Laboratory parameters characterizing chronically infected HCV patients attending viral hepatitis clinic at Benghazi Medical Centre. Benghazi - Libya

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Abstract

Background: Chronic hepatitis C is one of the leading causes of chronic liver disease. There are approximately 71 million chronically infected individuals worldwide, many of whom are unaware of their infection, with important variations according to the geographical area. Hepatitis C is predominantly or almost exclusively acquired through percutaneous exposure to blood. Genotype 4 is closely related to Egypt and northern Africa while there is a predominance of genotype 1b in Western Europe and 1a in North America. The severity of disease varies from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC). Currently, hepatitis C is the leading cause for liver transplantation worldwide. Out of 100 people that contract the infection, 75–85 people will develop chronic infection, 60–70 people will develop chronic liver disease, and 5 to 20 people will develop cirrhosis over the course of their chronic infection and one to five people will die of complications including hepatocellular carcinoma (HCC). Treatments for hepatitis C have progressed to the point that more than 90% of the people who take the treatments can be cured, and, for many people, the treatment duration is much shorter than before. Now, we have interferon-free therapy. Importantly, the side effects of the newer treatments will be much less than the side effects of interferon-based therapies. Antiviral therapy now allows many patients infected with hepatitis C virus (HCV) to achieve a sustained virological response (SVR) which is namely the clearance of serum HCV RNA after therapy withdrawal, which is equivalent to cure.

Aim: To evaluate the Laboratory parameters characterizing chronically infected HCV patients attending viral hepatitis clinic at Benghazi Medical Centre.

Patients and method: The cohort in our study was chronic HCV infected patients followed viral hepatitis clinic at Benghazi medical center. Inclusion criteria were age, sex, mode of infection, ALT, AST, PT, INR, Serum albumin, Alpha-fetoprotein, HCV RNA level and grade of fibrosis using ultrasonography (USS), Fibroscan or Fibro-ACTi test. Patients co-infected with HBV or HIV were excluded. Data were analyzed using the SPSS Statistics (SPSS Inc., Chicago,US)version17.0.

Results: Out of 161 patient, 77 are male (47.8%) and 84 are female (52.2%). The people are grouped into two groups, cirrhotic and non-cirrhotic based on USS abdomen, fibro scan or fibro-ACTi test. The mean PCR for cirrhosis is 5.99 log and for non-cirrhosis 5.97 log (P=0.82). The mean albumin for cirrhosis is 3.73gm% and for non-cirrhosis 4.1gm% (P<0.001). The mean platelet for cirrhotic 168,108/micro litter and for non- cirrhotic 223,808/micro litter (P<0.001).The INR for cirrhotic 1.23 and for non -cirrhosis 1.16 with (P=0.89). The mean bilirubin for cirrhotic 0.75 mg/dl and 0.73 mg/dl for non-cirrhosis (P=0.87). The mean fasting blood glucose for cirrhosis 119.4 mg% and for non-cirrhosis102mg%(P=008).

Conclusion: Because of the big burden of HCV infection world- wide where millions of people have chronic HCV infection with a significant number of them go to cirrhosis and liver cancer, therefore the efforts to encourage HCV testing, access to care and the improvement in HCV therapy have a positive impact on patients presenting for care.

Keywords:: - HCV, Liver cirrhosis, Risk factors, Libya

Introduction:

Chronic hepatitis C is one of the leading causes of chronic liver disease. There are approximately 71 million chronically infected individuals worldwide, (1), (2) many of whom are unaware of their infection, with important variations according to the geographical area. HCV was identified in 1989, is an enveloped virus with a 9.6 kb single-stranded RNA genome, a member of the Flaviviridae family, genus Hepacivirus. There is no doubt, however, that the prevalence of HCV infection varies globally, with the highest prevalence being reported in Egypt, especially in the Nile delta, and with more than 50% of the people born before 1962 being HCV positive and the overall prevalence reported at well above 10%. Despite differences between countries, the overall prevalence is mostly between 1% and 5 %.)3(Hepatitis C is predominantly or almost exclusively acquired through percutaneous exposure to blood. The six major genotypes differ in their geographic location.

For example, there is a predominance of genotype 1b in Western Europe and 1a in North America. Among western European IDU patients, genotype 3 predominates. Genotype 4 is closely related to Egypt and northern Africa. Genotype 5 is common in southern Africa, and genotype 6 is common in Southeast Asia and in Southeast Asian immigrants in Australia. The severity of disease varies from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC). (1) The HCV lifecycle begins with virion attachment to its specific receptor. The HCV RNA genome serves as a template for viral replication and as a viral messenger RNA for viral production. It is translated into a polyprotein that is cleaved by proteases. (4) Currently, hepatitis C is the leading cause for liver transplantation worldwide. Out of 100 people that contract the infection, 75–85 people will develop chronic infection, 60–70 people will develop chronic liver disease, and 5 to 20 people will develop cirrhosis over the course of their chronic infection and one to five people will die of complications including hepatocellular carcinoma (HCC). (5) At that time 1996, HCV treatment consisted of standard interferon monotherapy that you would inject under the skin three times a week for six months. For the most common strain of hepatitis C—genotype 1—there was only a 9% chance of being cured of hepatitis C. Nowadays, treatments for hepatitis C have progressed to the point that more than 90% of the people who take the treatments can be cured, and, for many people, the treatment duration is much shorter than before. Now, we have interferon-free therapy. Importantly, the side effects of the newer treatments will be much less than the side effects of interferon-based therapies. Antiviral therapy now allows many patients infected with hepatitis C virus (HCV) to achieve a sustained virological response (SVR) which is namely the clearance of serum HCV RNA 12weeks after therapy withdrawal.(6), which is equivalent to cure.(7) The moderate response rates was achieved with a combination of pegylated interferon-alpha (PEG IFN-α) and ribavirin (RBV), then protease inhibitors was added to the standard of care for treating genotype 1 HCV.(8) New, simpler therapeutics using direct-acting antivirals that target various stages of the HCV life cycle are introduced to eradicate HCV without concomitant interferon, furthermore Patients with HIV-HCV coinfection and patients with compensated cirrhosis (i.e., cirrhosis but preserved synthetic liver function) should receive the same treatment as HCV-mono-infected patients.(9)

Methodology:

It is a retrospective study involving 161 chronic HCV patients who follow viral hepatitis clinic at Benghazi medical center. All the following data had been collected from patient's files involving: age, sex and mode of infection. Patients were exposed to investigations including Alanine aminotransferase (ALT), Aspartate transaminase (AST), Prothrombin time (PT, INR), Serum albumin, Alpha-fetoprotein, HCV RNA level. The people are grouped into two groups, cirrhotic and non-cirrhotic based on ultrasonography of abdomen, fibro scan or fibro-ACTi test. Exclusion criteria include co-infection with HBV or HIV and end stage renal disease patients. Data were analyzed using the SPSS Statistics (SPSS Inc., Chicago, US) version 17.0. Discrete variables were expressed as numbers and percentages, and continuous variables as the mean and standard deviation (SD). Differences in variables between different groups were analyzed using the Student's t-test. A p-value less than 0.050 were considered statistically significant.

Results:

Out of 161 patient, 77 are male (47.8%) and 84 are female (52.2%). The mean age for cirrhotic 49.7years and 43.3 years for non-cirrhotic (P=0.012). The mean PCR for cirrhosis is 5.99 log and for non-cirrhosis 5.97 log (P=0.82). The mean albumin for cirrhosis is 3.73gm% and for non- cirrhosis 4.1gm% (P<0.001). The mean platelet for cirrhotic 168,108/micro litter and for non- cirrhotic 223,808/micro litter (P<0.001).The INR for cirrhotic 1.23 and for non -cirrhosis 1.16 with (P=0.89). The mean bilirubin for cirrhotic 0.75 mg/dl and 0.73 mg/dl for non-cirrhosis (P=0.87). The mean fasting blood glucose for cirrhosis 119.4 mg% and for non-cirrhosis 102 mg% (P=008) Table 1. Changing ALT in regard to PCR was estimated (P=0.132)Table 2.The major risk factor for transmission was Post- surgery (45.3%), Blood transfusion (24%), after Tattooing (2%), after Needle stick (0.6%). No clear risk factor found in (27%) of patient. (Figure 1)

Table 1: Laboratory characteristics of study patients according the presence or absence of cirrhosis

	Cirrhosis	No cirrhosis	P
Age	49.33	43.38	0.012 =
PCR	996587.83	940538.25	0.826 =
ALT	94.54	67.75	0.069 =
Albumin	3.75	4.12	0.001 >
INR	1.16	1.15	0.896 =
Bilirubin	0.73	0.74	0.873 =
Platelets	165333.33	224743.59	0.001 >
FBS	121.91	102.02	0.008 =

Table.2: Characteristics of study patients according to PCR log

	Log 5 >	Log 5 <	P
Age	50.47	43.45	0.006 =
ALT	55.81	79.95	0.132 =
Albumin	3.84	4.09	0.015 =
INR	1.28	1.12	0.085 =
Bilirubin	0.69	0.74	0.515 =
Platelets	219906.25	208958.68	0.471 =
FBS	109.79	105.70	0.617 =

Discussion:

Hepatitis C virus (HCV) causes both acute and chronic infection. Acute HCV infection is usually asymptomatic, and is only very rarely associated with life-threatening disease. About 15–45% of infected persons spontaneously clear the virus within 6 months of infection without any treatment (WHO).The remaining 55–85% of persons will develop chronic HCV infection. Of those with chronic HCV infection, the risk of cirrhosis of the liver is 15–30% within 20 years (WHO). In this study we tried to identify laboratory parameters characterizing HCV chronic infection. The results obtained from our study showed that cirrhotic patients had low level of albumin (mean 3.75) compared to non -cirrhotic (mean 4.12) (p<0.001). Albumin synthesis appears to parallel liver function, i.e. the more compromised is the liver, the less is the albumin production rate. (10) Platelet count in cirrhotic patient (mean165333.33/ micro litter) compared to non- cirrhotic (mean 224743.59 /micro litter) (p < 0.001). Thrombocytopenia is a common finding in advanced liver disease. It is predominantly a result of portal hypertension and platelet sequestration in the enlarged spleen, but reduced thrombopoietin (TPO) production (synthesized in liver) further reduces measurable serum platelet counts (14).Regarding INR there was no significant statistical difference between cirrhosis and non- cirrhotic (p= 0.896). It is of the utmost importance to realize that PT and INR have substantial intra-laboratory variation in these patients. The intra -laboratory variation of the PTs is well known and was shown by Robert et al. (11) also to exist in patients with liver failure. Kovacs et al. (12) concluded that different reagents that act as thromboplastin do not result in the same INR from the same samples. The INR has been reported in several studies to be unsuitable for standardization in patients with liver disease. Instead of reflecting most injuries to liver cells, INR is typically abnormal only in patients with advanced liver disease. This test is not sensitive enough to detect a minor impairment in liver function. (15) However, a new INR specific for liver diseases (INR "LD") be more helpful than INR in this setting, as INR LD could be used to standardize PT in liver disease, according to Laurent et al M.(13) The mean FBS for cirrhotic (121.91) and (102.02) for non- cirrhotic. (p=0.008) Recent evidence suggests that chronic hepatitis C virus (HCV) infection is associated with an increased risk for the development of type 2 diabetes. Indeed, the increased prevalence of diabetes in HCV has been shown to be predominately among genotype 1– and 2-infected Subjects. (16) This study showed no significant statistical relation between HCV-RNA and ALT with cirrhosis (P= 0.826) Table 2(P = 0.069) Table 1 respectively. Neither serum HCV-RNA titer nor serum ALT level can reflect the histological liver change accurately. As a result, liver biopsy or other noninvasive procedures that measure liver stiffness (transient electrography "Fibroscan") remain essential for accurate staging of liver fibrosis in patients with chronic HCV (genotype 4) infection. (17) HCV viral load and ALT had no significant relation (P= 0.132) viral load or HCV genotype does not accurately predict the degree of liver injury in chronic HCV carriers,

Figure.1 The major risk factors for HCV transmissin

- Post-surgery
- Blood transfusion
- Tattooing
- Needle stick
- No clear risk factor
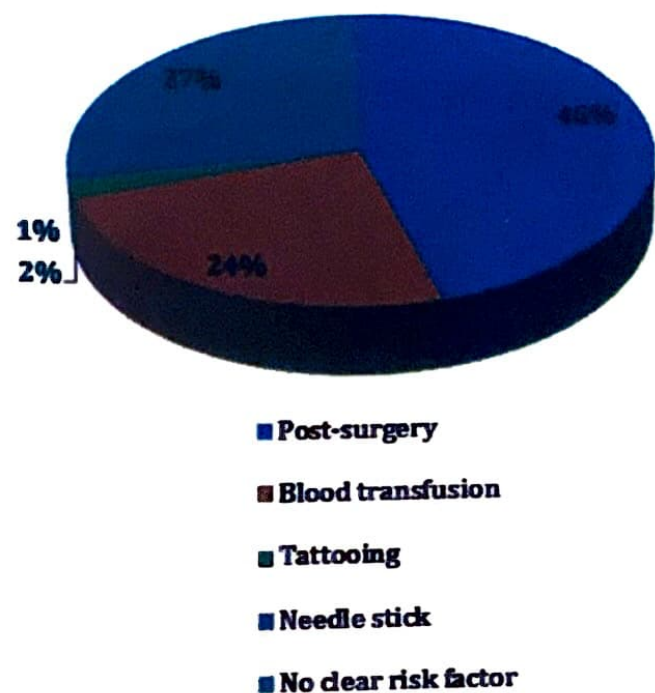
although serum ALT levels weakly correlate with portal inflammation and per portal necrosis. Thus, the histological evaluation would be the gold standard to accurately assess the degree of liver damage and to decide therapeutic plan in patients chronically infected with HCV (18). The interval between HCV infection and the subsequent development of significant liver disease is long, often measured in decades rather than years. In a Japanese cohort the mean interval between blood transfusion and diagnosis of HCC was approximately 29 years [19]. Similar observations were made in the United States with a mean interval between 19 blood transfusion and cirrhosis of 20.6 years and HCC 28.3 years [20]. Fibrosis progression is, therefore, highly variable and is influenced in particular by several host factors, such that some patients are without significant liver disease after many decades, while others rapidly develop cirrhosis. Several factors can accelerate fibrosis progression in a given individual. Age is the key factor enhancing fibrosis risk, with a stepwise reduction in the median duration to cirrhosis with advancing age at infection. Several factors can accelerate fibrosis progression in a given Individual. Age is the key factor enhancing fibrosis risk, with a stepwise reduction in the median duration to cirrhosis with advancing age at infection. Age per se also appears to be important during infection with HCV, with the rate of fibrosis progression suggested to increase in those over the age of 50 years whatever the duration of infection [21] Male sex has been consistently shown to increase fibrosis progression. Poynard et al showed male sex to be an independent risk factor for fibrosis progression with the median duration to cirrhosis reduced from 36 years for women to 26 years for men. (22) A protective role for oestrogen, possibly by inhibiting the proliferation of stellate cells and fibrogenesis is proposed as an explanation for this difference. The route of transmission of HCV has been proposed as an import-

ant factor in disease progression. In a large French study of 5,749 patients a significant increase in the prevalence of cirrhosis was found in transfusion recipients (23.4%) compared to the IDU group (7.0%). [23] This difference was independent of age at infection and duration of disease. The authors proposed that this difference could be due to either the HCV genotype or an increased amount of injected viral inoculum associated with blood transfusion. Patients with PNALT (persistent elevation ALT) were found to have lower Ishak fibrosis and necro-inflammatory scores on their first liver biopsy than patients with an elevated ALT [24, 25].

Conclusion:

Because of the big burden of HCV infection world- wide where millions of people have chronic HCV infection with a significant number of them go to cirrhosis and liver cancer, therefore the efforts to encourage HCV testing, access to care and the improvement in HCV therapy have a positive impact on patients presenting for care. Since a Substantial progress has been made in the development of treatment regimens, screening and early treatment is needed to prevent liver-related complications and mortality as current anti-HCV medications can cure more than 90% of patients with subsequent reduction of the risk of death from liver cirrhosis and cancer.

References:

1 Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017; 2: 161–176

2 European Union HCV Collaborators. Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study. Lancet Gastroenterol Hepatol. 2017; 2: 325–336

3 Antaki N, et al. The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. Liver Int 2010;30:342–355.

4 Asselah T, Bièche I, Sabbagh A, et al. Gene expression and hepatitis C virus infection. Gut 2009; 58: 846–58.

5 Alter H, Aragon T, AuBuchon J, et al. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. Morb Mortal Wkly Rep 1998; 47 (RR19):139

6 De Franceschi L, Fattovich G, Turrini F, e al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage: Hepatology 2000 Apri; 31(4); 997-1004

7 Sherman M, Bain V, Villeneuve JP, et al. Management of Viral Hepatitis: A Canadian Consensus Conference 2004. The Public Health Agency of Canada. http://www.phac-aspc.gc.ca (Version current at September 21, 2005).

8 Grattglinano I, Russmann S, Palmieri VO, Lauterburg BH, et al. Low membrane protein sulfhydril but not G6PD deficiency predict ribavirin-induced hemolysis in hepatitis C: Hepatology 2004 May; 39(5):1248-55.

9 Kohli A, Shaffer A, Sherman A, Kottilil S.Treatment of hepatitis C: a systematic review. JAMA. 2014 Aug 13 312(6):631-40.

10 Tessari, P. (2003). Protein metabolism in liver cirrhosis: from albumin to muscle myofibrils. Current Opinion in Clinical Nutrition Metabolism Care 6:79-85.

11 Tripodi A, Baglin T, Robert A, et al. Reporting pro thrombin time results as international normalized ratio for patients with chronic liver disease.J Thromb Haemost 2010; 8(6):1410-1412.

12 Kovacs MJ, Wong A, MacKinnon K, et al. Assessmen of the validity of the INR system for patients with liver im pairment. Thromb Haemost 1994; 71: 727–30

13 Laurent B, Valrerie E, Raoul P, Olivier C, Annie R.. Modified International Normalized Ratio as an Effective Way of Prothrombin Time Standardization in Hepatology Hepatology 2007;46:528-534

14 F.POORDAD, Review article: thrombocytopenia in chronic liver disease. Alimentary Pharmacology& Thera peutics. Blackwell Publishing Ltd.2007; 1365-2036.2007

15 M Sajjadieh, L Viunytska. Prothrombin time in pa tients with and without fibrotic chronic liver disease. The internet journal of Pathology . 2008 volume 8 Number 1

16 Jason M Hui et al . Insulin resistance is associated with chronic hepatitis C and virus infection fibrosis pro gression Gastroenterology ,Volume 125, Issue 6, Pages 1695–1704.

17 Reham Al Swaff Correlation between alanine aminotrans ferase level, HCV-RNA titer and fibrosis stage in chronic HCV genotype 4 infection. Egyptian Journal of Medical Human Genetics, Volume 13, Issue 2, June 2012, Pages 207–212

18- Young Sok Lee et al ,The Relationship of Histological Activity to Serum ALT, HCV genotype and HCV RNA titers in Chronic Hepatitis C, J Korean Med Sci 2001; 16: 585-91.

19 Kiyosawa, K., et al., Interrelationship of blood transfu sion, non-A, nonB hepatitis and hepatocellular carcino ma: analysis by detection of antibody to hepatitis C virus. Hepatology, 1990. 12(4 Pt 1): p. 671-5. 106

20 Tong, M.J., et al., Clinical outcomes after transfu sion-associated hepatitisC. N Engl J Med, 1995. 332(22): p.1463-6.

21 Poynard, T., et al., Rates and risk factors of liver fi brosis progression in patients with chronic hepatitis c. J Hepatol, 2001. 34(5): p. 730-9. 117

22 Poynard, T., P. Bedossa, and P. Opolon, Natural history of liver fibrosis progression in patients with chronic hep atitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOS VIRC groups. Lancet,1997.349(9055):p.825-32.

23 Roudot-Thoraval, F., et al., Epidemiological fac tors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6,664 patients. The Study Group for the Prevalence and the Epidemiolo gy of Hepatitis C Virus. Hepatology,1997.26(2):p.485-90.

24- Pradat, P., et al., Predictive value of ALT levels for his tologic findings in chronic hepatitis C: a European collab orative study. Hepatology, 2002. 36(4 Pt 1): p. 973-7

25 Alberti, A., et al., Prevalence of liver disease in a pop ulation of asymptomatic persons with hepatitis C virus infection. Ann Intern Med, 2002. 137(12): p. 961-4