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INTER-DEPENDENCE OF HOST LIPID METABOLISM INSCRIPTION AND THEIR CRITICAL ROLE IN THE REGULATION OF HEPATITIS C VIRUS REPLICATION

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ABSTRACT

BACKGROUND: Hepatitis C Virus (HCV) is defined as a lethal human pathogen affecting around 170 million individuals whole world. HCV remains one of the leading chronic liver diseases such as chronic hepatitis, cirrhosis, fibrosis and hepatocellular carcinoma. For the current study hundred (n=100) healthy individuals and hundred (n=100) patients of HCV treated with Interferon (IFN) were substituted in two groups. About 5ml blood was drawn and serum was separated for the evaluation of various biochemical variables i.e., ALT, MDA, IL-6, TNF-α and lipid profile (TCH, Tg, LDL and HDL) with the help of their respective ELISA and spectrophotometric methods. Results of current study show that there was a significant high levels of ALT, MDA, IL-6, TNF-α, Tg, TCH and LDL were increased significantly (p=0.001, p=0.011, p=0.012, p=0.014, p=0.026, 0.001 and 0.023 respectively) in patients (87.21±7.12 IU/L, 7.16±0.99 nmol/ml, 7.19±1.13 pg/ml, 32.20±4.55 pg/ml, 3.26±0.17 mmol/l, 5.19±1.25 mmol/l and 7.19±1.08mmol/l respectively) as compared to healthy controls whereas levels of HDL were decreased significantly (p=0.016) in HCV patients (1.45±0.195 mmol/l) contrast with healthy controls (1.67±0.331 mmol/l). Current study concludes that increased viral load in HCV patients treated with IFN therapy has direct correlation with oxidative stress, hepatic injury and lipid profile. Increased viral load results in increased TCH, Tg, and LDL levels.

Keyword: Interferon-α, HDL, LDL, IL-6, TNF-α

INTRODUCTION

Hepatitis C virus (HCV), a small member of Flaviridae family is one of the most important single stranded RNA positive virus affecting human health. It is estimated hepatitis C virus affects over 170 million people of the world's population [1]. Long term HCV infection may results in the development of liver cirrhosis and hepatocellular carcinoma (HCC) [2]. As usual suppression of chronic HCV infection is enormously infrequent, interferon (IFN) based antiviral therapy had been carried out but with limited efficacy [3]. HCV infection is exclusively affects lipid metabolism and cholesterol homeostasis at various strategic phases of HCV life cycle [4]. In clinical practice the association of HCV with lipid metabolism has been perceived extensively as an increase in cytosolic lipid droplets of neutral lipids have been presented by infected patient's liver biopsies [3]. Tremendously close association between lipoprotein metabolism and infection of HCV has been indicated by the secretion of HCV by liver as highly infectious lipoviral particles (LVPs) which shows surface expression of apolipoprotein E (apo E) and apolipoprotein C (apo C) that are the hallmarks of lipoprotein [5]. Better understanding about the close association of lipoprotein metabolism and the life cycle of HCV is quiet essential because in the model of HCV infection virus causes chronic infection while simultaneous with lipoprotein and taking over the lipoprotein metabolism. Each step of the life cycle of HCV is the potential target for anti HCV therapy that is associated with lipoprotein metabolism, there is the need to identify such targets arises.

The inconsistent lipid metabolism triggered by HCV infection may results in liver damage and hepatocarcinogenesis. It is reported that the membrane of endoplasmic reticulum (ER) has membranous web which contains non-structural HCV proteins including NS3/4A, 4B, NS5A/5B and newly synthesized HCV-RNA is responsible for the replication of HCV [6,7]. Membranous web assembly is effected by Phosphatidylinositol-4-kinase IIIa (PI4K IIIa) mediated alteration in the phosphorylation of HCV NS5A protein that is responsible for membranous web generation [8]. Initial step of membranous web generation occur in the cytosolic side of endoplasmic reticulum membrane which work together with LDs of cytosol. Crucial protein of HCV is connected with lipid droplets (LDs) which is then interact with NS2 and NS3-4A and recruited to the site of HCV assembly. In the next step there would the formation of lipid bilayer consisting of two glycoproteins i.e E1 & E2 and then assimilated into completely infective form of virus with a core of RNA and a capsid [9].

Interaction of glycoproteins i.e E1/E2 with juvenile particles is mediated by NS2, a transmembrane protein having perilous role in assembly of infectious virus [10]. During maturation newly synthesized virus particles combine with pre-very low density lipoproteins (pre-VLDLs) to fuel the contribution of luminal lipids droplets, apo B-100. Apo E and apo C-1in the development of lipo-viral particles (LVPs) that is responsible for the formation of HCV and VLDL hybrid particles. Apo B-100 is not an indispensable for morphogenesis of hepatitis C virus-lipo viral particle (HCV-LVP) but apo C-1 and E are absolutely necessary for intracellular morphogenesis of hepatitis C virus-lipo viral particle (HCV-LVP) [11]. In any case maturation of infectious virus is facilitated by engagement with the pathway for the assembly of very low density lipoproteins (VLDL). In this way the progression for the secretion and maturation of HCV particles is associated with the secretion and assembly of VLDL. Inhibition of microsomal TG transfer protein (MT) is considered the suppressor of HCV replication as it has very significant role in VLDL progression [12].

HCV restrict the production of Apo B-100 and over storage of TG results in liver disease i.e hepatic steatosis. Secretion of infectious HCV-LVP diminished by the inhibition of Apo E, as it is a key molecule of HCV-LVP and VLDL. These outcomes specify close association between HCV replication and VLDL production and suggest the implication of VLDL as a potential target of anti-HCV therapy. Some studies suggest that HCV replication is associated with fatty acid and phospholipid metabolism. During HCV infection fatty acid synthase is upregulated and required for the promotion of HCV replication because the formation of new membrane during the progression of HCV replication likely require the synthesis of phospholipids. Expression of the genes that are involved in lipid metabolism is moderated in HCV replication and this moderation is controlled by Sterol regulatory element-binding proteins (SREBP) [13]. Poly unsaturated fatty acid (PUFAs) inhibits the replication of HCV while it is stimulated by monounsaturated fatty acids [14]. Vitamin E or decreased cellular level of cholesterol may modulate the peroxidation of Poly unsaturated fatty acid which ultimately results in the inhibition of HCV replication. On the other hand HCV replication is inhibited by peroxidation of poly unsaturated fatty acid that is carried out by HCV replication induced sphingosine kinase-2 [15]. These outcomes intensely recommend that influence of lipid metabolism on liver cells may be an imperative therapeutic strategy to hinder the replication of HCV.

MATERIALS AND METHODS

Present study was performed to evaluate the role of interferon therapy in hepatitis C patients. Demographic data was collected to initiate this study. Various demographic variables including Weight, Age, BMI and Viral load before and after treatment with interferon therapy were analysed. 100 patients having Hepatitis C and receiving interferon treatment from last six months were included in the study. Whereas 100 age and sex matched healthy individuals with no congenital disease were taken as controls. Patients were excluded if they were infected with hepatitis B virus (HBV), decompensated liver disease or human immunodeficiency virus (HIV) infection, consuming alcohol more than 50 grams/day, or liver disease because of other causes (hemochromatosis, steato-hepatitis). Informed consent was taken from each individual before including him in the study. An approval was taken from the Research Ethical Committee of The Institute of molecular biology and biotechnology (IMBB), The University of Lahore to perform various biochemical assays. Blood was drawn from the cubital vein for the biochemical analysis that is centrifuged and serum was separated and stored at -70˚C in refrigerators.

BIOCHEMICAL ASSAYS

Levels of Alanine Transferase (ALT) were measured by ELISA Kit method to evaluate the liver damage. Whereas Lipid Profile (TCH, Tg, HDL and LDL) and Inflammatory markers including Interleukin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-α) were also measured through ELISA Kit. Significant stress marker that is Malondialdehyde (MDA) was measured through spectrophotometer through the method of Ohkawa *et al.,* 1979 [16].

RESULTS

Present study provides both demographic variables and prognostic variables among control and subjects. Demographic data showed that there was insignificant (p=0.231 and 0.512) difference of weight and age among healthy controls (79.76±5.216 Kg and 45.98±2.19 yrs) and patients (89.98±6.34 Kg and 49.77±3.26 yrs) whereas age and viral load after and before treatment were differ significantly between controls (17.26±1.01, 0 and 0) and subjects (26.32±3.01, 1.56±0.09 and 0.12±0.18) respectively.

Results presenting prognostic variables in table-02 showed that there was significant increase (p=0.001) in the levels of ALT (87.21±7.12 IU/L) that leads to increase oxidative stress and inflammation in patients infected with HCV receiving IFN therapy. Levels of MDA, IL-6 and TNF-α were increased significantly (p=0.011, p=0.012 and p=0.014 respectively) in patients (7.16±0.99 nmol/ml, 7.19±1.13 pg/ml and 32.20±4.55 pg/ml) as compared to healthy controls (1.06±0.156 nmol/ml, 4.16±0.88 pg/ml and 21.16±3.26 pg/ml). Moreover lipid profile were also affected due IFN therapy that was assessed by measuring the levels of Total cholesterol (TCH), Thyroglobulin (Tg), Low density lipoprotein (LDL) and High density lipoprotein (HDL). Results presented in table 02 showed that levels of TCH, Tg and LDL were increased significantly (p=0.001, p=0.026 and p=0.023 respectively) in HCV patients (5.19±1.25 mmol/l, 3.26±0.17 mmol/l and 7.19±1.08 mmol/l) as compared to healthy controls (2.16±0.25 mmol/l, 1.21±0.095 mmol/l and 2.28±0.512 mmol/l). Whereas, HDL levels were decreased significantly (p=0.016) in patients (1.45±0.195 mmol/l) as compared to healthy controls (1.67±0.331 mmol/l).

Table 1: Demographic Variables Of HCV Patients Receiving Interferon Therapy

VARIABLES	CONTROL	SUBJECTS	P(<0.05)
Weight	79.76±5.216	89.98±6.34	0.231
Age	45.98±2.19	49.77±3.26	0.512
BMI	17.26±1.01	26.32±3.01	0.013
VLBT	0	1.56±0.09	0.000
VLAT	0	0.12±0.18	0.000

VLBT (Viral Load before treatment). VLAT (Viral load after treatment)

Table 2: Variables Of Prognostic Importance Of HCV Receiving Interferon Therapy

VARIABLES	CONTROL	SUBJECTS	P(<0.05)
ALT (IU/L)	17.19±3.22	87.21±7.12	0.001
MDA (nmol/ml)	1.06±0.156	7.16±0.99	0.011
IL-6 (pg/ml)	4.16±0.88	7.19±1.13	0.012
TNF-α (pg/ml)	21.16±3.26	32.20±4.55	0.114
TCH (mmol/l)	2.16±0.25	5.19±1.25	0.001
Tg (mmol/l)	1.21±0.095	3.26±0.17	0.026
LDL(mmol/l)	2.28±0.512	7.19±1.08	0.023
HDL(mmol/l)	1.67±0.331	1.45±0.195	0.016

Table 3: Correlation S' Coeeficients Matrix Of Hcv Patients Receiving Interferon Therapy

VARIABLES	ALT	MDA	IL-6	TNF-α	TCH	Tg	LDL	HDL	VLBT	VLAT
ALT	0	0.816	0.746	0.661	0.589	0.416	0.649	-0.641	0.456	0.615
MDA		0	0.561	0.661	0.674	0.516	0.845	-0.671	0.562	0.567
IL-6			0	0.726	0.426	0.326	0.745	-0.526	0.126	0.326
TNF-α				0	0.645	0.216	0.641	-0.651	0.619	0.715
TCH					0	0.452	0.465	-0.5612	0.756	0.819
Tg						0	0.326	-0.625	0.415	0.319
LDL							0	-0.745	0.669	0.749
HDL								0	-0.684	-0.819
VLBT									0	-0.516
VLAT										0

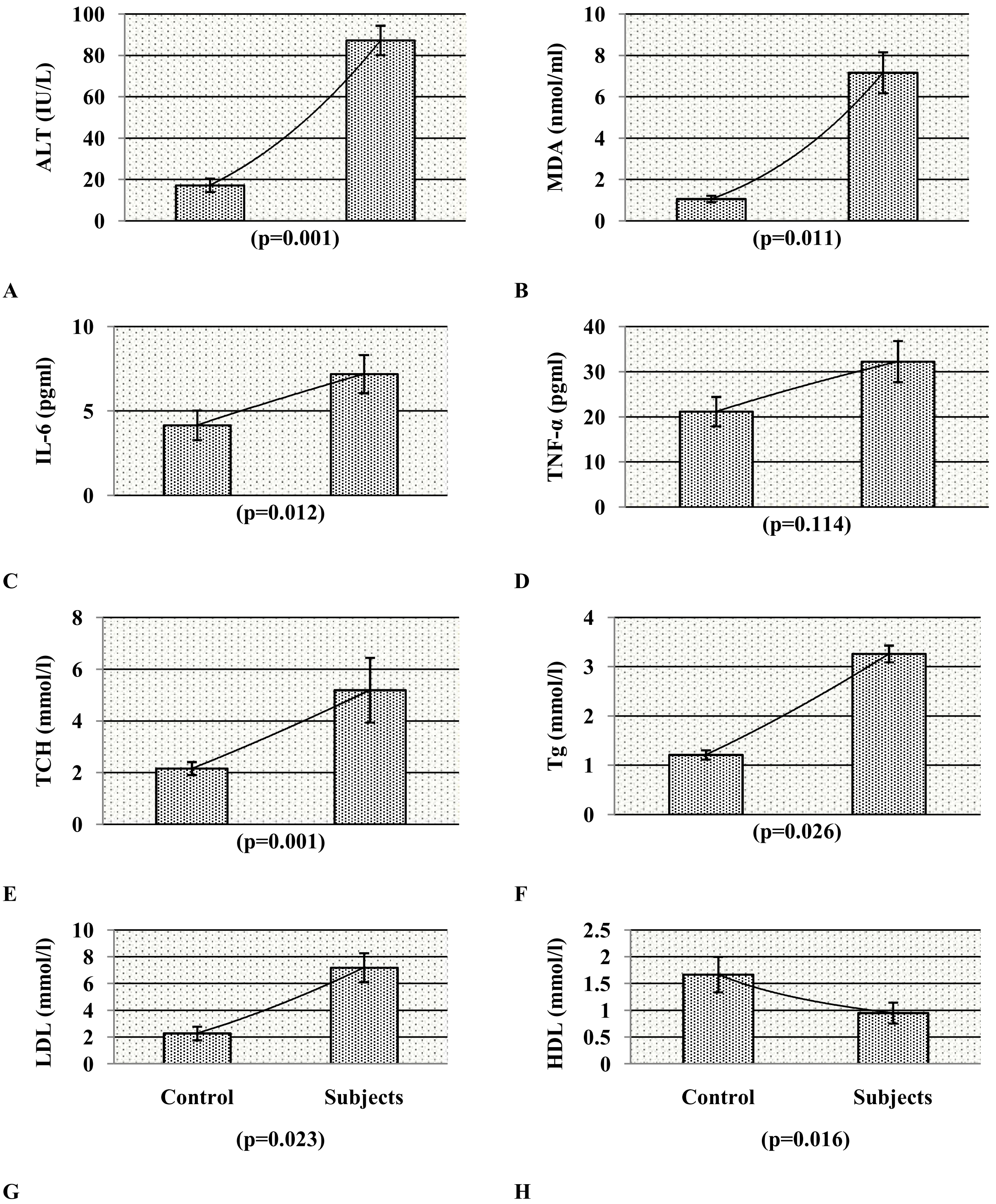

Figure 1: Variables Of Prognostic Importance Of Hcv Receiving Interferon Therapy

DISCUSSION

Present study was performed to evaluate the role of viral load and lipid profile in HCV patients receiving IFN therapy. HCV belongs to hepacivirus genus that links with flaviviridae family that composed of GB virus, pestivirus and flavivirus [17]. HCV can be divided in to seven major genotypes numbers accordingly 1, 2, 3 etc. but most prevalent are genotype 1 that shows poor response against IFN-α therapy [18]. Viral infection of hepatitis C leads to the cirrhosis of liver and resulting in significant hepatic damage. Hepatic damage can be assessed by measuring the liver profile and through biopsy of liver [19]. In present study there was a significant increase in the levels of ALT due to increase oxidative stress that results in liver injury as shown in table 03 (ALT vs. MDA, r=0.816***). Increased in the liver profile was due to viral infection of hepatitis C that is due to IFN induction through IFN Receptors (IFNRs) present on the cell membrane of all organs specifically on liver that induces liver toxicity [20]. Severe toxicity will leads to the production of reactive oxygen species (ROS). ROS including superoxide radical, oxygen radical and hydrogen peroxide attacks on the lipid content of the cell membrane and generate Malondialdehyde (MDA) that is a significant marker of oxidative stress and can be measured from serum through its specific protocol [16]. Oxidative stress will lead to the inflammation of liver those results in production of various inflammatory cytokines including IL-6 and TNF-α [21]. IL-6 specifically produced in results of lethal infection and tissue injuries that results in production of TNF-α (IL-6 vs. TNF-α, r=0.726**). These ILs were produced in acute response against stress condition due to the activation of defence system that is either through the haematopoiesis or immune reactions. IL-6 production and expression is controlled by transcriptional and posttranscriptional mechanism that plays a key role in ILs production, any disturbance in the mechanism will leads to severe pathological conditions that might induce autoimmunity or inflammation [22]. Stress markers that are generated in response of heavy viral load leads to the activation of a NF-kB pathway that is present in the cytosol and after activation moves inside the nucleus results in activation of TNF-α [23].

HCV has direct association with the lipoproteins of the host through taking up LDL and VLDLs from specific receptors (LDL receptors) present on the cell membrane [24] moreover MDA levels directly increased the LDL levels (MDA vs. LDL, r=0.845***). Antibodies that are produced from the HCV envelope may showed its significant effect on HCV lipid containing envelope, moreover these HCV antibodies provide effective pathway to the virus to enter in to the host hepatocyte [25]. HCV core protein confines with apolipoprotein AII at the surface of lipid droplets that shows a strong relationship between the protein expressed by HCV and cellular lipid metabolism. Moreover, various HCV encoding proteins have exclusive membrane anchors, localize at cytoplasm. HCV replication is associated with frequent cellular lipid factors and lipid droplet [26]. It's still unclear that either lipid factors enhanced the HCV entry in the cell or increased the viral replication. In this study, present results demonstrated a positive correlation between HCVs with Tg, TCH, LDL levels, whereas there is depletion of HDL as the viral infection increases. Depletion of HDL and increase in LDL, Tg and TCH showed that there is a lethal disruption in the lipid profile of patient infected due HCV (LDL vs. HDL, r=-0.745**). Moreover significant increase in the BMI levels of HCV patients also showed that there is a significant increase lipid content in HCV patients, as the HDL is good lipoproteins and high levels of HDL is beneficial for the body [27].

CONCLUSION

Present study suggests the role of IFN therapy over the viral load in the HCV patients. It shows increased liver damage and oxidative stress in the patients of HCV. It concludes that viral load in the HCV patients increases with the IFN therapy and leads to the increased liver damage and oxidative stress. Moreover HCV infection leads to disruption of lipid profile by increasing TCH, Tg and LDL levels and depletion of HDL.

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CONFLICT OF INTEREST

Authors declares no conflict of interest

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