4, platelet-derived growth factor (PDGF), von willebrand factor (VWF), fibrinogen, factor V, and thrombospondin. α-Granules are the most numerous, with about 80 per platelet and contain a rich diversity of proteins and membrane receptors that support many processes in hemostasis and in host defence. Dense bodies contain ADP, ATP, calcium, and serotonin. Dense granules contain high levels of small molecules that support platelet activation and mediate vasoconstriction. Dense granules are present at a 10-fold lower level that for α-granules, with approximately
seven per platelet. Lysosomes also release their contents on activation, although the significance of this is unclear (*George, 2000 and Hiller, 2007*).

Platelets have a dense network of intracellular membranes known as the dense tubular system, which rapidly liberate their stores of the intracellular messenger Ca+2. in response to the generation of the second messenger, inositol 1,4,5-trisphosphate (IP3)(*Nieswandt and Watson, 2003*).

**Platelet functions**

1) In hemostasis

Platelets play an important role in both the formation of a primary plug as well as the coagulation cascade. The formation of a plug at the site of a cut vessel serves as the initial mechanical barrier. The lumen of the vessel is lined with endothelial cells; a break in this will initiate a series of reactions (*Ciesla, et al., 2007*).

There are four phases to platelet function:

**REACTION 1 (adhesion):** Platelets adhere to collagen and undergo shape change from disc to spiny spheres. Glycoprotein (GP) Ib and vWF aid in adhesion. This is primary aggregation, and is reversible. This reaction is mediated by the release of platelet grannies.

**REACTION 2 (aggregation):** In response to chemical changes, these events lead to platelet aggregation in which platelets adhere to other platelets. Platelet shape change occurs.

**REACTION 3 (release):** Platelets release the contents of their dense granules. The release of these granules constitutes a secondary aggregation that is irreversible. Thromboxane A2 is released by platelets, which promotes vasoconstriction. ADP amplifies the process.
REACTION 4 (stabilization of the clot): This reaction is responsible for thrombus formation. The adherent and aggregated platelets release factor V and expose platelet factor 3 to accelerate the coagulation cascade and promote activation of clotting factors and ultimately stabilize the platelet plug with a fibrin clot.

When there is a defect in any of these functions and/or platelet number, hemostasis is usually impaired and there may be an associated increased risk of bleeding, in contrast, a marked increase in platelet number or reactivity can lead to inappropriate thrombus formation. Arterial thrombi can also develop within atherosclerotic lesions resulting in stroke and myocardial infarction, two of the major causes of morbidity and mortality in the Western World (Rugeri, 2002).

2) In host defense

There is a growing body of evidence to suggest that platelets contribute significantly to antimicrobial host defense. Platelet interactions with leukocytes are proposed to be integral to defense against infections; activated platelets are a chemostatic stimuli for monocytes and neutrophils and participate in recruiting these cells to the site of infection (Drake et al., 1992).

Platelets also potentiate cell-mediated effector cell antimicrobial function. The antimicrobial activity of platelets is believed to be mediated by platelet microbial proteins (PMPs) and thrombin-stimulated PMPs (Yeaman et al., 1993). These molecules have been shown to disrupt microbial cytoplasmic membranes and exert microbicidal activities in vitro against a number of pathogens, including Candida albicans and cryptococcus (Yeaman, 1997). Although platelets interact with viruses, their role in
antiviral host defense has not been established.

3) **In inflammation**

Platelets play a critical role in acute and chronic inflammation. Major advances have been achieved in the understanding of the molecular mechanisms involved in platelet interaction with inflammatory cells (Ruggeri, 2002). The interaction of platelets with leukocytes and endothelial cells results in substantial activation of these cell types that changes their chemotactic, adhesive and proteolytic properties significantly (Nieswandt and Watson, et al, 2003).

Specific cell adhesion receptors such as selectins, immunoglobulin-type receptors, and integrins are involved in regulation of platelet adhesion with inflammatory cells (Ruggeri, et al, 2000). Thereafter, release of platelet-dependent proinflammatory mediators substantially triggers the inflammatory response in the microenvironment of the site of platelet adhesion. Thus, platelet adhesion and subsequent release reaction are critical mechanisms involved in platelet-mediated inflammation and might represent targets for future therapeutic strategies in a variety of inflammatory diseases (Hood and Cheresh, 2002).
Thrombocytopenia

Thrombocytopenia is an abnormally low platelet count of less than 150,000/mm³. It is the most common cause of hemorrhagic disorders. It may be congenital or acquired; the acquired form is more common. Because platelets are needed for coagulation, this disease poses a serious threat to hemostasis (Arnold et al., 2001).

Possible causes of thrombocytopenia include: Decreased or detective platelet production in the bone marrow (as in leukemia, a plastic anemia, or drug toxicity), increased platelet destruction outside the marrow due to an underlying disorder (such as cirrhosis of the liver, I disseminated intravascular coagulation, or severe infection), sequestration (increased amount of blood in a limited vascular area, such as the spleen) and Blood loss (Ogle, 2001).

Pathophysiology

in thrombocytopenia, lack of platelets can cause inadequate, hemostasis. Four mechanisms are responsible: decreased platelet production, decreased platelet survival, pooling of blood in the spleen, and intravascular dilution of circulating platelets. Platelet production decreases when the number of megakaryocytes is reduced or when platelet production becomes dysfunctional (Cairns et al., 1998).

Clinical manifestations

Clinical manifestations are mild; often limited to easy bruising. Below 10,000/µL, the risk of spontaneous mucocutaneous bleeding (gingival bleed, epistaxis, menorrhagia, petechiae and ecchymoses) and life threatening,
spontaneous intracranial hemorrhage or gastrointestinal bleeding increases rapidly (Cines, 2000).

**Diagnostic approach**

Asymptomatic patient with a low platelet count, the clinician should initially seek to exclude artifactual or “pseudothrombocytopenia” as the etiology. This is caused by in vitro clumping of platelets when ethylenediaminetetraacetic acid (EDTA) is used as an anticoagulant. The presence of platelet clumps on examination of the peripheral blood smear and normal repeat platelet count using citrated blood confirms pseudothrombocytopenia as the cause.

If thrombocytopenia is confirmed, a stepwise evaluation should be undertaken to assess the causes and the urgency of treatment. If diagnoses such as thrombotic thrombocytopenic purpura (TTP) or heparin-induced thrombocytopenia (HIT) are suspected, immediate intervention is required. In addition, any patient with severe thrombocytopenia or evidence of hemorrhage should receive immediate attention and possible platelet transfusion.

A detailed comprehensive history can provide valuable diagnostic information, physical examination; a diligent neurologic examination should indicate the need for imaging in patients with a suspected intracranial bleed and the presence of lymphadenopathy and splenomegally can also provide clues to the diagnosis.

Initial laboratory evaluation should include a peripheral blood smear, serum creatinine, DIC panel, LDH, total and direct bilirubin, AST and ALT. This allows an initial determination of whether thrombocytopenia is an isolated
abnormality or part of a constellation of abnormalities that may suggest a specific diagnosis. If hemolysis is suspected then a direct antiglobulin test (Coombs test), reticuloeyte count and haptoglobin should be checked. The presence of schistocytes on the smear is suggestive of TTP or D1C. Differentiation is usually made by the presence of normal coagulation parameters in TTP and elevated PT, PTT, fibrin split products; and low fibrinogen in DIC.

An HIV test is indicated in patients with any risk factors. Vitamin B12 and folate levels can help diagnose nutritional causes of thrombocytopenia. Suspicion of connective tissue disorders should lead to appropriate seroiogie testing (Seklion and Roy, 2006).

Role of Bone Marrow Exam:

A bone marrow biopsy may help differentiate inadequate production versus excessive destruction/consumption as the predominant cause of thromboeytopenia. In general, a bone marrow biopsy is indicated when a platelet production problem is suspected to help delineate the cause of underproduction. If peripheral destruction/sequestration is suspected a bone marrow biopsy is unlikely to provide useful additional information. However, in elderly patients, a bone marrow examination can edicidate the presence of a primary marrow disorder, such as myelodysplasia, leukemia or lymphoma. The incidence of these conditions generally rises with age (Baldwin, 2003).
Relation between liver and platelet

The liver and platelets display a very intimate interconnection. A number of proteins which induce opposing effects on liver regeneration are present in platelets. For instance, platelets harbour important growth factors for execution of liver regeneration, although platelets contain TGF-β which is required for termination of liver regeneration. Thus, platelets may participate in orchestrating liver regeneration through harmonized stimulation and inhibition of growth-related signals (Clavien, 2008). Until 2006 it was unclear whether platelets are promoters, inhibitors of, or not even active contributors to liver regeneration. Many in vitro studies demonstrated that platelets contain several growth factors which may theoretically contribute to the process of liver regeneration (Rozman and Bolta, 2007). However, the only in vivo study on the role of platelets in liver regeneration in rats failed to identify a correlation between platelets and liver regeneration. In a study on regeneration of liver in rats it was noted that splenectomy increases platelet counts and accelerates liver regeneration via an unclear mechanism (Tomikawa et al., 1996). In another study thrombocytotic mice exhibited increased liver regeneration while a thrombocytopenic group showed impaired regeneration. Matsuo et al., (2008) reported that direct contact between platelets and hepatocytes is necessary for the proliferative effect.

Platelets contain many growth factors, such as platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and tissue growth factor (TGF) -β that are required for tissue regeneration (Matsuo et al., 2008).
**Thrombocytopenia in chronic liver disease**

Thrombocytopenia is one of the most common hematological abnormalities in patients with chronic liver disease. Its prevalence and severity increase with increasing hepatocellular damage (Giannini, 2006), and is considered an indicator of advanced disease (Bashour et al, 2000).

Patients with chronic liver disease due to infection with the hepatitis C virus (HCV) who have thrombocytopenia (<75,000 platelets per cubic millimeter) have been routinely excluded from clinical trials of interferon and ribavirin and few published reports have described the treatment of chronic HCV infection in patients with platelet counts of less than 50,000 per cubic millimeter (Shiffman et al, 2007).

Severe thrombocytopenia could be a life-threatening side effect of interferon therapy if patients are not adequately diagnosed and treated. In almost all patients administered interferon, the platelet count typically decreases by 30% to 50%, but a decrease of more than 90% is quite rare (Hoofnagle JH,1991).

Thrombocytopenia is common in chronic liver disease, although thrombocytosis may arise when liver disease occurs with one or more known causes of “reactive” increases in platelet production (e.g., acute bleeding, iron deficiency, bacterial infection, or malignancy), asplenia, or hyposplenia (Peck-Radosavljevic et al 2000).

**Incidence of thrombocytopenia**

Patients with advanced chronic liver disease frequently experience thromboeytopenia with increasing incidence correlating with severity of liver disease. In cirrhotic patients, thromboeytopenia occurs in up to 64% of
patients and is independent of the cause of cirrhosis (Wang et al. 2004).
Patients with more advanced end-stage disease tend to have a higher degree of thrombocytopenia than those with compensated chronic liver disease (Giannini et al., 2005).

Platelet counts of less than 150,000 /µL are more frequently observed in patients with cirrhosis compared to patients without cirrhosis (64% vs. 26%, respectively); approximately one-quarter to one-half of cirrhotic patients have counts <100,000/µL. Platelet counts <50,000/µL occur in approximately 1% of patients with chronic HCV infection (Bashour et al., 2000).

Pathophysiology

The pathophysiology of thrombocytopenia in patients with chronic liver disease is not completely understood. However, it is universally agreed that multiple pathogenetic mechanisms that are complementary and often act in concert are involved. Thrombocytopenia in these patients is often attributed to splenic sequestration (hypersplenism), but may also occur as a result of platelet destruction mediated by platelet associated immunoglobulin (PAIgG) (Sanjo et al., 2003) and impaired hepatic synthesis and/or increased degradation of thrombopoietin (TPO) by platelets sequestered in the congested spleen (Rios et al., 2005).

Severe thrombocytopenia can occur as a consequence of a plastic anemia, a rare complication of acute hepatitis (Zeldis et al., 1983). Idiopathic immune thrombocytopenia (ITP) has also been reported in patients with hepatitis C (Narita et al., 2003). Cocaine may cause thrombotic thrombocytopenic purpura associated with toxic acute hepatitis (Balaguer et al., 2005). Ethanol directly suppresses platelet Formation and
decreases the life span of platelets, both of which contribute to thrombocytopenia commonly seen with alcohol-related liver disease (Scharf and Aul, 1988).

Other etiologies including medications, folate and vitamin B12 deficiencies, severe infections and DIG should also be considered in evaluating thromboeytopenia in patients with liver disease within the appropriate clinical setting.

Factors affecting the pathogenesis of thrombocytopenia in patients with chronic liver disease:

1. Disease-related factors
   - Hypersplenism.
   - Bone marrow suppression.
   - Immune dysfunction.
   - Decreased thrombopoietin levels/activity.

2. Treatment-related factors
   - Anti-viral therapy (Dienstag and McHutchison, 2006).

1. Disease-related factors

Hypersplenism

Hypersplenism was the first mechanism proposed for the pathogenesis of thrombocytopenia in chronic liver disease. Many patients with advanced liver disease (especially cirrhotic patients) develop portal hypertension that results in an enlarged spleen and subsequent platelet sequestration. The increase in resistance to portal blood flow (i.e. increased
portal pressure) causes redistribution of blood to the spleen subsequent pooling of platelets, and the increased clearance of platelets from the circulation (Weksler, 2007).

Splenomegaly may reduce peripheral platelet count through two routes: (i) a shift from the peripheral platelet pool to the splenic pool would occur, and (ii) because of massive portal hypertension and hemostasis, peripheral platelets would be removed in the splenic flow bed. In fact, non-viral liver disease was associated with lower platelet counts when the spleen size was enlarged, but a direct impact of splenostasis on platelet count is uncertain (Banks, 2000).

Thus, portal hypertension and hypersplenism probably play a role in the development of thrombocytopenia in combination with other pathogenic factors (Goulis et al., 1999).

**Bone marrow suppression**

Underproduction of platelets secondary to bone marrow suppression caused by HCV infection and other viral infections [e.g. varicella, Epstein-Barr virus (EBV), human immunodeficiency virus (HIV) and parvovirus] is thought to be a contributing factor in the development of thrombocytopenia (Drews, 2003).

Also, the decrease in HCV viral load following IFN-a treatment correlates with significant increases in platelet count in the absence of hypersplenism or serological evidence of an lo immunity (platelet autoantibodies) (Iga et al., 2005). In addition to the suppressive effects of HCV on the bone marrow, excessive consumption of alcohol by chronic liver disease patients can Slave direct toxic effects on megakaryocytes
resulting in decreased platelet production and ineffective thrombopoiesis (Ballard, 1989).

**Immune dysfunction**

In patients with HCV-related liver disease; autoantibodies directed against platelet surface antigens can promote platelet sequestration and destruction by cells of the reticuloendothelial system in the spleen and liver, as observed in patients with chronic immune thrombocytopenic purpura (ITP) (Sandler, 2004).

64% of patients with chronic liver disease of diverse aetiology were found to have platelet-associated antiglycoprotein (GP) antibodies, primarily against the GPIb-IX complex, either alone or in combination with anti-GPIIb-IIIa antibodies. It has been suggested that the binding of HCV to platelets may induce the development of neoantigens on the platelet surface, or alter the conformation of platelet membrane GPs, thereby contributing to autoantibody formation against target platelet GPs (Panzer and Seel, 2003).

Immune-complex associated platelet clearance and reticuloendothelial destruction have also been proposed to contribute to thrombocytopenia in patients with chronic viral liver disease (Doi et al., 2002).

Elevated titers of platelet associated immunoglobulin G (PAIgG), which could represent immune complex-coated platelets, have been detected in as many as 88% of patients with chronic HCV infection. PAIgG levels have been shown to increase gradually as the severity of liver disease increases; suggesting that prolonged HCV infection causes marked immune system abnormalities (Nagamine et al., 1996).

An inverse correlation between PAIgG and platelet count has also
been documented in study populations completely or predominantly composed of patients with HCV-related chronic liver disease (Sanjo et al., 2003). ITP develops in patients with HCV infection more frequently than in healthy persons; one study documented the incidence of ITP to be 1:500 in patients with HCV infection (Pockros et al., 2002).

Chronic HCV infection has been associated with the development of thrombocytopenia. Thrombocytopenia may be present even in the absence of clinically evident liver disease or splenomegaly and may be mistakenly diagnosed as primary CITP (Rajan et al, 2005)

Treatment with corticosteroids should be avoided as long as possible, because it can increase the viral load and worsen liver damage. If possible, treatment should be directed at the HCV infection. In patients without clinically evident liver disease, treatment with IFN- combination therapy should be considered. (Rajan et al, 2005)

**Decreased thrombopoietin levels or activity**

Thrombopoietin (TPO), also known as c-Mpl ligand, is the primary cytokine governing megakaryocyte maturation and platelet formation (Kaushansky, 1998).

TPO is produced mainly by hepatocytes, and is normally released at a constant rate into the circulation. Under normal conditions, if platelet production decreases, the circulating platelet count subsequently falls, less TPO is bound to platelets, and consequently the plasma TPO concentration increases. As a result, megakaryocytopoiesis increases to restore platelet homeostasis, resulting in more platelets produced and released. Once the platelet count increases, excess TPO is bound by circulating platelets, and
TPO levels decrease to normal levels (Jelkmann, 2001).

However, in patients with extensive liver cirrhosis and/or fibrosis and subsequent reduction in functioning hepatocytes (clinically manifested as severe impairment of liver function), production of TPO can be reduced (Peck-Radosavljevic et al., 2000).

Increased degradation of TPO, mediated by the binding of TPO to platelets sequestered in the enlarged spleen, may also contribute to thrombocytopenia in patients with cirrhosis. Serum TPO levels and platelet counts can increase significantly after partial splenic embolization and the normal physiological relationship between TPO and platelet count has been restored following this procedure (Rios et al., 2005).

Serum TPO levels have been variously reported to be low, normal or elevated, in the presence of thrombocytopenia (Sanjo et al., 2003). Patients with high grade (grades 3-4) liver fibrosis have significantly lower TPO levels than patients with less severe fibrosis (liver fibrosis grades 0-2), reflecting decreased production of the TPO by the damaged liver (Adinolfi et al., 2001).

2. Treatment-related factors

Anti-viral therapy

Development of thrombocytopenia is a well-known side effect of PEG-IFN, which together with ribavirin is the current treatment of choice for hepatitis C (Dienstag and McHutchison, 2006)

Several studies have suggested that IFN-α induces thrombocytopenia by inhibiting the proliferation of human megakaryocytes, as the number of
colony-forming units of megakaryocytes (CFU-MK) is reduced by relatively high concentrations of IFN-α in vitro. (Wang Q, et al 2000)

In clinical studies, however, the administration of human IFN-α to patients with chronic hepatitis, solid tumors, and myeloproliferative disorders does not affect the number of megakaryocytes in bone marrow. (Kreft A et al 2000)

We speculate that this discrepancy is caused by differences in the dose of IFN-α and hematopoietic microenvironment between CFU-MK in vitro assays and in vivo studies. Clinical studies have also proposed autoimmune reaction and capillary sequestration as causes of IFN-α–induced thrombocytopenia. (Dormann H et al 2000)

Megakaryocytes differentiate from hematopoietic stem cells and undergo endomitosis followed by cytoplasmic maturation. (Sekkai D, et al 2007)

IFN-a treatment may also suppress the production or secretion of TPO (Peck-Radosavljevic et al., 1998)

Treatment with PEG-IFN has been shown to reduce platelet counts by up to 33% (Homoncik et al., 2005).

Concomitant ribavirin therapy appears to have a protective effect against IFN-induced reductions in platelet count (Sulkowski, 2005). Because a less pronounced decrease in platelet count has been observed during combination therapy with IFN plus ribavirin compared with IFN alone (Poynard et al., 1998). The size and position of the PEG molecule used in the two forms of Peg-
IFN, Peg-IFN alpha-2a and Peg-IFN alpha-2b, differ significantly in their respective physical-chemical characteristics (Grace et al, 2004).

Peg-IFN-alpha-2a (40 kD) is a conjugate of recombinant interferon-alpha-2a and a 40 kD branched Peg moiety while Peg-IFN alpha-2b (12 kD) is a conjugate of recombinant interferon alpha-2b and a 12 KD branched Peg moiety. With regard to time-concentration profiles following subcutaneous administration of Peg-IFN alpha-2a 180 µg or Peg-IFN alpha-2b 1.5 µg/kg, there was a -16-fold greater exposure in the serum of patients treated with Peg-IFN alpha-2a than in those treated with Peg-IFN alpha-2b and the biological half-life of interferon activity was longer in patients receiving Peg-IFN alpha-2a than in those receiving Peg-IFN alpha-2b (Silva et al, 2006).

Reflecting these facts, a significantly greater reduction in white blood cell and neutrophil counts is associated with Peg-IFN alpha-2a treatment. Interferon therapy can induce multiple autoantibodies and approximately 2% of patients receiving chronic hepatitis treatment with IFN develop an autoimmune condition, such as thyroiditis, Sjogren’s syndrome, lupus-like syndrome, hemolytic anemia, and thrombocytopenic purpura. Although an autoimmune mechanism that induces thrombocytopenic purpura with peripheral consumption of platelets is speculated, the precise mechanism of IFN-induced autoimmune thrombocytopenia in this patient, it is very interesting that although severe thrombocytopenia did not occur during 48 weeks of Peg-IFN alpha-2b plus ribavirin therapy, it occurred after a single subcutaneous administration of Peg-IFN alpha-2a. The thrombocytopenia seen in this patient was likely autoimmune, as suggested by the lack of spontaneous recovery after IFN withdrawal and the rapid
response to prednisolone therapy. It is not clear whether prior administration of Peg-IFN for 48 weeks played any role in the subsequent severe thrombocytopenia induced by a single administration of Peg-IFN alpha-2a. However, it can be speculated that exposure over a longer time, due to the longer biological half life, and higher concentration of Peg-IFN alpha-2a would have contributed to the development of severe thrombocytopenia. Treatment of thrombocytopenia induced by IFN therapy by steroids and intravenous immunoglobulin are reported to be effective. In addition, the effectiveness of rituximab, an anti-CD20 humanized monoclonal antibody has been also reported (Weitz IC, 2005)

Some lines of evidence have indicated that eradication of H. pylori could increase the platelet count in ITP patients pathogenesis remain unknown. Therefore, eradication of H. pylori in addition to steroid and intravenous immunoglobulin therapy could be considered to have contributed to recovery. (Stasi R et al, 2009)

This prompted us to undertake this study, which investigates the mechanism of human IFN-α–induced thrombocytopenia. Thrombopoietin (TPO) is a lineage-specific cytokine that regulates both proliferation and differentiation of megakaryocytes (Kaushansky K, et al, 1995).

The receptor for TPO is c-Mpl and c-mpl–deficient mice demonstrated severe thrombocytopenia, with an 85% reduction in the number of platelets and megakaryocytes (Gurney et al, 1994)

Although several clinical trials of TPO have demonstrated that recombinant human (rh) TPO and pegylated recombinant human megakaryocyte growth and development factor (PEG-rhMGDF) are
effective for treating thrombocytopenia associated with nonmyeloablative chemotherapy, some individuals treated with PEG-rhMGDF have demonstrated thrombocytopenia because of the development of neutralizing antibodies to endogenous TPO. (Li, et al, 2001)

Recently, second-generation of thrombopoietic growth factors such as TPO peptide (AMG 531) and TPO nonpeptide mimetics (eltrombopag, AKR501) have been shown to increase platelet counts in healthy volunteers and patients with chronic idiopathic thrombocytopenic purpura. (Kuter DJ, 2007)

In a recent Phase II study, eltrombopag, an oral thrombopoietin receptor agonist, increased platelet counts in the majority of patients with cirrhosis associated with HCV infection to >100 x 10^9/L and allowed for effective IFN-based therapy. Thrombopoietin receptor agonists may prove effective in preventing IFN-suppression of platelet production, therefore, preventing treatment interruption and IFN-dose reduction that can result in a reduction in the efficacy of antiviral therapy. Improvements in platelet count should parallel a reciprocal suppression of viral load and eradication of HCV infection should result in remission of the thrombocytopenia. (Hutchison JG et al, 2007)

The use of Urodoxycholic Acid in autoimmune thrombocytopenia that has developed due to PEG-IFN treatment in chronic HCV infection is a favorable option (Koike et al, 2006) reported two cases in whom liver dysfunction with immune thrombocytopenia was detected and whose platelet counts increased after UDCA treatment was initiated. They suggested that UDCA treatment was effective in these cases by decreasing
immunoglobulin production in B lymphocytes and cytokine production in T cells. (Koike M et al, 2006)

Functional platelet defects in chronic liver disease

Defective platelet function may be a result of an acquired storage pool defect, defective transmembrane signal transduction, decreased levels of arachidonic acid required for thromboxane A2 production in the membrane, decreased levels of functional platelet receptors as a result of proteolysis by plasmin the presence of abnormal high-density lipoprotein, or a reduced hematocrit (Escolar et al., 1999).

One important compensatory mechanism is the substantially elevated level of von Willebrand factor (vWF) in patients with liver disease. Because of the high levels of vWF, the overall primary haemostatic function in patients with liver disease appears less disturbed than what would be expected on the basis of the platelet count and functional defects as observed in diagnostic tests. It has been demonstrated that vWF plasma levels can be elevated more than 10-fold in patients with cirrhosis. Despite subtle functional defects, these extremely high plasma levels support platelet adhesion under conditions better than plasma from healthy volunteers, thus compensating for reduced platelet numbers and function (Lisman et al., 2006).

Complications of thrombocytopenia in chronic liver disease

Severe thrombocytopenia is associated with life-threatening complications that occur in end-stage liver disease. The presence of moderate thrombocytopenia (platelet count of 50,000 - 75,000 / μL) can
increase the risk of bleeding during invasive diagnostic procedures such as liver biopsy, particularly in patients with coexistent coagulopathy (Sharma et al., 1982), and can limit treatment of chronic hepatitis C virus (HCV) infection with pegylated interferon (PEG-IFN) (Dienstag and McHutchison, 2006).

Furthermore, in patients with cirrhosis, severe thrombocytopenia has been identified as an independent risk factor for developing complications of cirrhosis (variceal bleeding) and death (Liangpunsakul et al., 2003).

**Impact of thrombocytopenia on clinical management**

The inability to initiate or maintain planned treatments because of low platelet counts may lead to increased morbidity or mortality, and can significantly impact patient care. Initiation of anti-viral therapy is generally contraindicated when platelet counts are below 75,000-100,000 /µL although actual clinical practice may vary (Poordad, 2007).

Patients with chronic liver disease frequently undergo medical procedures for diagnosis and treatment, some of which are invasive. Thrombocytopenia can complicate or postpone routine care by increasing the risk of bleeding from such procedures. These procedures include liver biopsies (percutaneous, laparoscopic and transjugular) (Friedman, 2004), banding; paracentesis for ascites; liver transplantation (Lin et al., 2005), central line insertion; endoscopy; prostate biopsy and elective surgery. Some studies have found no increase in the risk of bleeding in patients with platelet counts above 50,000 / µL undergoing these procedures (Ito et al., 2005). The risk of bleeding complications can cause postponement of necessary procedures and therapy, interfere with planned medical care, significantly add to healthcare costs in these patients.
Patients and methods

This is a retrospective study included 1000 patients with chronic hepatitis C virus infection who were treated by interferon and ribavirin in Liver Research Centre, Tanta fever hospital during the period from June 2007 to June 2009, the data of all patients were collected from their files and statistically analyzed.

Inclusion criteria:

1. Patients confirmed to have chronic hepatitis C infection (positive serological test for HCV antibodies and HCV RNA)

2. Both male and non pregnant female.

3. Only adult patients (older than 18 years)

4. Patients whose their primary and pretherapy data was completed.

Exclusion criteria:

1. Other causes of chronic liver disease (by liver biopsy)

2. Children and patients younger than 18 years

3. Alcoholic intake (current or previous history of intake)

4. Pregnant or breast feeding (by history)

5. Drug related liver disease.

6. Decompensated liver disease or impaired liver functions (clinical, examination and lab investigation)

7. Coinfection with hepatitis B (HBsAG and HBeAG)
8-autoimmune liver disease.(liver biopsy)

9-heimochromatosis,Wilson and alpha 1-antitrypsin defiency.(liver biopsy).

Data of 1000 patients were collected from their files included :-

1-Age ,gender and body weight of all patients.

2-physical finding suggesting decompensated liver disease( jaundice, ascites,flapping tremors,lower limb oedema, heptomegally or splenomegaly )

3-laboratory investigations :-

- Complete blood count before and during the therapy includig haeamoglobin, red blood cell count ,white blood cell count ,platelet count and erythrocyte sedimentation rate .
- Liver functions test(ALT, AST , alkaline phosphates ,prothrombin time,total bilirubin and serum albumin)
- serum HCV RNA by PCR
- HepatitisC virus antibody by ELIZA.
- Hepatitis B surface antigen be ELIZA.
- Parasitic liver diseases(stool analysis and anti-schitosomal antibodies)
- ANA
- Interferon dose and duration
- Ribavirin dose and duration
- Pregnant test for women at child-bearing period.

4- abdominal ultrasonography for assessment liver, spleen, ascites and portal hypertension.

5- liver biopsies (percutaneous biopsy )of all patients for assessment of grade of liver inflammation and stage of liver fibrosis in chronic hepatitis c

Table No.(4) Necroinflammatory score (Ishak et al., 1995).
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<thead>
<tr>
<th>Change</th>
<th>Score</th>
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<tr>
<td><strong>Periportal or periseptal interface hepatitis</strong></td>
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<tr>
<td>(piecemeal necrosis)</td>
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<tr>
<td>Absent</td>
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</tr>
<tr>
<td>Mild (focal, few portal areas)</td>
<td>1</td>
</tr>
<tr>
<td>Mild/ moderate (focal, most portal areas)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate (continuous around &lt; 50% of tracts or septa)</td>
<td>3</td>
</tr>
<tr>
<td>Severe (continuous around &gt; 50% of tracts or septa)</td>
<td>4</td>
</tr>
<tr>
<td><strong>Confluent necrosis</strong></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Focal confluent necrosis</td>
<td>1</td>
</tr>
<tr>
<td>Zone 3 necrosis in some areas</td>
<td>2</td>
</tr>
<tr>
<td>Zone 3 necrosis in most areas</td>
<td>3</td>
</tr>
<tr>
<td>Zone necrosis + occasional portal-central</td>
<td></td>
</tr>
<tr>
<td>(P-C) bridging</td>
<td>4</td>
</tr>
<tr>
<td>Zone 3 necrosis + multiple (P-C) bridging</td>
<td>5</td>
</tr>
<tr>
<td>Panacinar or multiacinar necrosis</td>
<td>6</td>
</tr>
<tr>
<td><strong>Focal (spotty) lytic necrosis, apoptosis and focal inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>One focus or less per 10 x objective</td>
<td>1</td>
</tr>
</tbody>
</table>
The field of view when using the 10x objective (100x total magnification) is 2 mm. If 8 plant cells extend across the field of view (2 mm), then each cell is 2/8 or 0.25 mm long.

**Staging of chronic hepatitis:**

Table No.(5) Architectural changes, fibrosis and cirrhosis (*Ishak et al.*, (1995)).

<table>
<thead>
<tr>
<th>Change</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Fibrosis</td>
<td>0</td>
</tr>
<tr>
<td>Fibrous expansion of some portal areas, with or without short fibrous septa</td>
<td>1</td>
</tr>
<tr>
<td>Fibrous expansion of most portal areas, with or without short fibrous septa</td>
<td>2</td>
</tr>
<tr>
<td>Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging</td>
<td>3</td>
</tr>
<tr>
<td>Fibrous expansion of portal areas with marked bridging (portal to portal (P-P) as</td>
<td></td>
</tr>
</tbody>
</table>
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis) | 4
Cirrhosis, probable or definite | 5
| 6
Maximum possible score | 6

## Statistical analysis

Descriptive: no, %, mean value and SD.

No=number

%=percentage

Mean value(X) The sum of observations divided by the number of observation.

\[
(X) = \frac{\Sigma x}{n}
\]

Where \(\Sigma\) = sum & n = number of observation

Standard Deviation (SD):

It measures the degree of scatter of individual varieties around their mean

\[
SD = \sqrt{\frac{\Sigma (X-X)^2}{N-1}} \quad (Davies, 1993)
\]

Analytical: This included,

A) One-way ANOVA (F test)
It is a parametric test used to indicate the presence of any significant difference between several groups for a normally distributed quantitative variable. (Blair, 1981)

B) Kruskal-Wallis test

It is the non-parametric version of ANOVA for not normally distributed interval dependent variable. (Kruskal and Wallis, 1952)

C) Wilcoxon signed rank test

The Wilcoxon signed rank sum test is the non-parametric version of a paired samples t-test. It is used when you have two related observations (i.e., two observations per subject) and you want to see if the means on these two not normally distributed interval variables differ from one another. (Fagerland, 2009)

D) Student's t-test

It is a single test used to collectively indicate the presence of any significant difference between two groups for a normally distributed quantitative variable. (Zimmerman, 1997)

E) Mann Whitney Test

It is a parameteric test of student's test for not normal disturbed between two group. (Herrnstein, 1976)

F) Chi-Squared ($\chi^2$)
It is used to compare between two or more qualitative variable.. (Mood, 1974)

G) P value

P < 0.05 indicates significant difference

P < 0.01 indicates high significant difference

P > 0.05 indicates insignificant difference

P < 0.001 indicates very high significant differences (Schervish, 1996).
Results

Table (8): descriptive data of the studied patients before start of therapy.

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Mean ±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>534732.9 ±107760.5</td>
<td>1450-1500000</td>
</tr>
<tr>
<td>Prothrombin(sec.)</td>
<td>83.5 ± 9.3</td>
<td>60-100</td>
</tr>
<tr>
<td>Albumin (gm/dL)</td>
<td>4.4 ± 0.68</td>
<td>3.1-5.4</td>
</tr>
<tr>
<td>Billirubin (mg/dL)</td>
<td>0.88 ± 0.31</td>
<td>0.19-1.92</td>
</tr>
</tbody>
</table>

Total number =1000
PCR=polymerase chain reaction of HCV RNA

Table (9): descriptive data of the studied patients before and during the therapy:

<table>
<thead>
<tr>
<th></th>
<th>Baseline Mean ±SD</th>
<th>Week 4 Mean ±SD</th>
<th>Week 12 Mean ±SD</th>
<th>Week 24 Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs(1000/mm³)</td>
<td>5884.9 ±2191.8</td>
<td>4260.2 ± 1454.2</td>
<td>3791.0± 1424.4</td>
<td>3629.5± 1279.9</td>
</tr>
<tr>
<td>Platelet</td>
<td>195.6 ±60.2X10³</td>
<td>170.5 ± 81.6 X10³</td>
<td>164.6± 82.4 X10³</td>
<td>164.8± 63.4 X10³</td>
</tr>
<tr>
<td>AST</td>
<td>50.3 ±87.6</td>
<td>38.4 ± 29.2</td>
<td>41.5± 40.1</td>
<td>39.5± 43.3</td>
</tr>
<tr>
<td>ALT</td>
<td>50.2 ±40.2</td>
<td>38.2 ± 28.1</td>
<td>40.2± 36.3</td>
<td>35.8± 37.1</td>
</tr>
</tbody>
</table>

WBCs=White blood cells
AST=Aspartate aminotransferase
ALT=Alanine aminotransferase
Fig 8: Mean value of platelets at different time sequence among the studied patients

Table (10): The response of patients to the therapy:

<table>
<thead>
<tr>
<th>Status</th>
<th>Male (n=750)</th>
<th>Female (n=250)</th>
<th>Total (n=1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Responder</td>
<td>380 (50.7%)</td>
<td>145 (58.0%)</td>
<td>525 (52.5%)</td>
</tr>
<tr>
<td>Resistant (12 w)</td>
<td>110 (14.7%)</td>
<td>25 (10.0%)</td>
<td>135 (13.5%)</td>
</tr>
<tr>
<td>Resistant (24 w)</td>
<td>115 (15.3%)</td>
<td>40 (16.0%)</td>
<td>155 (15.5%)</td>
</tr>
<tr>
<td>Missed</td>
<td>145 (19.3%)</td>
<td>40 (16.0%)</td>
<td>185 (18.5%)</td>
</tr>
</tbody>
</table>

no=number
%=percentage
Table (11): Comparison between the classified groups regarding the measured Platelets at different time sequence:

<table>
<thead>
<tr>
<th>Platelet</th>
<th>Responder Mean ±SD (n=525)</th>
<th>Resist 12 Mean ±SD (n=135)</th>
<th>Resist 24 Mean ±SD (n=155)</th>
<th>Kruskal-Wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>197.5 ± 65.9 x 10^3</td>
<td>195.9 ± 44.6 x 10^3</td>
<td>199.9 ± 56.9 x 10^3</td>
<td>0.95</td>
<td>0.62</td>
</tr>
<tr>
<td>W4</td>
<td>165.2 ± 58.8 x 10^3</td>
<td>180.1 ± 58.4 x 10^3</td>
<td>179.6 ± 150.1 x 10^3</td>
<td>8.35</td>
<td>0.02</td>
</tr>
<tr>
<td>W12</td>
<td>158.1 ± 68.3 x 10^3</td>
<td>170.7 ± 57.4 x 10^3</td>
<td>164.2 ± 121.9 x 10^3</td>
<td>11.32</td>
<td>0.004</td>
</tr>
<tr>
<td>W24</td>
<td>161.7 ± 64.2 x 10^3</td>
<td>180.4 ± 60.2 x 10^3</td>
<td>158.3 ± 63.8 x 10^3</td>
<td>10.33</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Table (12): patients response according to histological activity index (HAI):

<table>
<thead>
<tr>
<th>Activity</th>
<th>Responder (n=525)</th>
<th>Resist 12 (n=135)</th>
<th>Resist 24 (n=155)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>0</td>
<td>0 (0.0%)</td>
<td>5 (3.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>1-4</td>
<td>397 (75.6%)</td>
<td>124 (91.9%)</td>
<td>125 (80.6%)</td>
</tr>
<tr>
<td>5-8</td>
<td>73 (13.9%)</td>
<td>6 (4.4%)</td>
<td>30 (19.4%)</td>
</tr>
<tr>
<td>9-12</td>
<td>55 (10.5%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

no=number  
%=percentage
Table (13): patients response according to fibrosity stage:

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>Responder (n=525)</th>
<th>Resist 12 (n=135)</th>
<th>Resist 24 (n=155)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>0</td>
<td>0 (0.0%)</td>
<td>10 (7.4%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>1</td>
<td>200 (38.1%)</td>
<td>40 (29.6%)</td>
<td>80 (51.6%)</td>
</tr>
<tr>
<td>2</td>
<td>180 (34.3%)</td>
<td>25 (18.5%)</td>
<td>35 (22.6%)</td>
</tr>
<tr>
<td>3</td>
<td>110 (21.0%)</td>
<td>55 (40.7%)</td>
<td>30 (19.4%)</td>
</tr>
<tr>
<td>4</td>
<td>30 (5.7%)</td>
<td>5 (3.7%)</td>
<td>10 (6.5%)</td>
</tr>
<tr>
<td>5</td>
<td>5 (1.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Table (14): platelet count and percentage before and after therapy in relation to patient respond:

<table>
<thead>
<tr>
<th>Platelet level</th>
<th>Responder (n=525)</th>
<th>Resist 12 (n=135)</th>
<th>Resist 24 (n=155)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Pretherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100-200 x10³</td>
<td>315 (60.0%)</td>
<td>60 (44.4%)</td>
<td>90 (58.1%)</td>
</tr>
<tr>
<td>200-400 x10³</td>
<td>210 (40.0%)</td>
<td>75 (55.6%)</td>
<td>65 (41.9%)</td>
</tr>
<tr>
<td>Post-therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-75 x10³</td>
<td>45 (8.5%)</td>
<td>7 (5.0%)</td>
<td>12 (8.0%)</td>
</tr>
<tr>
<td>75-100 x10³</td>
<td>52 (9.9%)</td>
<td>0 (0.0%)</td>
<td>19 (12.0%)</td>
</tr>
<tr>
<td>&gt;100 x10³</td>
<td>428 (81.7%)</td>
<td>128 (95.0%)</td>
<td>124 (80.0%)</td>
</tr>
</tbody>
</table>
**Table (15): Comparison between and within the studied groups regarding platelets pre and post therapy:**

<table>
<thead>
<tr>
<th>Platelet level</th>
<th>Responder Mean ±SD (n=525)</th>
<th>Resist 12 Mean ±SD (n=135)</th>
<th>Resist 24 Mean ±SD (n=155)</th>
<th>Kruskal-Wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-therapy</td>
<td>197.5 ± 65.9 x10^3</td>
<td>195.9 ± 44.6 x10^3</td>
<td>199.9 ± 56.9 x10^3</td>
<td>0.95</td>
<td>0.62</td>
</tr>
<tr>
<td>Post-therapy</td>
<td>161.7 ± 64.2 x10^3</td>
<td>180.4 ± 60.2 x10^3</td>
<td>158.3 ± 63.8 x10^3</td>
<td>10.33</td>
<td>0.006</td>
</tr>
<tr>
<td>Wilcoxon signed rank test</td>
<td>7.64</td>
<td>2.29</td>
<td>6.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig 9:** Mean value of platelets pre and post therapy within the studied groups
Table (16): Mean value distribution of the platelets level Pre-therapy and the primary investigations:

<table>
<thead>
<tr>
<th></th>
<th>Platelet pre therapy</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100-199 x10^3 Mean ±SD (n=590)</td>
<td>200-400 x10^3 Mean ±SD (n=410)</td>
<td># 2.16</td>
</tr>
<tr>
<td>PCR(pre)</td>
<td>502504.2 ± 114808.6</td>
<td>581110.8 ± 160816.7</td>
<td># 2.16</td>
</tr>
<tr>
<td>Prothrombin(sec.)</td>
<td>72.1 ± 9.7</td>
<td>85.6 ± 8.2</td>
<td>6.03</td>
</tr>
<tr>
<td>Albumin(gm/dL)</td>
<td>4.1 ± 0.8</td>
<td>4.4 ± 0.5</td>
<td>2.79</td>
</tr>
<tr>
<td>Billirubin(mg/dL)</td>
<td>0.87 ± 0.3</td>
<td>0.89 ± 0.2</td>
<td># 2.69</td>
</tr>
</tbody>
</table>

# Mann-Whitney test
PCR=polymerase chain reaction of HCV RNA

Table (17): Mean value distribution of the platelets level Pre-therapy and baseline investigations:

<table>
<thead>
<tr>
<th></th>
<th>Platelet pre therapy</th>
<th>Mann-Whitney test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100-199 x10^3 Mean ±SD (n=590)</td>
<td>200-400 x10^3 Mean ±SD (n=410)</td>
<td></td>
</tr>
<tr>
<td>WBCs(1000/mm^3)</td>
<td>5194.3 ± 1887.7</td>
<td>6313.2 ± 2499.2</td>
<td>3.56</td>
</tr>
<tr>
<td>AST</td>
<td>56.2 ± 111.4</td>
<td>48.7 ± 27.5</td>
<td>1.32</td>
</tr>
<tr>
<td>ALT</td>
<td>50.9 ± 44.6</td>
<td>47.3 ± 32.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

WBCs=White blood cells
AST=Aspartate transeaminase
ALT=Alanine transeaminase
Table (18): Mean value distribution of the platelets level (Post-therapy) and the primary investigations:

<table>
<thead>
<tr>
<th>Platelet post-therapy</th>
<th>Kruskal-wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>50000-75000 Mean ±SD (n=55)</td>
<td>194760.6 ± 156574.7</td>
<td># 0.63</td>
</tr>
<tr>
<td>≥75000-100000 Mean ±SD (n=65)</td>
<td>327986.7 ± 153459.3</td>
<td>0.03</td>
</tr>
<tr>
<td>&gt;100000 Mean ±SD (n=615)</td>
<td>558139.8 ± 117233.0</td>
<td>0.63</td>
</tr>
</tbody>
</table>

PCR(pre.) 194760.6 ± 156574.7
Prothrombin(sec.) (pre.) 75.5 ± 8.2
Albumin(gm/dL) (pre.) 3.9 ± 0.4
Billirubin(mg/dL) (pre.) 0.84 ± 0.2

# F test
PCR=polymerase chain reaction of HCV RNA

Table (19): Mean value distribution of the platelets level (Post-therapy) and week 24 investigations:

<table>
<thead>
<tr>
<th>Platelet post-therapy</th>
<th>Kruskal-wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>50000-75000 Mean ±SD (n=55)</td>
<td>2865.5 ± 830.9</td>
<td>0.73</td>
</tr>
<tr>
<td>≥75000-100000 Mean ±SD (n=65)</td>
<td>3016.9 ± 1020.2</td>
<td>0.39</td>
</tr>
<tr>
<td>&gt;100000 Mean ±SD (n=615)</td>
<td>3762.6 ± 1295.9</td>
<td>0.73</td>
</tr>
</tbody>
</table>

WBCs=White blood cells
AST=Aspartate transferase
ALT=Alanine amino transferase
Table (20): Number and percent distribution of platelet level (post-therapy) regarding Necro-inflammatory score:

<table>
<thead>
<tr>
<th>Activity grading</th>
<th>Platelet post-therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50000-75000 (n=55)</td>
</tr>
<tr>
<td>no</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>1-4</td>
<td>35 (63.6%)</td>
</tr>
<tr>
<td>5-8</td>
<td>10 (18.2%)</td>
</tr>
<tr>
<td>9-12</td>
<td>10 (18.2%)</td>
</tr>
</tbody>
</table>

Table (21): Number and percent distribution of platelet level (post-therapy) regarding Fibrosis scores:

<table>
<thead>
<tr>
<th>Fibrosis staging</th>
<th>Platelet post-therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50000-75000 (n=55)</td>
</tr>
<tr>
<td>no</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>1</td>
<td>20 (36.4%)</td>
</tr>
<tr>
<td>2</td>
<td>20 (36.4%)</td>
</tr>
<tr>
<td>3</td>
<td>5 (9.1%)</td>
</tr>
<tr>
<td>4</td>
<td>10 (18.2%)</td>
</tr>
<tr>
<td>5</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>
This retrospective study included 1000 patients with chronic hepatitis C virus infection who were attended and treated with interferon and followed up for a minimum of six months in Liver Research Centre, Tanta Fever Hospital.

As shown in table (8), descriptive data of the studied patients before start of therapy shows screened quantative HCV, RNA PCR that the average was (534732.9 ±1077609.5) from a minimal level 1450 IU/ml to a maximum level 1500000IU/m in the patients serum, shows screened prothrombin per second that the average was (83.5 ± 9.3) from a minimal level 60 secod to a maximum level 100 second in the patients serum, shows screened albumin per gm/dl that the average was (4.4 ± 0.68) from a minimal level 3.1 gm/dl to a maximum level 5.4 gm/dl in the patients serum and shows screened bilirubin per mg/dl that the average was (0.88 ± 0.31) from a minimal level 0.19mg/dl to a maximum level 1.92 mg/dl in the patients serum.

As shown in table (9), descriptive data of the studied patients before start and during the therapy shows

1- WBCs, before therapy was (5884.9 ±2191.8) ,4weeks after therapy was(4260.2 ± 1454.2),12 weeks after therapy was(3791.0± 1424.4) and 24 weeks after therapy was(3629.5± 1279.9).

2- platelets, before therapy was (195.6 ±60.2) ,4weeks after therapy was(170.5 ± 81.6),12 weeks after therapy was(164.6± 82.4) and24 weeks after therapy was(164.8± 63.4).

3 -AST, before therapy was (50.3 ±87.6) ,4weeks after therapy was(38.4 ± 29.2),12 weeks after therapy was(41.5± 40.1) and24 weeks after therapy was(39.5± 43.3)

4- ALT, before therapy was (50.2 ±40.2) ,4weeks after therapy was(38.2 ± 28.1),12 weeks after therapy was(40.2± 36.3) and24 weeks after therapy was(35.8± 37.1).
Table (10) shows the response of the patients to the therapy. It was revealed that the total patients who respond to therapy are 525 (380 males, 145 female), those who resist the therapy at week 12 were 135 patients (110 males, 25 females), those who were resistant at week 24 were 155 patients (115 males, 40 females), the number of missed patients were 185 (145 males, 40 females).

Table (11) shows the comparison between the classified groups regarding the measured platelets at different time sequence, it was revealed that there is no statistically difference between different groups platelet's count at the baseline measurement (p-value >0.05), but revealed that at 4th week the responder group platelet's (165.2±58.8) was statistically significant lower than other resistant12 (180.1±58.4) and resistant24 groups (180.1±58.4) (p-value <0.05), and the resistant 12 platelet (170.7±57.4) was statistically higher than other responder (158.1±68.3) and resistant24 (164.2±121.9) groups at the 12th week (p-value <0.05) and also shows that the platelets was significantly higher of the resistant12 (180.4±60.2) group than either responder (161.7±64.2) and resistant 24 (158.3±63.8) groups at the 24 weeks (p-value <0.05).

Table (12) shows patients respond in relation to histological activity index. It was revealed that 397 (75.6%) patients out of 646 patients with grade 1-4 HAI showed response to therapy and 124 (91.9%) patients were resistant at 12 week, 125 (80.6%) patients were resistant at week 24.

Also it was revealed that 73 (13.9%) patients out of 109 patients with grade 5-8 HAI showed response to therapy and 6 (4.4%) patients were resistant at 12 week, 30 (19.4%) patients were resistant at week 24.

Also it was revealed that 55 (10.5%) patients out of 55 patients with grade 9-12 HAI showed response to therapy.
Table(13) shows the patients response in relation to fibrosis stages. It was revealed that 200(38.1%) patients out of 320 with fibrosis stage 1 showed response to therapy, 40(29.6%) patients were resistant at week 12 and 80(51.6%) patients were resistant at week 24.

Also, It was revealed that 180(34.31%) patients out of 240 with fibrosis stage 2 showed response to therapy, 25(18.5%) patients were resistant at week 12 and 350(22.6%) patients were resistant at week 24.

Also, It was revealed that 110(21.0%) patients out of 195 with fibrosis stage 3 showed response to therapy, 55(40.7%) patients were resistant at week 12 and 30(19.4%) patients were resistant at week 24.

Also, It was revealed that 30(5.7%) patients out of 45 with fibrosis stage 4 showed response to therapy, 5(3.7%) patients were resistant at week 12 and 10(6.5%) patients were resistant at week 24.

Also, It was revealed that 5(1.0%) patients out of 5 with fibrosis stage 5 showed response to therapy.

Table(14) shows the platelet count and patients percent before and after therapy in relation to patients respond:

Pre therapy with platelet count 100000-200000, responded patients (no=315) (60.0%), resistant 12 patients (no=60) (44.4%), resistant 24 patients (no=90) (58.1%). with platelet count 200000-400000, responded patients (no=210) (40.0%), resistant 12 patients (no=75) (55.6%), resistant 24 patients (no=65) (41.9%).

Post therapy with platelet count 50000-75000, responded patients (no=30) (8.5%), resistant 12 patients (no=5) (5.0%), resistant 24 patients (no=10) (8.0%). with platelet count 75000-100000, responded patients (no=35) (9.9%), resistant 12 patients (no=0) (0.0%), resistant 24 patients (no=15) (12.0%). with platelet count >100000, responded patients (no=290) (81.7%), resistant 12 patients (no=950) (95.0%), resistant 24 patients (no=100) (80.0%).
Table (15) shows that there is no statistically significant difference between the responder, resistant 12 and resistant 24 groups (197.5 ± 65.9)(195.9 ± 44.6)( 199.9 ± 56.9) regarding the pre-therapy platelet level but there was significant (p-value<0.05) lower platelet count in resistant 24 group(158.3 ± 63.8) than other groups(161.7 ± 64.2)( 180.4 ± 60.2) in the post-therapy level, Also it shows that the platelet level was significantly lower in post-therapy(161.7 ± 64.2)( 180.4 ± 60.2)( 158.3 ± 63.8) patients than the pre-therapy patients(197.5 ± 65.9)( 195.9 ± 44.6)( 199.9 ± 56.9) in all the groups respectively(p-value<0.05)( p-value<0.05)( p-value<0.05).

Table (16) shows that the mean value distribution of platelet level pre-therapy and primary investigations ,This table revealed that the PCR count was significantly lower in the patients group with platelet count (100-199 x10$^3$) than patients with platelet count (200-400x 10$^3$) (p-value<0.05) also it shows that the prothrombin by second was significantly lower in patients with platelet count (100-199 x10$^3$)than patients with platelet count (200-400x 10$^3$) (p-value<0.05) also it shows that the albumin by grams was significantly lower in patients with platelet count (100-199 x10$^3$)than patients with platelet count (200-400x 10$^3$) (p-value<0.05) and it shows that the billirubin by mg was insignificantly lower in patients with platelet count (100-199 x10$^3$)than patients with platelet count (200-400x 10$^3$) (p-value<0.05).

Table (17) shows the mean value distribution of the platelets level pre-therapy and the base line investigations, This table shows that WBCs(1000/mm3) was statistically significant lower in patients group with platelet count (100-199x 10$^3$)than patients with platelet count (200-400x 10$^3$) (p-value<0.05) but there is no significant difference between AST and ALT of both groups in the pre-therapy groups(p-value>0.05) ( p-value>0.05).
Table (18) shows the mean value distribution of the platelets level post-therapy and the primary investigations. This table revealed that the PCR count was significantly lower in patients group with platelet count (50-75x $10^3$) than both patients group with platelets count (75-100x $10^3$) and ($>100x 10^3$) (p-value>0.05), but the table shows that the prothrombin by second was insignificantly lower in patients with platelet count (50-75x $10^3$) than patients with platelet count (75-100x $10^3$) ($>100x 10^3$) (p-value<0.05), also it reveals that the albumin by grams was significantly lower in patients with platelet count (50-75x $10^3$) than patients with platelet count (75-100x $10^3$) ($>100x 10^3$) (p-value<0.05) and it shows that the billirubin by mg was insignificantly higher in patients with platelet count (50-75x $10^3$) than patients with platelet count (75-100x $10^3$) ($>100x 10^3$) (p-value>0.05).

Table (19) shows the mean value distribution of the platelets level post-therapy and week 24 investigations, the table shows that WBCs(1000/mm3) was insignificantly lower in patients group with platelet count ($>100x 10^3$) than patients with platelet count ($<100x 10^3$) (p-value>0.05) but it revealed that ALT and AST was significantly lower in patients group with platelet count ($>100x 10^3$) than other patients group with platelet count ($<100x 10^3$) in post-therapy groups (p-value<0.05).

Table (20) shows subdivided patients according to the platelet count at the end of therapy in relation to histological activity index. it was revealed that 35 (36.6%) patients out of 586 patients with grade 1-4HAI showed platelet level 50000-75000, 54 (83.1%) patients were platelet level 75000-100000 and 497 (80.8) patients with platelet level more than 100000.

Also it was revealed that 10 (18.2%) patients out of 996 patients with grade 5-8HAI showed platelet level 50000-75000, 11 (16.9%) patients were
platelet level 75000-100000 and 78(12.7) patients with platelet level more than 100000.

Also it was revealed that 10(18.2%) patients out of 45 patients with grade 9-12HAI showed platelets level 50000-75000, no(0.0%) patients were platelet level 75000-100000 and 35(5.7) patients with platelet level more than 100000.

Table (21) shows subdivided patients according to the platelet count at the end of therapy in relation to fibrosis stages. It was revealed that, 20(36.4%) patients out of 270 patients with fibrosis stage 1 showed platelets 50000-75000, 30(46.2%) patients with platelets level 75000-100000 and 220(35.8%) patients with platelets level more than 100000.

Also it was revealed that, 20(36.4%) patients out of 255 patients with fibrosis stage 2 showed platelets 50000-75000, 30(46.2%) patients with platelets level 75000-100000 and 205(33.3%) patients with platelets level more than 100000.

Also it was revealed that 5(9.1%) patients out of 150 patients with fibrosis stage 3 showed platelets 50000-75000, 5(7.7%) patients with platelets level 75000-100000 and 140(22.8%) patients with platelets level more than 100000.

Also it was revealed that 10(18.2%) patients out of 45 patients with fibrosis stage 4 showed platelets 50000-75000, no patients with platelets level 75000-100000 and 35(5.7%) patients with platelets level more than 100000.

Also it was revealed that, 10(1.6%) patients out of 10 patients with fibrosis stage 5 showed platelets level more than 100000.
Discussion

The prevalence of hepatitis C infection in Egypt increases steadily with age and high rates of infection are observed among persons in all age groups \(\textit{Abdel-Aziz et al., (2000)}\). This pattern indicates an increased risk in the distant past followed by an ongoing high risk for acquiring HCV infection although there are regional differences in average overall prevalence \(\textit{Perz et al., (2006)}\).

Hepatitis C virus (HCV) infection affects about 170 million people worldwide. Chronic hepatitis develops in up to 80% of individuals who contract acute infections and may cause liver fibrosis and cirrhosis.\(\textit{Wong,( 2006)}\)

HCV infection is an inflammatory disease characterized by the enhanced expression of various pro- and anti-inflammatory cytokines in the liver. The initiation of several intracellular signal pathways that involve apoptosis, proliferation, and extracellular matrix synthesis constitutes the major impetus for the development of hepatic injury, fibrosis, and cirrhosis.3-5 During the past decade. \(\textit{Wong, (2006)}\)

The current standard treatment for chronic hepatitis C is the combination of pegylated interferon (IFN) alfa and ribavirin. The efficacy endpoint of hepatitis C treatment is the "sustained virological response" (SVR), defined by the absence of detectable HCV RNA in serum as assessed by an HCV RNA assay with a lower limit of detection of 50 IU/ml or less 24 weeks after the end of treatment. \(\textit{Enomoto, et al, (2010)}\).

Thrombocytopenia is one of the most common hematological abnormalities in patients with chronic liver disease. Its prevalence and
severity increase with increasing hepatocellular damage (Giannini, (2006), and is considered an indicator of advanced disease (Bashour et al, (2000).

Immune thrombocytopenia after interferon-α therapy has been noted, but this effect is uncommon. We believe that interferon therapy for chronic hepatitis C need not be systematically avoided just because of thrombocytopenia. Moreover, we reported that interferon treatment may be useful in thrombocytopenia associated with HCV infection . (Jean Marc Durand,(1996).

According to available data, the association between HCV and autoimmune thrombocytopenia requires further confirmation. However, we suggest that this association should be considered, given its possible implications for therapeutic options in patients with chronic HCV infection. (Jean Marc Durand,(1996)

Patients with chronic liver disease due to infection with the hepatitis C virus (HCV) who have thrombocytopenia (<75,000 platelets per cubic millimeter) have been routinely excluded from clinical trials of interferon and ribavirin and few published reports have described the treatment of chronic HCV infection in patients with platelet counts of less than 50,000 per cubic millimeter (Shiffman et al, (2007).

Thrombocytopenia is likely the most common and prevalent haematological abnormality that can be found in patients affected by chronic liver disease . A recent systematic review has shown that, in patients chronically infected with the hepatitis C virus (HCV), the prevalence of thrombocytopenia may vary from 0.16% in a cohort study that identified patients with severe autoimmune cytopenia alone to 45.4% in a study that included only patients with compensated cirrhosis.

Several studies have suggested that IFN-α induces thrombocytopenia by inhibiting the proliferation of human megakaryocytes, as the number of colony-forming units of megakaryocytes (CFU-MK) is reduced by relatively high concentrations of IFN-α in vitro. (Wang, et al (2000)

Treatment of thrombocytopenia induced by IFN therapy by steroids and intravenous immunoglobulin are reported to be effective. In addition, the effectiveness of rituximab, an anti-CD20 humanized monoclonal antibody has been also reported(Weitz, (2005)

This study was done on 1000 patients of chronic hepatitis C aiming to monitor the problem of thrombocytopenia pre and post therapy and for how extent it is present.

The majority of patients were males (75%) and this came in agreement with Poynard et al., (2000); Abo Alazm and El Sheikh (1996) whose studies showed significant association between the male gender and fibrosis progression. Moreover, Pinzani (2004) reported that male gender (for groups of age >50 years) has been shown to be a predictor of the development of significant fibrosis or, at least, of a faster progression to cirrhosis.

The peripheral platelet count decreased more significantly in females with chronic hepatitis C. (Zeki Karasuet al (2007)

Univariate analysis of baseline data for the anti-HCV positive participants revealed that those who were >65 years old (OR, 3.1; 95% CI, 1.29.5) or had an ALT level of >40 U/L (OR, 2.2; 95% CI, 1.05.4) were more likely to have thrombocytopenia. There were no significant
differences with respect to sex, BMI, habitual smoking, or habitual alcohol consumption (Chong-Shan Wang et al, (2004))

In the present study, the response of patients in females (58.0%) which is significantly higher than males (50.7%) that agreed with John et al, (2009) who suggests that women may have higher rates of sustained virologic response with standard-dose than with low-dose peginterferon alfa-2b, also Jun Hayashi, (1988) found that women aged 39 years and younger are responsive to interferon alfa treatment suggests that hormonal activity, in particular the level of estrogen, may be associated with the sustained elimination of HCV.

Response rates are reported lower with initial levels of HCV RNA >600,000 IU/ml, male gender, high body weight, and advanced liver fibrosis. (Bressler, et al (2003))

Other data demonstrates that chronic hepatitis C may be associated with variable degrees of thrombocytopenia. There is no connection between gender and the severity of thrombocytopenia. There is a correlation between thrombocytopenia and the severity of liver disease (ALT and liver fibrosis stage) and the viral load (HCV RNA). Older age has a minimal influence. (Mihai Olariu,(2010))

In this study, table(9) showed the accumulative effect of therapy on the platelets revealing a progressive decline of both with time from baseline up to the 24 week.

Table (15) revealed that there is significant decline in the post-therapy platelets than the pre-therapy indifferent groups.

These results go inline with Renault, (1989) Mild to moderate thrombocytopenia is common in patients with chronic viral hepatitis,
especially those receiving interferon. The latter, in its usual recommended dose for viral hepatitis treatment, may cause bone marrow suppression in treated patients, and in some, the platelet count may decrease in the range of 25-50% from the base-line reading within the first month of therapy.

Also, in some situations, patients who are otherwise eligible for HCV treatment with interferon and ribavirin cannot be so treated because their platelets counts are low, which jeopardizes the treatment. Nevertheless, hepatitis C patients treated with interferon and ribavirin also present a drop in the platelet count as a side effect. (Fried, (2002)

Also, thrombocytopenia: Roughly 4-6% of patients receiving Peginterferon alfa-2a and ribavirin required dose reductions for thrombocytopenia during treatment. In general, dose reduction is recommended when platelet counts fall below 50,000. Although discontinuation of therapy is usually unnecessary, it would be recommended if platelet counts fell below 30,000. (Marinos, (2001)

Also, Cirrhosis and hypersplenism perse can cause thrombocytopenia, and this may be exacerbated by interferon. The low platelet counts are generally well tolerated, and the interferon effect is reversible on lowering the dose or stopping the medication. Another type of thrombocytopenia, caused by autoimmune-mediated accelerated platelet destruction, can infrequently be seen with viral hepatitis, especially hepatitis C treated with interferon. This complication is unpredictable, develops rapidly, and results in extremely low platelet levels. Fortunately, it is a rare complication, having been reported in eight cases of hepatitis C and two of hepatitis B since 1992 (Dourakis et al., (1996).
Also, thrombocytopenia either pre-exists and prevents the initiation of treatment with pegylated interferon (PEG-IFN) or develops as a consequence of PEG-IFN treatment, leading to dose modification in 19% of cases and discontinuation in 2% of cases. In patients with cirrhosis, thrombocytopenia complicates antiviral treatment much more frequently than in patients with HCV infection without cirrhosis.

Pathophysiology: A variety of pathogenic mechanisms are reported to be implicated in thrombocytopenia related to chronic HCV infection. These include: 1) sequestration of platelets in the enlarged spleen secondary to portal hypertension (hyper-splenism); 2) reduced hepatic production of thrombopoietin; 3) bone marrow suppression by HCV or antiviral treatment; and 4) increased platelet destruction mediated by immune mechanisms involving anti-platelet autoantibodies and platelet-associated immune complexes. (Sulkowski, (2005) (Bashour et al, (2000)

Shiota et al (1997) measured serum thrombopoietin (TPO) in chronic hepatitis C treated with interferon (IFN). The platelet count before the therapy was 161.9 x 10(9)+/- 64.1 x 10(9)/l, which decreased to 116.3 x 10(9)+/- 48.4 x 10(9)/l 1 week after IFN therapy (P < 0.01). On the other hand, serum TPO increased from 1.96 +/- 0.60 fmol/ml to 2.68 +/- 0.69 fmol/ml (P < 0.02). Contrary to a recent report that serum TPO was not altered in liver cirrhosis, these data indicate that serum TPO was increased in chronic hepatitis C in response to thrombocytopenia by IFN therapy.

Table(11) revealed that there is no significant difference between the base line platelets in different groups, however there is significant decrease in the responder group at 4th and 12week than other groups and platelets decrease more in resistant 24 group at the week 24.
Table (9) revealed that there is a progressive decline in WBCs in the therapy course till the 24 week, that was agreed by soza, who found that absolute neutrophil and lymphocyte count decrease by 30-50% of the baseline values during interferon alfa therapy but is usually not associated with infections. (soza A, 2002)

According to soza et al (2002), neutropenia is common during treatment of chronic hepatitis C with combination therapy (interferon alfa and ribavirin). Their results showed that neutropenia occurred about 8% of cases at 12 weeks of therapy. The neutrophilic count returned to the baseline 24 weeks after the end of interferon and ribavirin therapy. Peripheral thrombocytopenia was found after 24 weeks of treatment in 45% of patients, and was so severe in 5% of patients that lead to either decrease the dose or discontinuation of the treatment.

Also, Dennis (2009) documented that the majority of patients treated with interferon alpha products develop side effects early in therapy that include fever, chills and flu-like symptoms, as well as hematopoietic side effects such as neutropenia, thrombocytopenia, and leukopenia.

Table (17) shows that WBCs (1000/mm³) was significantly lower in patients with platelet count (100-199x 10³) than patients with platelet count (200-400x 10³) pre-therapy (p-value < 0.05).

Table (19) shows that WBCs (1000/mm³) was insignificantly lower in patients with platelet count (>100x 10³) than patients with platelet count (<100x 10³) post-therapy (p-value > 0.05).

Neutropenia and thrombocytopenia were more common among patients treated with regimens that contained peginterferon alfa-2a, and
anemia was more common among patients treated with regimens containing ribavirin. (Francesca et al, (2004)

In this study, In table (17) revealed that there is no significant difference between AST and ALT in different platelet groups in the pre-therapy, and table (19) showed that ALT and AST was significantly lower in group of patients with higher platelet count in post-therapy groups.

Univariate analysis of baseline data for the anti-HCV positive participants revealed that those who were >65 years old (OR, 3.1; 95% CI, 1.29.5) or had an ALT level of >40 U/L (OR, 2.2; 95% CI, 1.05.4) were more likely to have thrombocytopenia. There were no significant differences with respect to sex, BMI, habitual smoking, or habitual alcohol consumption (Chong-Shan Wang et al, (2004)

Multiple logistic regression analysis revealed that anti-HCV positivity (odds ratio, 6.0; 95% confidence interval, 3.211.2), an alanine aminotransferase level of 40 U/L, and age of 65 years were significantly associated with thrombocytopenia. The prevalence of thrombocytopenia among anti-HCV positive subjects increased as the severity of liver disease increased, but, in HBsAg-positive subjects, thrombocytopenia presented only in those with advanced liver disease. (Chong-Shan Wang et al, (2004)

Serum ALT levels are well-known indicators of hepatocellular damage. In the current study, an elevated serum ALT level was strongly associated with thrombocytopenia. (Dennis et al, (2009)

Elevated ALT levels are good predictors of fibrosis progression in chronic HCV infection. This indicates that individuals with HCV
infection might have more hepatic inflammation and might be more likely to progress to advanced liver disease (Chong-Shan Wang et al., 2004).

There was a statistically significant relationship between the platelet count and the serum ALT levels. We found higher ALT levels in patients with a low platelet count; in these patients, hepatitis C virus is active and can generate thrombocytopenia by one of the mechanisms described above (Dai CY, 2010).

Table (16) shows that the Prothrombin by second was significantly lower in patients with platelet count (100-199 x10^3) than patients with higher platelet count (200-400x 10^3)(p-value<0.05). Table (18) shows that the prothrombin has no significant difference between different platelet groups in post-therapy.


Table (18) shows that the Albumin by grams was significantly lower in patients with platelet count (50-75x 10^3) than patients with platelet count (75-100x 10^3)( >100x 10^3) (p-value<0.05).

In the present study, the serum albumin level significantly decreased as activity grade increased, which came in agreement with Friedman et al., (2002) who reported that hypoalbuminemia was common in chronic liver diseases.

Table (16) and table (18) show that PCR was significantly lower with lower platelets groups in both pre-therapy and post-therapy.
A significant correlation was found between the degree of thrombocytopenia and the level of viral load. The same association was reported recently by (Dai, (2010)].

Viral load does not correlate with the severity of the hepatitis or with a poor prognosis (as it seems to in HIV infection); but viral load does correlate with the likelihood of a response to antiviral therapy. Rates of response to a course of alpha interferon and ribavirin are higher in patients with low levels of HCV RNA. There are several definitions of a "low level" of HCV RNA, but the usual definition is below 2 million copies per milliliter (mL)(Strader, (1996).

Table (12) shows that the response to therapy is higher in early activity changes, that agreed by Shindo, et al (1997) who found the staging score, but not the grading score, appears to be associated with a long-term response, but the viral level and genotype are more important predictors than the staging score; and both the grading and staging scores decreased significantly with interferon therapy, but the staging score appeared to take longer to improve than the grading score.

Jármay et al (1998), found a significant serum alanine aminotransferase (ALT) level improvement in both groups, but a significant histological improvement in necroinflammatory activity (grade) only in the 12-month group. The Chevallier stage scores demonstrated a significant progression in both groups.

Table (13) showed that there is negative correlation between the response and the fibrosis stage, these result go inline with John et al (2009). Stepwise multivariable logistic-regression analyses identified several baseline factors as independent predictors of sustained virologic
response: baseline HCV RNA level (≤600,000 IU per milliliter), race (nonblack), minimal fibrosis (METAVIR score of F0, F1, or F2), absence of steatosis, normal baseline fasting glucose level, and elevated baseline serum alanine aminotransferase level.

Also, although the time between biopsies partly affected the patient's clinical course, the differences observed here suggest that in patients with chronic hepatitis C, regression of fibrosis is associated with sustained virologic response to interferon therapy. (Yasushi Shiratori, et al (2000))

Also, risks factors for the presence of extrahepatic manifestations (univariate analysis) were: age ≥ 60 years, female gender, virus transmission by blood transfusions, longstanding infection (≥20 years), and extensive liver fibrosis. The most significant risks factors (multivariate analysis) were longstanding infection and extensive liver fibrosis. (Diana et al, (2007))

Table (20) showed higher prevalence of patients (post-therapy) with HAI score 1-4 plus that there is positive correlation between patients number and platelet count at HAI score.

HCV infection is strongly associated with thrombocytopenia, which is correlated with hepatocellular damage and hepatic fibrosis. It is advisable to further check the hepatic condition of the patient, especially for HCV infection, if thrombocytopenia is present, this indicates that thrombocytopenia might be highly correlated with hepatocellular damage. Therefore, it is reasonable to check the liver condition of patients with thrombocytopenia to determine whether they have viral hepatitis or other liver-associated diseases. (Dennis et al, (2009))
Table (21) showed higher prevalence of patients (post-therapy) with fibrosis stage 1 and 2 than other HAI scores in relation to platelets. Platelet count are more sensitive than conventional tests for indicating fibrotic change in chronic hepatitis C. Both could be used to reliably identify those who do not have fibrotic progression, and platelet count also has a high positive predictive value for disease progression (Shirley et al, 1998).

Although the majority of patients evaluated (97.5%) had at least significant fibrosis and half of them had significant fibrosis – and increasing fibrosis stages have been associated with more prevalent thrombocytopenia (Adinolfi, et al, 2001).

A decrease in peripheral platelet count may be a sign of an increase in the degree of fibrosis during the course of chronic viral hepatitis B and C and factors other than hypersplenism may play a role in this decrease in the peripheral platelet count. (Zeki Karasu et al, 2007).

Overall, evaluating the association between decreased platelet count and demographic, biochemical, virological, and histological parameters the authors found that the severity of thrombocytopenia increased with increasing age and severity of fibrosis stage. Interestingly enough, they also found that all patients with thrombocytopenia had evidence of fibrosis at histology, and up to 18.5% of the study cohort had a platelet count below 100×10^9/L. (Roomer, 2010).

Platelet count are more sensitive than conventional tests for indicating fibrotic change in chronic hepatitis C. Both could be used to reliably identify those who do not have fibrotic progression, and platelet
count also has a high positive predictive value for disease progression (Shirley et al, 1998).

Mihai Olariu et al, (2010) demonstrates that chronic hepatitis C is associated with a variable degree of thrombocytopenia. As the disease advances, the platelet count decreases and, in most cases both mechanisms are involved. The stage of fibrosis is one of the major determinants of thrombocytopenia. Our data demonstrates that chronic hepatitis C may be associated with variable degrees of thrombocytopenia. In most cases, both a central (bone marrow suppression) and a peripheral (platelet antibodies) mechanisms are involved. There is no connection between gender and the severity of thrombocytopenia. There is a correlation between thrombocytopenia and the severity of liver disease (ALT and liver fibrosis stage) and the viral load (HCV RNA). Older age has a minimal influence.

The stage of fibrosis was directly related to the decrease in platelet count. This fact was also pointed out by Giannini et al in (2002). In our study, the Sperman’s rank correlation test (rho= -0.86485) demonstrated an inverse relationship between the platelet counts and the stage of fibrosis. As the liver disease advances, the platelet count decreases and this fact may be related to a decrease in thrombopoietin (TPO) production in the hepatocytes. A decreased TPO could thus explain thrombocytopenia as an independent factor.
**Summary**

Chronic hepatitis C is a major medical issue because it is a world wide progressive preventable and treatable disease with a lot of major complications and problems of the disease and therapy ,HCV is a global health problem approximately 3% of word population, Egypt has the highest HCV prevalence in word 10-20% of general population are infected , the transmission of HCV is primarily through exposure to infected blood.

Chronic hepatitis C infection can cause cirrhosis, liver failure, HCC and extra hepatic manifestation like hematological disorder ,autoimmune disorder, diabetes mellitus, renal disease, ocular disease , dermatolological disease and musculoskeletal disease.

Diagnosis of chronic HCV infection by full history taking, general and local abdominal examination and investigation by biochemical assessment of liver function included (serum aminotransferase enzymes, serum albumin, serum bilirubin, prothrombin time), virological assay for HCV included(enzyme immunoassay, RIBA and PCR for HCV RNA),liver biopsy for histological grading and fibrosis stages and recently fibroscan for evaluation of liver stiffness measurement.

The currant standard treatment of HCV is the combination of pegylated interferon (IFN) alfa and ribavirin. The efficacy endpoint of hepatitis C treatment is the "sustained virological response" (SVR), defined by the absence of detectable HCV RNA in serum as assessed by an HCV RNA assay with a lower limit of detection of 50 IU/ml or less 24
weeks after the end of treatment. Responses to therapy in chronic hepatitis C can be categorized as biochemical as shown by

1- normal alanine aminotransferase (ALT) levels
2- virological as shown by absence of detectable HCV RNA
3- histological as shown by improvements in liver biopsy results.

The interferons have multiple effects like antiviral effect, immunomodulatory effect, antiproliferative effect and antifibrogenic effect.

Interferon-α has a myelosuppressive effect and decrease in peripheral blood counts occur in almost all patient, Thrombocytopenia is one of the most common hematological complication with chronic liver disease and also to antiviral therapy.

This study aims to monitor occur thrombocytopenia after interferon therapy so should measure platelets level before, at weekly intervals for 4 weeks, then every 3 months during treatment and 6 months latter.

This study included 1000 patients with chronic hepatitis C virus infection who were treated by interferon and ribavirin in Liver Research Centre, Tanta Fever Hospital during the period from June 2007 to June 2009, the data of all patients were collected from their files included age, sex, liver biopsy, laboratory investigation included (serum aminotransferase enzymes, serum albumin, serum bilirubin, prothrombin time, WBC, platelet count, PCR for HCV RNA).

This study shows majority in 750 males and minority in 250 females and response to interferon therapy in females higher than males.
Also, that the response to treatment with interferon is significant higher in patients with low necro-inflammatory score, response to treatment with interferon is significant higher in patients with low fibrosis stage, there is significant post-therapy thrombocytopenia in all patients groups(responder, resistant 12 and resistant 24), there positive correlation between the platelet count and the viral load an both pre and post-therapy groups there positive correlation between WBCs and platelet count in the pre-therapy treatment and also there is significant hypoalbuminemia in low platelet count.

And in this study proved occurrence post therapy thrombocytopenia by comparison platelet count before and during and after interferon therapy.

**Recommendation**

We recommend further studies on the interferon induced thrombocytopenia, its relation with WBCs and its relation with other liver enzymes and functions (ALT,AST,bilirubin, albumin and prothrombin)

Also we recommend to put a clear cut off point when to start the interferon and at what time or platelet level we should stop.

Also we recommend further study about the activity and fibrosis and the level and degree that can predict the patients with high incidence of treatment failure and and patients with high incidence of complications.
References


**Bressler BL, Guindi M, Tomlinson G. et al.(2003)** High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. Hepatology. 2003;38:639-44.


**Cantin, E.; Tanamachi, B.; Opensgaw, H.; Jmann and Clarke, K. (1999):** Gamma interferon (IFN-gamma) receptor null-mutant mice are more susceptible to herpes simplex virus type I infection than IFN gamma ligand null-mutatDmice. J. Virol., 73;5196-5200.

**Causse, X.; Godinot, H. and Chevallier, M. (19991):** Comparison of 1 or 3 Mu of interferon alfa-2b and placebo in patients with chronic non-A, non-B hepatitis. gastroenterology; 1 c1: 497-502.


**Layden, J.E. and Layden, T.J. (2001):** How can mathematics help us understand HCV? Gastroenterology; 120:1546-1549.


**Lurie, Y; Dusheiko, G. and Rouzier-pains, R.and Glue, P(2000):** Assessment of optimal dosing frequency of pegylated interferon alfa-2b (peg intorn) in chronic hepatitis C. Hepatology; 32:444A.

**Mangia, A; Santoro, R; Pittelli, M; et al.(2002):** High does of interferon in combination with ribavirin are more effective than the standard regimen in patients with HCV genotype 1 chronic hepatitis J. Hepatol,37(1):109-16


Mihai Olariu1, Cristina Olariu2, Dan Olteanu2(2010)
Thrombocytopenia in Chronic Hepatitis C ,University of Medicine and Pharmacy „Carol Davila” Bucharest,University Hospital Bucharest, RomaniaJ Gastrointestin Liver Dis, December 2010 Vol.19 No 4, 381-385


Hepatitis C: Value for Predicting Fibrotic Progression, The American Journal of Gastroenterology 1998, 1384–1390


Takashi Himoto, Mikio Nishioka(2008) ;Autoantibodies in Hepatitis C Virus-Related Chronic Liver Disease,Hepatitis Monthly FALL 2008; 8(4): 295-303,


**Wack KE, Ross MA, Zegarra V, et al:** Sinusoidal ultrastructure evaluated during the revascularization of regenerating rat liver. Hepatol 2001;33:363-78.


Yasushi Shiratori, MD; Fumio Imazeki, MD; Mitsuhiko Moriyama, MD ET AL (2000); Histologic Improvement of Fibrosis in Patients with Hepatitis C Who Have Sustained Response to Interferon Therapy. Ann Intern Med April 4, 2000 132:517.


Zeki Karasu, Fatih Tekin, Galip Ersoz, et al 2007: Liver Fibrosis Is Associated with Decreased Peripheral Platelet Count in Patients with Chronic Hepatitis B and C Digestive Diseases and Sciences. Volume 52, Number 6, 1535-1539


الملخص العربي

يعتبر مرض الالتهاب الكبدي الوبائي سي المزمن من الأمراض ذات الهمية الطبية وذلك نظرا لانتشاره عالميا. أمكانيه الوقاية والعلاج منه وكذلك تزايد نسب حدوث مضاعفات نتيجة للمرض أو أثار جابية لعلاج، تمكن اهمية الالتهاب الكبدي الوبائي سي المزمن في انه مرض عالمي يصيب نسبة 3% من سكان العالم وتزداد هذه النسبة خصوصا في مصر حيث تصل الي 10-20% ويعتبر التعامل مع الدم الملوث اهم اسباب العدوى.

يسبب التهاب الكبدي شي المزمن الكثير من المضاعفات مثل فشل وظائف الكبد، تشع الكبد، أمراض الكلي، أمراض العيون، الأمراض الجلدية، الأمراض المناعية وكذلك أمراض الدم.

يجب الاهتمام بالتشخيص المبكر للمرض وتشمل طرق التشخيص التاريخ المرضي، الفحص الطبي العام وكذلك الفحص السريري للبطن، تحليل وظائف الكبد (إنزيمات الكبد، نسبة الاليبيمون، نسبة الصفراء ومعدل سرول الدم) تحليل الفيروسات النوعي والكمي واخذ عينه من الكبد (قياس معدل نشاط الالتهاب ونسبة التليف).

يعتبر العلاجات، فيروسات و الوبائي ين استمرار الاستجابة الفيروسية للعلاج والتي تحتاج للمتابعة المستمرة بتحليل الفيروسات.

الفيروسات يعود كثير من المضاعفات المناعية وكذلك تأثيره المثبط للخلايا النشطة وخصوصا نخاع العظام، حيث يعتبر نقص الصفائح الدموية من المشاكل الهامة كثيرة خصوصا للمرضى الخاضعين للعلاج بالفيروسات.

اهتمت هذه الدراسة ببحث حدوث نقص الصفائح الدموية قبل العلاج وبعد العلاج بشهرين وثلاثة أشهر ثم سته اشهر حيث تم عمل هذه الدراسة علي عدد 1000 مريض من الخاضعين للعلاج.
بالالترفيرون في معهد أبحاث الكبد في مستشفى حميات طنطا، وقد تم تجميع البيانات من ملفات
المرضى والتي شملت النوع، العمر، نتيجة عينه الكبد، تحليل وانزيمات الكبد، نسبة الصفراء، نسبة
الألبومين، عدد كرات الدم البيضاء وكذلك عدد الصفائح الدموية قبل وبعد العلاج شهر و3
شهر و6 شهور.

لقد أثبتت الدراسة وجود نقص الصفائح الدموية لمرضى الالتهاب الكبدى الوبائي في المزم
للمرضى المعالجين بالالترفيرون بنسبة أعلى من قبل العلاج، كما وجد أن نسبة الحدوث أعلى في
النساء منه في الرجال.

وتوصي الدراسة بمتابعة حالات العلاج بالالترفيرون ومتابعة مضاعفاته وكذلك البحث في
العلامات والدلالات السابقة للعلاج والتي ترتبط بصورة ما بالمضاعفات، حيث يتم تقييم المرضى
الي مجموعات حسب التوقع بنسبة حدوث المضاعفات ومحاولة التنبؤ السريع والوقاية منها.