Assessment of Serum Lipid Profile in patients with chronic Hepatitis C

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Abstract*:* Chronic Hepatitis C is the most common cause of liver disease and liver cirrhosis and it is due to common spreading of HCV by liver transplantation in the United State (U.S) Australia and most of Europe. Approximately 170 million people are affected by HCV worldwide and 3% are in global population. HCV is the most common chronic blood born infection in the U.S and its involved 40% in chronic cases of liver disorders (Wasley & Alter, 2000)). HCV was first time isolated from a person who has not affected with hepatitis A and hepatitis B in 1989 (QL, et al., 1989). Shortly after the cloning of HCV this new-found virus was discovered to be the cause of approximately 90% of non-A and non-B hepatitis in the U.S. (Lauer & Bruce, 2001)

Objectives: To investigate the serum lipid profile in patients with chronic Hepatitis C. To explore the variation in lipid profile due to Hepatitis C.

Methodology: Fasting blood samples was collected from patients that are already diagnosed with Hepatitis C virus infection in a cold Lithium heparin anticoagulant and immediately centrifuged at 8000xg 4^{o}C for 5 minutes. To minimize the losses of lipid metabolism the sample was stored at -80^{o}C and examined the all parameter of lipid profile on Hitachi 912 (routine chemistry analyzer).

Results: In this study 100 subjects were taken in this 59 (59%) were male and 41 (41%) were female. in this study HCV positive patients were included which were already diagnosed by using Bio Molecular technique PCR and then blood specimen of these 100 patients were examined for assessment of serum lipid levels. All the parameters (Cholesterol, Triglycerides, LDL Cholesterol, HDL Cholesterol, and VLDL Cholesterol) of lipid profile were performed on automatic Hitachi 912 (Instrument which is used for routine chemistry analysis). In these 63 % patients was normal, 3% of hyperlipidemic and 34% were hypolipidemic patients.

Conclusion: This study indicates that the advancement of Hepatitis C viral infection can lead to decrease level of lipid profile**.**

Keywords.: HCV (Hepatitis C Virus), HDL (High density lipoproteins), LDL (Low density lipoproteins), VLDL (Very low density lipoproteins), HBV (Hepatitis B Virus), RIBA (The Recombinant immunoblot Assay), PCR (Polymerase Chain Reaction), NHANES (National Health and Nutrition, Examination Survey).

Introduction: HCV infection is a public health problem. Approximately 170 million people worldwide and 200,000 in UK are infected with HCV (An RNA virus of the Flavivirus family up to 2% of people have been expressed to HCV with high rate in Asia and Africa)(Marks , Webster, & Baloom, 2002).Recent studies indicate that HCV accounts for 70% of all cases of chronic Hepatitis and is responsible for 30% of all liver transplants performed in developed countries. Persistent HCV infection triggers immune response resulting in intrahepatic inflammation.(Feng, et al., 2014).Chronic Hepatitis C is the most common Cause of liver disease and liver cirrhosis and it is due to common spreading of HCV by liver transplantation in the United State (U.S) Australia and most of Europe. Approximately 170 million people are affected by HCV worldwide and 3% are in global population. HCV is the most common blood borne infection in the U.S and it is involved 40% in chronic cases of liver disorder (Wasley &

Alter, 2000).HCV was first time isolated from a person who has not affected with hepatitis **A and hepatitis B** in 1989.(QL, et al., 1989). Shortly after the cloning of HCV this newfound virus was discovered to be the cause of approximately 90% of non-A, non-B Hepatitis in the U.S. (Lauer & Bruce, 2001)

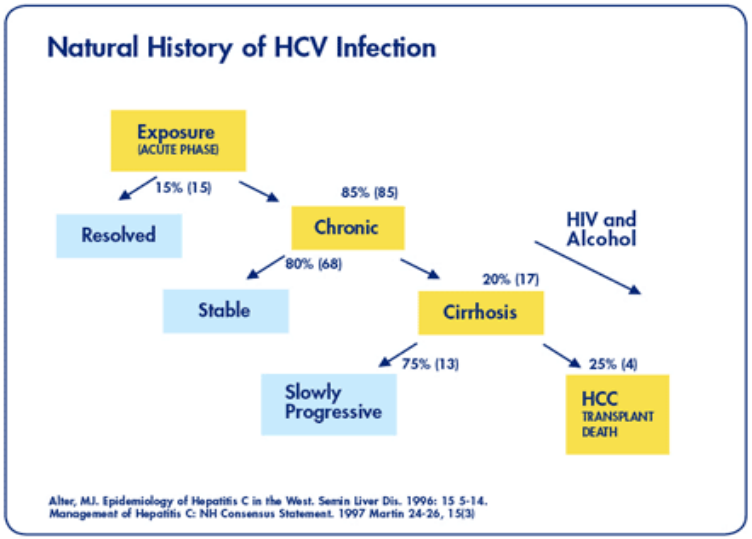

Figure 1

Natural history of HCV in the form of diagram (Alter, 1997)

What is hepatitis?

"Hepatitis" means inflammation of the liver. Toxins, certain drugs, some diseases, heavy alcohol use, bacterial and viral infections can all cause hepatitis. Hepatitis is also the name of a family of viral infections that affect the liver; the most common types are hepatitis A, hepatitis B. and hepatitis C.

What is Hepatitis C?

Hepatitis C is a contagious liver disease that range in severity from a mild illness lasting a few weeks to a serious, lifelong illness that attacked the liver. It results from infection with the Hepatitis C virus (HCV) which is spread primarily through contact with the blood of an infected person Hepatitis C can be either "acute" or "chronic".

HEPATITIS C VIRUS

Important properties: HCV is a member of Flavivirus family. HCV is an enveloped virion containing a genome of single standard positive polarity RNA. It has no virion polymerase. HCV is replicates in the cytoplasm of hepatocytes but is not directly cytopathic.

Replication Cycle: The replication of HCV is not exactly known because it has not been grown in the cell culture. Other viruses which are related to the Flavivirus family replicate in the cytoplasm of hepatocytes and then translate the genome RNA into large polypeptides, from which functional proteins are cleaved by virion encoded protease. This protease is the main target to treat the HCV. This replication of HCV in the liver is enhanced by a liver specific micro-RNA. The principle of this micro-RNA is to increase the synthesis of mRNA. Micro-RNA is also known as to enhance the synthesis of mRNA in cells of the tissue (Levinson, 1989).

Routes of Transmission: Human is a primarily reservoir of HCV. Transmission of HCV is primarily via infected blood; risk of transmission includes blood transfusion before 1992. The other than transmission factors are

- Uses of intravenous drugs
- High risk sexual activity
- Solid organ transplantation from infected Donor
- Occupational exposure
- Haemodialysis
- Birth to an infected mother
- Intra nestle cocaine use

Chronic hepatitis C infection: Chronic hepatitis C is noticeable by the persistence of HCV polymer in infected patients for a minimum of 6 months after the exposure of acute infection. HCV is self-limiting in exactly 15%-25% of patients in whom HCV polymer within the blood serum becomes undetectable. Approximately 75%-85% of infected patients don't clear the virus in 6 months, and chronic infectious disease develops. The rate of chronic HCV infection is suffering from several factors, including the age at time of infection, gender, ethnicity, and the development of jaundice throughout the acute. (Stephen & Timothy, 2006)

Risk factors for developing chronic HCV infection

Risk factors:

- Male gender
- Immunosuppression
- African American race

- Age at the time of infection>25 years
- HIV infection
- No jaundice for symptoms during acute infection

Pathogenesis and immunity: HCV taints liver cells (hepatocytes) fundamentally however there is no proof for an infection incited cytopathic consequences for liver cells. The demise of hepatocytes is presumably caused by resistant assault by cytotoxic T cells. HCV contamination unequivocally inclines to hepatocellular carcinoma however there is no confirmation for an oncogene in viral genome or for an inclusion of duplicate of viral genome into DNA of a tumor cells (Levinson, 1989). Higher admission of liquor enormously upgrades the hepatocellular carcinoma in HCV contaminated individual so we can state that the disease is caused by drawn out harm of liver and resulting strange development of liver cells (Levinson, 1989). Antibodies against HCV are made however around 75% of patients are incessantly tainted and keep on producing infection for no less than multi year. Unending carriage of HCV is higher than HBV. Perpetual dynamic hepatitis and cirrhosis happen around 10% of these patients. In once the disease clear then it isn't known precisely whether reinfection can happen or it is a long lasting invulnerability (Levinson, 1989)

Clinical findings: Clinically the acute infection of HCV is mild rather than infection HBV. Patients presents with these findings:

- Fever
- Anorexia
- Nausea
- Vomiting
- Jaundice

Dark urine, pale faces and elevated transaminase level are seen (Levinson, 1989). HCV infection also leads to significant autoimmune reactions including:

- Vasculitis
- Arthralgia
- Purpura
- Membranoproliferative glomerulonephritis.(Levinson, 1989)

Laboratory diagnosis: HCV infection can diagnose by detecting the antibodies against this virus by Elisa because false positive result can occur in an Elisa technique then we use RIBA (recombinant immunoblot assay) for confirmation, if the result of RIBA is positive then a PCR base test perform that detects the presence of viral RNA in the serum of infected individual.(Levinson, 1989)

Isolation of virus from the patients cannot do. A chronic HCV infection diagnose by elevated level of Transaminase. (Levinson, 1989)

Lipids

What are lipids?

Any classes of organic compounds that are fatty acids or their derivatives are insoluble in water but soluble in organic solvents. They include many natural oils, waxes and steroids are called lipids.

What is lipid profile?

Lipid profile or lipid panel is a panel of blood tests that serves as an initial board. Medical screening tool for abnormalities.

Parameters of lipid profile test:

- Cholesterol
- Triglycerides
- High density lipoproteins (HDL Cholesterol)
- Low density lipoproteins (LDL Cholesterol)
- Very low-density lipoproteins (VLDL)

Lipid metabolism: Liver plays a central role in lipid metabolism as several pathways are at least in part dependent to this site. Major metabolic processes take place at this level involving the production transportation and storage of apoproteins and lipoproteins as well as catabolism of various lipids and excretion of cholesterol and phospholipids and alteration in liver function resulting from cellular injury leads to change in the serum concentration of cholesterol and lipoproteins (Criston , Costin , Lilana S, & Ion, 2012).Infection with (HCV) leads to hepatic damage which in turn relates to change in alterations of lipid metabolism. Different mechanisms involved dependent on the stage of the liver disease and the metabolic state. Low levels of plasma cholesterol and lipoproteins as well as lower triglyceride (TG) values are usual in chronic liver disease. However, the number of studies which included patients with advanced cirrhosis remains low.(Criston , Costin , Lilana S, & Ion, 2012)

Replication of the hepatitis C virus (HCV) involves several important host lipid interactions. Hepatitis C virion complex with have lipoproteins to from Lipoviroparticles in the host flow. Lipoviroparticles may then utilize low thickness lipoprotein (LDL) receptors on hepatocytes as one component of cell section furthermore the HCV envelope glycoprotein E2 has been exhibited in vitro to bind to

lipoprotein and lead to enhanced LDL receptor and CD81 binding. Once inside the hepatocytes, HCV replication requires geranylation of the host protein FBL2 a process dependent on the host cholesterol synthesis pathway. Interruption of this pathway results in dissolution of HCV replication complex. Further, HCV secretion appears to be tied to host apolipoprotein B secretion. Interaction between HCV and host lipids has been shown in clinical studies. Lower serum cholesterol and LDL levels are found in patients infected with hepatitis C when compared with compared with patients with hepatitis B or without infection,(Corey, et al., 2011)

Methodology:

Technique for collecting Venous Blood:

The steps involved in this technique are described below:

1. The patient should sit in a chair. Position the patient and extend the patient's arm.
2. Apply the tourniquet 3-4 inches above the selected puncture site. Don't place too tightly or leave on more than 2 minutes (and no more than a minute to avoid increasing risk for haemo concentration). Wait 2 minutes before reapplying the tourniquet.
3. The patient should make a fist without pumping the hand.
4. Select the venepuncture site.
5. Prepare the patient's arm using alcohol swab. Cleanse in circular fashion, beginning at the site and work outward. Allow to air dry.
6. Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the Vein. The needle should form a 15 to 30-degree angle with the surface of the arm. Swiftly insert needle through the skin and into lumen of the vein. Avoid trauma and excessive probing.
7. When the last tube to be drawn is filling, remove the tourniquet.
8. Remove the needle from patient's arm using a swift backward motion.
9. Press down on the gauze once the needle is out of the arm, applying adequate pressure to avoid formation of hematoma.
10. Dispose of contaminated materials/supplies in designated containers.
11. Mix and label all appropriate tubes.

Fasting blood sample was collected from patients that are already diagnosed with Hepatitis C virus infection in a cold lithium heparin anticoagulant and immediately centrifuged at 8,000xg 4 °C for 5 minutes. To minimize the losses of lipid metabolism, the samples was stored at -80 °C.

Polymerase chain Reaction (PCR): Polymerase chain reaction is basic molecular technique which is used for amplification of the required gene in three steps.

1. Denaturation
2. Primers Annealing
3. Extension

Qualitative PCR: In this study HCV qualitative is performed on automatic Abbot Molecular m2000 real-time system (Abbot Molecular, Wiesbaden, Germany) for this purpose 800 ul of patient sample is loaded on to the instrument which quantifies the simple and gives results in international units per milliliter (IU/ml).

Genotyping PCR: HCV genotyping is performed using multiplex PCR containing different primers for different genotypes as described by Company kit for this purpose, HCV RNAis extracted using RNA extraction kit from Qiagen (Qiagen, Hidden, Germany).Extracted RNAis subjects to reverse transcription to make cDNA. This cDNA is amplified with type specific primers to get HCV genotypes.

New Hepatitis C virus (HCV) Genotypes System that Allows for identification of HCV genotypes la, 1b,2a, 2b,3a,36,4,5a, and 6a.

Lipid profiling: Lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. All the parameter (Cholesterol, Triglycerides, HDL Cholesterol LDL Cholesterol, VLDL Cholesterol)

Of lipid profile were performed on automatic Hitachi 912 (Instrument which is used for routine Chemistry analysis) for this purpose 400 ul of patient sample is loaded on to the instrument which analyze the sample and give result in milligram per deciliter (mg/dl).

Results: In this study 100 subjects were taken, out of which 59 (59%) were male and 41 (41%) were female. In this study HCV positive patients were included which was already diagnosed by using. Bio Molecular technique PCR and then blood specimen of these one hundred patients were examined for assessment of Serum lipid level. All the parameters (Cholesterol, Triglycerides, HDL Cholesterol LDL Cholesterol, VLDL Cholesterol) of lipid profile were performed on automatic Hitachi 912 (Instrument which is used for routine Chemistry analysis).In these one hundred 63% patients were normal, 3% had raised level of lipid profile and 34% had low level of lipid profile.

Table No 1.1: Age of patients

	N	Minimum	Maximum	Mean	Std. deviation
Patients	100	30	72	54.82	8.365

age					

In these 100 subjects maximum age of patient is 72 year and minimum age is 30year

Table No 1.2: Gender distribution

Gender	Frequency	Percentage%
Male	**59**	**59.0%**
Female	**41**	**41.0%**
Total	**100**	**100.0%**

Out of 100 subjects 59 (59%) were male and 41 (41%) were females.

Table No 1.3: Frequency distribution of lipid profile results

Results	Frequency	Percentage%
Normal	**63**	**63%**
Raised lipids	**03**	**3.0%**
Lowered lipids	**34**	**34%**
Total	**100**	**100.0%**

Out of 100 patients 63 (63%) were normal 3 (3%) had high level of lipids and 34 (34%) low level of lipid profile.

Discussion: HCV infection is a public health problem. Approximately 170 million people worldwide and 200,000 in UK are infected with HCV (an RNA virus of the Flavivirus family up to 2% of people have been expressed to HCV with high rates is Asia and Africa (Marks , Webster, & Baloom, 2002).

Chronic hepatitis C is the most common cause of liver disease and liver cirrhosis and it is due to common spreading of HCV by liver transplantation is the United State (U.S), Australia and most of Europe. Approximately 170 million peoples are affected by HCV worldwide and 3% are in global population. HCV is the most common chronic blood borne infection in the U.S and it is involved 40% in chronic cases of liver disorders. (Wasley & Alter, 2000)

The spectrum of severity of chronic hepatitis C varies widely as done the rate of its progression to the cirrhotic stage. This heterogeneity largely depends on host and environmental factors, although the contributing role of viral features such as the HCV genotype has recently been revisited. Cofactors influencing hepatitis C severity and progression include age, gender, and excess alcohol consumption, co infection with other hepatotropic viruses and/or HIV and the metabolic syndrome. The role of the latter in the pathogenesis of hepatitis C has attracted considerable attention in recent years.

HCV virion circulate in serum bound to lipoproteins; lipids have been shown to modulate (and indeed are essential for) the HCV life cycle and an occasionally severe accumulation of triglycerides in hepatocytes is observed in a distinct subgroup of patients in the form of fatty liver. In summary lipid metabolism shows widespread alteration conferring an idiosyncratic profile to HCV infection. This review will discuss these aspects focusing on both their molecular mechanisms and their clinical consequences. ((Criston , Costin , Lilana S, & Ion, 2012)

Conclusion: This study indicates that the advancement of Hepatitis C viral infection can lead to decrease level of lipid profile.

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