Introduction

Viral hepatitis C is a major cause of liver related morbidity and mortality and represent a major health problem in Egypt and world wide ***(Alberti and Benvegnu, 2003)*** and there is a large underlying reservoir of HCV caused liver disease ***(Strickland et al., 2002).***

There is two to three fold increase for the prevalence of steatosis in chronic hepatitis C infected patients, HCV genotype 3 is associated with a higher prevalence and more severe grades of steatosis than non genotype 3.HCV load and body mass index are associated with steatosis in HCV -3 and HCV –non -3 patients respectively ***(Lornado et al., 2006).***

The prevalence of steatosis in liver biopsy specimens from patients with chronic HCV has been reported, further studies found a role for steatosis in the progression of chronic HCV. The pathogenesis of steatosis in patients with HCV is not well understood ***(Jean–Michel et al., 2005).***

Alcohol intake and type 2 Diabetes mellitus are primary causes of steatosis in the general population, However, most studies of HCV–associated steatosis have excluded alcohol drinkers and individuals with diabetes and thus have not addressed the relative contribution of known causes of steatosis to liver injury in HCV–associated disease ***(Alexander et al., 2005).***

In this study we will assess for the correlation between serum HCV load and degree of steatosis detected in liver histology.

Aim of the Work

The aim of the present work is to assess the correlation between serum HCV load detected by quantitative PCR in chronically infected patients with genotype (4) commonly presents in Egyptian population and degree of hepatic steatosis detected in histopathological examination of their liver biopsies.

Hepatitis C Virus

HCV is a major health problem. Global Prevalence of chronic Hepatitis C is estimated to be 3% of average. Ranging from 0.1 to 5% in different countries, HCV associated chronic liver disease results in 8000 to 10000 deaths per year and the annual cost of acute and chronic hepatitis C exceeds $ 600 million.

There are 175 millions chronic Hepatitis C throughout the world HCV account about;

- 20 % of cases of acute hepatitis.
- 40 % of end stage liver disease.
- 30 % of liver Transplants**.**

(Theodor and Mazen, 2006)

- 60% of cases with Hepatocellular Carcinoma ***(Hoofangle, 1995)***.

It is the most frequent indication for hepatic transplantation. Symptomatic infection is 1-3/100.000 cases. Annually the actual incidence is much higher. As the majority of cases (25%) is asymptomatic.

The incidence is now declining being now reduced to near zero, Universal precautions have been markedly decrease the incidence of transmission in medical settings; Intravenous

Drug use remains the main mode of Transmission in united states ***(Sutton et al., 2008)***.

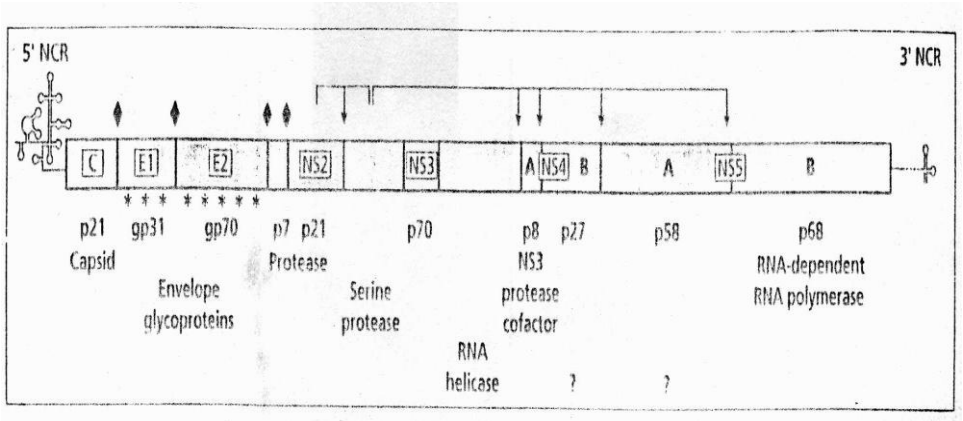

Figure (I): The hepatitis C viral genome. Asterisks in the E1 and E2 region indicate glycosylation of the envelope proteins. Diamonds denote cleavages of the HCV polyprotein precursor by the endoplasmic reticulum signal peptidase. Arrows indicate cleavages by HCV NS2-3 and NS3 proteases. NCR, non-coding region ***(Murphy et al., 2000).***

Molecular Biology:

The structure and replicating cycle are still incompletely under stood due to lack of an efficient cell culture system, HCV is a member of flavivirdae family.

All members of this family are small sized enveloped viruses containing antisense single stranded RNA encoding viral polyprotien in the viral genome is composed of 5 non coding region, along open reading frame encoding poly protein precursor of about 3000 amino acids and non-coding region ***(DeDerancisco, 1999)***.

HCV quasi-species:

HCV exists in a mixture of closely related, yet heterogeneous viral sequences known as quasi-species although mutations occur throughout the entire genome regions. hypervariable region ($HVO_{1)}$ located at the end terminus of E_2 NS1 region (figure -I). The degree of diversity is related to the progression of liver disease. Mutations follow therapy allowing HCV to escape antiviral effects; lower heterogeneity increases the response to antiviral therapy ***(Hassoba et al., 1999)***.

Genotypes:

HCV shows considerable heterogenicity, particular in the viral envelop. Using sequence comparison known variants of HCV collected form different parts of the world, can be divided into eleven main genotypes, *designated from (1-11),* and subtypes (designated a, b, c, etc.) There are at least 50 more closely related variants with considerable **geographical variation** in the prevalence of the various genotypes.

In *England* 50% of all infections are due to *genotype 1* it implies worse results, but there is higher percentage of genotype 3 among intravenous drug users. *Genotype 4* is largely found in the *Middle East* ***(Thomoson and Finch, 2005)***.

In *Beijing* and *China* 52% of 63% RNA samples categorized as *genotype 2* and 29% as genotype 3. *In* Indian subcontinent *and Thailand*, *genotype 3a* was the most common 50-60% ***(*Apichartpiy *et al., 1999*** and ***Bdour, 2002)***.

Genotype 5 can be found in *South Africa*, *Genotype 6-11* are distributed in Asia **(*Bdour, 2002)***. *Genotype 6* in *south East Asia* ***(Huy and Abe, 2004)***.

Serological tests:

To detect antibodies to viral antigens for routine screening ELISA is satisfactory particularly for blood donors, listless sensitive in haemodialysis and immune-compromised patients. ELISA is positive as early as 11 weeks after infection, and always within 20 week of the onset in patients with acute hepatitis of unknown cause ELISA should be performed first ***(Adrian et al., 2006)***.

In low-risk settings such as blood banks and other general screening situations approximately 25% of ELISA-positive tests may be false ***(Adrian et al., 2006)***.

In this case supplemental specificity test such as strip immune blot assay (RIBA) is recommended then quantitative HCV RNA should be performed if anti-HCV positively is confirmed in high-risk populations and in clinical settings where HCV is suspended, positive ELISA should be confirmed by quantitative HCV RNA ***(Adrian et al., 2006)***.

If hepatitis A and B are negative, Quantitative HCV RNA must be performed, ELISA-negative patient with chronic hepatitis of unknown cause, particularly in haemodialysis and immune compromised patients, Quantitative HCV RNA test is essential ***(Martinet et al., 1989)***.

Polymerase chain reaction (PCR) is supersensitive technique which is complicated and time consuming and costly listless subjected to inter laboratory error it will not achieve routine general use. Quantitative method is a branched DNA (b DNA) signal amplification ***(Farci et al., 1992)***. Generally available and easy to perform although less sensitive than qualitative reverse-transcription-polymerase chain reaction (PCR) ***(Lunel et al., 1999)***.

The bDNA signal amplification method is based on hybridization with specific probes in the 5 non-coding region which are used to capture the HCV bDNA on the surface of the Tube ***(Lau et al ., 1994)***. The lower limit of detection is 2 $X10^5$ HCV genome equivalent/mL. The bDNA method is less sensitive than Amplicor method and may not be sensitive enough to detect virus in all pre-treatment samples. The amplicor HCV lists amplifies HCV RNA in single reaction using fairy stable enzyme rTth DNA polymerase. The detection limit is 1000 genome equivalents / ml ***(Martinet et al., 2001)***.

Immune response:

The virus specific CD4 and Th1 T cell response, that eliminate the virus during the acute stage, has to be maintained permanently to achieve long-term control of the virus ***(Gerlach et al., 2000)***.

There is no association between the course of the disease and the HLA class I alles (HLA A, B, C) which present viral antigens to CD8 cytotoxic T-cells ***(Naoumov ., 2000)***.

However, there is significant association between HLA class II alleles (DR, DQ and DP) and protection from HCV chronicity. HLA class II alleles DRBI*1101and DQB10301 are associated with viral clearance. They may be weak positive, but have not a causative role ***(Lunel et al.,1999 and Thurs et al., 1999)***.

Epidemiology of HCV:

Hepatitis C virus is carried by about 0.01-2% of blood donor's world wide ***(Alter, 1995)***.

Hepatitis C virus (HCV) has been estimated by the World Health Organization (WHO) to infect 170 million patients worldwide, with the highest prevalence rate among Egyptians (14% - 18%; approximately 10 fold greater than in the United States and Europe) **(*Mohamed, 2005)***.

The risk factors associated with acute-hepatitis C are present or past injection of drugs, previous blood or blood products transfusion, healthcare employment, sexual and house hold contact and low socioeconomic status. Egypt seems to be the highest prevalence in blood donors ***(Abdel-Wahab et al., 1994)***.

Anti-HCV was found in 12% of rural primary children 22.1% of army recruits and 16.4% in children with hepatosplenomegaly. Thalassamics patients because of repeated blood transfusions, have an anti HCV prevalence between 10-50%. Until about 1964 Therapeutic coagulation factors contained HCV this has resulted in prevalence of nearly 100%

HCV in hemophiliac patients receiving unsterilized large-pool coagulation factors ***(Makris et al., 1990)***.

Introduction of vapour-heated and recombinant clotting factors has controlled this method of spread; patients with primary hypogammaglobuinemia have developed hepatitis C after treatment with contaminated immunoglobulin. Contaminated anti-rhesus Immunoglobulin has caused large out brakes in Ireland and Germany ***(Bijoro et al., 1994)***.

Parentral exposure:

The chances of HCV after a needle-stick exposure to patients with positive HCV is 3-10% ***(Mitsuit et al., 2000)***. Dentists are at risk of acquiring HCV presumably from blood and saliva of patients. Oral surgeons are at particular risk ***(Klein et al., 1991)***. An infected surgeon can transmit HCV to patients.

Dialysis patients develop HCV, not only from blood transfusions, but also from neglected dialysis techniques ***(Okuda et al., 1995)***.

The chances of infection increases with years on dialysis injecting drug user using shared needles and syringes accounts for most HCV in USA, the infection may have occurred many years and forgotten by the patient ***(Murphy et al., 2000)***.

Sexual and interfamilial spread:

This is believed to be very low in most population studies, anti HCV doesn't appear until the age of 16 years this would suggest sexual transmission ***(Tedder et al., 1991)*** and

there are geographical differences in reported prevalence of sexual transmission. However, HCV has been linked with multiple sexual partners, prevalence in homosexuals is 3%, in prostitutes 6% and in heterosexuals attending asexually transmitted disease clinic 4%. *Interfamilia*l spread is rare but has been reported with the same strain of HCV ***(Honda et al., 1994)***. *Vertical transmission* is infrequent. It is greater if the mother is serum HCV RNA positive ***(*Ohoto *et al., 1994)***. Transmission may be increased by concomitant maternal HIV. The infection is more likely if the mother suffers an acute attack in the last trimester ***(Zanetti et al., 1995)***.

Breast milk does not transmit HCV. Babies borne to anti HCV positive mothers usually have circulating antibody for 6 months, presumably due to passive transfer. But HCV RNA is absent ***(Manzini et al., 2005)***.

While in those with no obvious risk factors family spread is rare but possible. Infection may be through sharing razors, tooth brushes or unsterile syringes and needles with people ***(Kiyoswa et al., 1994)***.

Other possibilities include post abuse of drugs and folk remedies such as acupuncture and cutting skin using non-sterilized knives. Direct questioning may reveal risk factor such as post blood transfusion or intravenous drug abuse. Hepatitis C is much less infectious than Hepatitis B as the passage of large quantities of infective material is necessary for transmission ***(Kiyosaw et al., 1994)***.

Natural history of HCV:

Hepatitis C is a disease with varying rates of progression, but is generally only slowly progressive about 15% of infected individuals recovers spontaneously ***(Consensus Panel EASL, 1999)***.

An additional 25% have an asymptomatic disease with persistently normal ALT and generally benign hepatic histology ***(Alter, 1995)***. Hence 40% of patients recover and will have a benign outcome. Where as the majority of those with raised ALT and evidence of chronic hepatic is only mild histological changes and long term out come is unknown about 20% of patients develop cirrhosis in 10-20 years. The incidence of hepatocelluar carcinoma higher in about 1-4% per year in patients with cirrhosis. Other co-factors such as hepatitis B and D are associated with more serious disease ***(Lau, 1994)***. Alcohol is also an important risk factor and intake should be recorded ***(Pession et al ., 1998)***.

Clinical course of Hepatitis C

Acute Hepatitis C:

The incubation period is about 7-8 weeks (range 2-26 weeks) prodromal symptoms are rare. Only 20% of patients become icteric. The symptoms resemble those of other forms of viral hepatitis. *Serum HCV RNA becomes positive* 1-2 weeks after infection. At 7-8 weeks, serum ALT is moderately increased to about 15 times the upper limit of normal. Clinical

diagnosis is rarely made and this depends on viral markers. Icteric hepatitis is rare and fulminate hepatic failure is controversial ***(Hoofnagle et al., 1995)***.

Those with self-limited disease develop normal serum ALT and HCV RNA become negative ***(Hoofnagle et al.,1995)***.

Chronic hepatitis C:

About 85% of those infected with HCV will not clear the virus and will develop chronic hepatitis of varying severity ***(Marcellin, 1999)***. Viral load fluctuates and in many patients declines with time**,** the disease is an indolent one extending over many years ***(Fanning et al.,2000)***.

A. Chronic hepatitis with Normal ALT:

This is seen in about one-third of patients despite detectable HCV RNA in serum. The patients are often diagnosed by change at the time of blood donation, routine medical check or during investigation for another condition. In most instances, hepatic histology shows only mild disease. HCV RNA is lower than in those with raised ALT, hepatic fibrosis progression and activity are also lower ***(Jamal et al.,1999)***.

B. Chronic hepatitis with elevated ALT:

The severity of liver disease varies considerably. Mild chronic hepatitis affects 50% of chronic cases ***(Marcellin, 1999)***.

The main symptoms are fatigue associated with musclo-skeletal pain **(*Barkhuizen et al., 1999)***. When HCV is diagnosed the quality of life falls ***(Rodger et al.,1999)***.

There is no correlation between symptoms, ALT levels and the hepatic histological score. The course is a slow one, marked by fluctuating transaminases over many years; early elevation probably represents an episode of HCV viraemia, perhaps due to quasi-species. Moderate to severe chronic hepatitis is seen in about 50% of the newly diagnosed patients with raised ALT ***(Healy et al., 1995)***.

There are no abnormal physical signs and the ALT is usually 2-10 times of the upper limit of normal but this is a poor marker of disease activity. Serum bilirubin, albumin and prothrombin time are usually normal. Serum HCV RNA values exceeding 10^5 genome equivalents per ml correlates with active disease if possible, viral genotype should be checked. Type Ib related to increased severity, worse prognosis to antivirals after liver transplantation and possible development of cancer. Liver biopsy remains the most accurate way of distinguishing mild form moderate to severe chronic hepatitis ***(Sherlock and Dooley, 2002).***

Hepatitis C in Egypt

Hepatitis C virus is considered the most common etiology of chronic liver disease in Egypt, which is the highest country wide prevalence of HCV in the world. An important cause for the high prevalence of the high exposure to HCV was the establishment of large reservoir of infection as a result of extensive Schistomiasis control programs that used intravenously administrated tartar emetic 20-50 yrs ago. The prevalence of anti HCV in adults living in rural areas especially Nile Delta is higher than the rest of Egypt ***(Abdel-Wahab et al., 1994).*** Interestingly, genotype 4 represent 90% of cases in Egypt. Chronic HCV is the main cause of liver cirrhosis and liver cancer in Egypt which is one of the top five leading causes of death in Egypt. The major route of exposure appears to be due to medical therapy and inadequate sterilization techniques and supplies in addition to blood transfusion but prior to 1994, the major risk factor associated with HCV infection is a history of antischistomal injection treatment ***(Franck et al., 2001)*.**

Genetic variability of hepatitis C virus in South Egypt and its possible clinical implication:

Egypt is one of the countries with very high rates of hepatitis C virus (HCV) related morbidity and mortality. However, little is known about geographical and clinical differences in genetic variability of HCV in Egypt. Using direct sequencing and phylogenetic analysis of partial core/E1 and

NS5B regions of the HCV genome, HCV genotype/subtype was determined in 129 HCV-infected patients residing in three governates in south Egypt: Assuit, Sohag, and Qena. According to clinical stage of infection, patients were categorized into four groups: asymptomatic carriers, chronic hepatitis C patients, liver cirrhosis, and hepatocellular carcinoma (HCC), Genotype 4a was detected in 80.6% .The prevalence of 4a differed regionally; from 88.5% (in Sohag) to 64% (in Assuit). In conclusion, geographical diversity of HCV was revealed in this study in southern Egypt ***(Elkady et al., 2006)***.

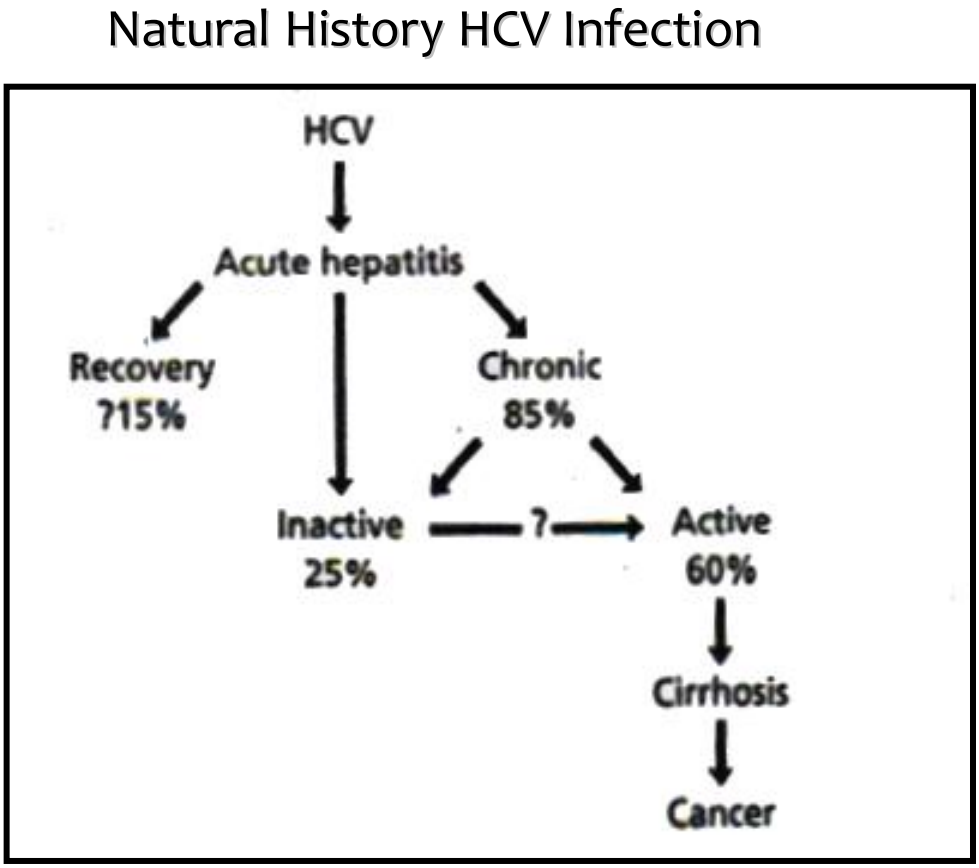

Figure (II): Natural history of HCV ***(Seeff et al., 2000).***