



Clinical Evaluation and Serum Lipid Profile between Individuals with Acute Hepatitis C

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SQA, FNT and NAC designed the experiment. Author RK helped in samples collection. Author SQA conducted the experiment and analyzed the data. Author FNT supervised experiment design, data analysis and reviewed manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBcRR/2015/14989

Editor(s):

(1) Carmen Lúcia de Oliveira Petkowicz, Federal University of Parana, Curitiba, Parana, Brazil.

Reviewers:

(1) Hasan Sedeek Mahmoud Ali, Tropical Medicine and Gastroenterology Department of South Valley University, Egypt.

(2) Julieta Trinks, Instituto de Ciencias Básicas y Medicina Experimental, Argentina.

(3) Anonymous, Brazil.

(4) Anonymous, Brazil.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=846&id=3&aid=7572>

Original Research Article

Received 30th October 2014
Accepted 13th December 2014
Published 31st December 2014

ABSTRACT

Aim: Hepatitis C virus (HCV) exerts an intense impact on host lipid metabolism. It has been shown that the synthesis of cholesterol and fatty acids (FA) is directly affected in HCV patients but serum FA profile of acute HCV patients have not been directly quantified in humans.

Methodology: In present study the serum lipid and FA's profile (free and total) of acute hepatitis C patients (n=50) is evaluated in comparison to healthy controls (n=50). The acute HCV patients were diagnosed by center of diseases control (CDC) criteria. Blood hematology, serum proteins, enzymes, waste metabolites and nutrition status were also assessed by standard methods.

Results: The acute HCV patients have significantly lower (P<0.05) lipid profile including triglycerides, cholesterol, HDL and LDL in relation to controls. Results of serum lipid FA's (total and

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free form) reveal elevated level of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), with lower content of polyunsaturated fatty acid (PUFA) in acute hepatitis C patient as compared to controls. Among SFA, myristic and palmitic acid were increased and five unsaturated FA including nervonic, linoleic, α -linolenic, docosahexaenoic and arachidonic acid were reduced than control subjects. Significantly higher ($P < 0.05$) alanine transaminase, alkaline phosphatase, direct bilirubin and globulin levels were found in acute HCV patients possibly due to viral infection. **Conclusion:** This work makes available direct proof that lipogenesis is elevated in acute HCV patients while as evident from reduced serum PUFA and elevated SFA as well as MUFA. Hence acute HCV patients have low PUFA levels, which put forward importance of PUFA supplementation.

Keywords: Complete picture of blood; proteins and enzymes; waste metabolites; lipids; GC-FID.

ABBREVIATIONS

HCV: Hepatitis C virus, FA's: fatty acids, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid; PUFA: poly unsaturated fatty acid, VLDL: very low density lipoprotein, LDL: low density lipoprotein, HDL: high density lipoprotein, TAG: triacylglyceride, ALT: transaminase, ALP: alkaline phosphatase, FAMES: FA methyl esters, GC: gas chromatography, FID: flame ionization detector, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MTIP: microsomal triglyceride transferase protein, SCD: stearyl-CoA desaturase.

1. INTRODUCTION

The hepatitis C virus (HCV) was identified in 1989, belongs to *flaviviridae* family. It is a linear, single strand RNA virus and recognized as a major causal agent of non-A and B hepatitis [1]. The World Health Organization (WHO) had estimated that every year, 3–4 million people are affected by HCV. About 150 million people are infected chronically will develop liver cirrhosis and/or liver cancer. More than 3.5 million people die from hepatitis C related liver diseases every year [2]. HCV is the one of the leading causes of chronic liver disease worldwide, affecting 3% of world population. Approximately 10 million people with prevalence 2.2-14% are infected with HCV infection reported in Pakistan [3].

Most of the endogenous lipids and lipoprotein are synthesized in liver. This depends on the integrity of liver cellular function, which ensures homeostasis of lipid and lipoprotein metabolism [1]. HCV is a unique virus targeted towards liver cells; this virus closely interacts with host lipoprotein metabolism. The particles of HCV exist in binding from with β -lipoprotein [low density lipoprotein (LDL) and very low density lipoprotein (VLDL)] and immunoglobulins. Complexing of the virus to VLDL or LDL could promote the endocytosis of HCV via the LDL receptor [4]. HCV-infected patient's diagnosis and treatment has become a clinical challenge due to association of severe autoimmune features. The HCV infection history in terms of

chronic toxicity and disease progression seems to be largely determined by the host immune response to virus-infected hepatocytes [5-7].

Serum alanine transaminase (ALT) has also been correlated with the degree of histological inflammation in the liver, particularly long-term elevation in serum ALT is considered as the principal indicator for HCV infection. Serum liver enzymes such as ALT, alkaline phosphatase (ALP) are tested routinely and automatically in current clinical settings [8].

Recently, fatty acids (FA) have been implicated in the pathogenesis of several diseases associated with metabolic disorders (such as obesity, diabetes and cardiovascular disease) [9,10] and in immunological response [11]. In liver diseases, especially in non-alcoholic steatohepatitis, the effect of impaired peroxisomal polyunsaturated fatty acid (PUFA) metabolism and nonenzymatic oxidation on FA constitution is associated with disease progression [12]. It has been reported that HCV core protein has effects on fatty acid synthesis, and that fatty droplets in the liver are related to development of disease [13,14]. It has been revealed that the synthesis of cholesterol and FA is directly affected in HCV patients; however serum FA profile is not reported previously in acute HCV patients.

These findings suggests that examination of serum biochemical and metabolic profile

including FA composition is important which may contribute in understanding biological feature of acute HCV infection and may possibly will help to select the suitable treatment.

2. MATERIALS AND METHODS

Acute hepatitis C infection is defined as the 6-month time period following acquisition of HCV. Acute HCV patients diagnosed by center of diseases control (CDC) criteria; the patients do not have symptomatic non-specific symptoms that may include malaise, anorexia, and abdominal pain. Five hundred patients came in civil hospital Hyderabad and Taluka hospital Tando Adam with these symptoms, their blood samples was subjected ELISA and ALT test. HCV positive patients with elevated ALT levels were further confirmed by PCR. All patients signed a written consent and were enrolled in the study. The study was approved by ethnic committee Institute of Biochemistry University of Sindh, Jamshoro.

Exclusion criteria were: Diabetic, hypertension and hepatitis co-infection and pregnancy or lactation.

Fifty acute HCV patients along with age and gender matched control subjects were included in the study. 10 ml intravenous blood was collected from the patients and healthy subjects; 5ml was transferred in cp vacate for complete blood count (CBC) and analyzed by CP analyzer MS43e. 5 ml blood was transferred in gel tube, serum was separated and immediately used for liver function test (ALT, ALP, Total, direct, and indirect bilirubin) and serum total protein (albumin, globulin) and lipid profile (cholesterol, triacylglyceride (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL) total lipid using kit method based on spectrophotometric and microlab measurements. The kits are manufactured by Daisy's diagnostic system, Germany. Fatty acid composition was analyzed by Perkin Elmer Gas chromatograph 8700 equipped with FID detector by derivatization as reported by Liebich [15]. All solvents and reagents were used analytical grade.

2.1 Sample Preparation

Fasting blood sample were instantly centrifuge at 3000 rpm for 15 minutes and stored at -80°C before analysis. Total fatty acids (TFA) were extracted from 200 μl serum by adding 4ml methanol/toluene (4:1, v/v) and 400 μl acetyl chloride. The mixture was reacted at 100°C for

60 minutes in screw-cap vials on the heating/stirring module, and then neutralized by shaking with 5 ml of 6% aqueous potassium carbonate. Following the addition of 2 ml toluene with subsequent mixing the sample was centrifuged at 2000 rpm for 10 minutes. The toluene phases with the FA methyl esters (FAMES) were subjected to gas chromatographic (GC) analysis.

The free fatty acid (FFA) were methylated and isolated from 200 μl serum by adding 5ml methanol and 200 μl acetyl chloride. The mixtures were reacted at 40°C for 45 minutes in screw-cap vials on the heating/stirring module, and were then neutralized by shaking with 3 ml of 6% aqueous potassium carbonate. Following the addition of 2 ml hexane with subsequent mixing the sample was centrifuge at 2000 rpm for 10 minutes. The supernatant collected was analyzed by GC.

2.2 Gas Chromatographic Analysis of the Total and Free Fatty Acid

FA composition was analyzed by Perkin Elmer Gas chromatograph 8700 (Buckinghamshire, England) fitted with nonbonded biscynopropyl siloxane stationary-phase, polar capillary column Rt-2560 (100 m \times 0.25 mm) 0.2 μm film thickness (Supelco, PA, USA) and an FID. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. The initial oven temperature was 120°C at rate of 4 min which was raised to 220°C held for 20 min. The injector and detector temperature were set at 260°C and 270°C , respectively. A sample volume of 2.0 μl was injected as reported earlier [16].

Peaks were identified by authentic standards supplied by Fluka Chemika (Buchs, Switzerland). The FA standards, myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1w9), α -linolenic acid (ALA (C18:3w3), stearic acid (C18:0), linoleic acid (C18:2w6), oleic acid and vaccenic acid (C18:1), eicosapentaenoic acid (EPA (C20:5w3), arachidonic acid (C20:4w6) arachidic acid (C20:0), docosahexaenoic acid (DHA (C22:6w3), docosenoic acid (C22:1), nervonic acid (C24:1), eicosatrienoic acid (C-20:3). The FA composition was reported as a relative percentage of the total peak area.

2.3 Statistical Analysis

All values are expressed as mean \pm SD. For the comparison between the groups (patients vs.

controls) student's t-test or the Mann- Whitney U test was used as appropriate with SPSS version 15 (SPSS Inc. Chicago, IL). *P* value less than 0.05 was considered statically significant.

3. RESULTS

One hundred one patients consented; fifty one patients were excluded after the enrolment for the reason that they met exclusion criteria. The median age of all patients was 34.16 (range, 17–57 years) and 50% were male.

3.1 Clinical Characteristics

Some clinical characteristics including random blood sugar, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leucocytes, lymphocytes, monocytes, platelets, serum ALT, ALP, direct and indirect bilirubin, urea, globulin, albumin globulin (A/G) ratio were severely affected by acute HCV, which reveals marked differences in the number of abnormal liver chemistry test. Significantly higher ($P < 0.05$) ALT and ALP levels were found and total and direct bilirubin, albumin was similar in HCV patients and controls. However direct bilirubin, globulin and total leucocytes including lymphocytes was found significantly higher ($P < 0.05$) with significantly lower level of A/G ratio, urea, random blood sugar, hemoglobin, MCV, MCH, MCHC and platelets counts in acute HCV patients as Compared to controls (Tables 1 and 2).

3.1.1 Lipid Profile

The acute HCV patients have significantly lower ($P < 0.05$) LDL, HDL, TAG and total lipid in relation to controls (Table 3).

3.1.2 TFA Profile

The Table 4 summarizes the TFA composition of acute HCV patients. Mean levels of individual FA in two groups were subjected to pair-wise comparison. This revealed that there were significant differences in the levels of seven FA's in acute HCV patients and controls. Two SFA myristic and palmitic acid were elevated and five unsaturated FA including nervonic, linoleic, α -linolenic, DHA and arachidonic acid were reduced in acute HCV patients than controls.

3.1.3 FFA Profile

The total FFA composition of acute HCV patient's serum in comparison to control is depicted in Table 5; the total free SFA and MUFA contents were higher in acute HCV patients, where as only one SFA arachidic acid and total free PUFA were found lower except eicosatrienoic acid in patients as contrast of controls. The significant difference occurred in three saturated and three unsaturated FA including myristic, palmitic, stearic, palmitoleic, linoleic acid and arachidonic acid.

Figs. 1 and 2 shows the detailed evaluation of total free form of fatty acids. The SFA, MUFA were elevated and PUFA was lower both total and free form in acute HCV patients with contrast of healthy subjects. The Significant variation ($P < 0.005$) was seen in total as well as free fatty acids including SFA, PUFA, and its n-3, n-6 form.

4. DISCUSSION

Our study has shown significantly elevated level of ALT and ALP in acute HCV patients as compared to controls. ALT is a cytosolic enzyme, which is specific to the liver function and disease. Elevations in ALT levels should be interpreted as indicative of liver disease with only rare exceptions: severe rhabdomyolysis or systemic myopathies. ALP being more common with superadded viral infection [17].

In present study patients shows lower A/G ratio and increased globulin level that may reflect an increased burden on the liver. Umer et al. [18] has also found elevated levels of α -1, β and γ globulin levels in serum for combined anti- HCV and HBsAg positive group as compared to seronegative and anti-HCV positive groups. The significantly lower urea, random blood sugar, hemoglobin, MCV, MCH, MCHC and platelets counts were detected in acute HCV patients; similarly abnormal level of these clinical characteristics have been reported previously [19,20]. The result shows higher total leucocytes including lymphocytes in patients in contrast to control. It has been established that the extracellular binding of HCV-E2, stimulates the proliferation of hepatocytes and lymphocytes [21].

The present study shows significantly lower lipid profile in acute HCV patients. HCV induced steatosis occurs via different mechanism that interfere in the host lipid production for its benefit.

The HCV interfere in the mevalonate pathway leading to decreased cholesterol production compensatory up regulation of LDL receptors leading to decrease LDL levels, comparable to the mechanism of HMG-CoA reductase inhibitors [22]. In addition hepatitis C associated with reduced level of microsomal triglyceride

transferase protein (MTIP), the enzyme responsible for the production of VLDL, which is in turn accountable for decreased circulating LDL and cholesterol levels. Thus the decrease in non HDL cholesterol levels, a composite of principally VLDL and LDL, could reflect viral inhibition of MTIP [23].

Table 1. Comparison of complete blood count (CBC) between HCV patients and healthy controls

Complete blood count	Healthy controls	HCV Patients
Age (years)	34.2±9.2 (17–57)	34.16±9.1 (17–57)
RBC (m/cmm)	4.7±0.6 (4.0–5.5)	4.7±0.6 (3.61–6.4)
Hemoglobin (g/dl)	14±1.4* (12–16)	12.1±1.9* (9.0–15.1)
MCV (μm3)	90±10* (80–100)	78.0±7.4* (53–86)
MCH (pg)	30±4.0* (26–34)	25.8±3.7* (15–32.2)
MCHC (g/dl)	34±3.0 (31–37)	32.7±2.4* (30–34.3)
Total Leucocytes count (m/cmm)	7x10 ⁹ /L ± 27* (4–10 x 10 ⁹ /L)	8 x 10 ⁹ /L±16* (6–11x10 ⁹ /L)
Neutrophil (%)	58±4.0*(54–62)	66.5±8.1* (40–78)
Esinophil (%)	2±1 (1–3)	2.2±1.4 (1–4)
Monocytes (%)	31.5±8.0* (24–44)	2.6±1.8* (1–9)
Lymphocytes (%)	4.5±1.5* (3–6)	28.8±7.7*(17–58)
Basophils (%)	0.6±0.4 (0–1)	ND
Platelets (m/cmm)	300x10 ⁹ /L±150 (150–450x10 ⁹ /L)	245x10 ⁹ /L ± 66* (116–314x10 ⁹ /L)

Values are median (range)*different from HCV patients with healthy controls, p<0.05 (T test)

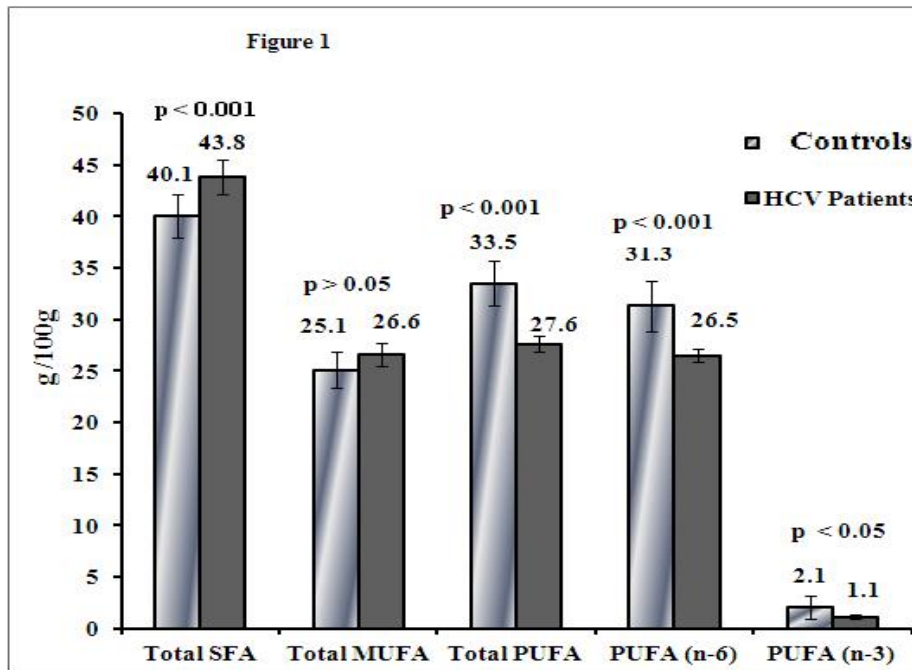


Fig. 1. Comparison of SFA, MUFA, PUFA including n-3 and n-6 fatty acids in total FA composition of controls and HCV patients

Table 2. Comparison of clinical profile between HCV patients and healthy controls

Clinical parameters	Healthy controls	HCV patients
Random Blood sugar (mg/dl)	140±30* (100–170)	104.8±26.4* (74–185)
Total Protein (g/dl)	7.1±0.9 (6.2–8.0)	7.451±0.7 (5.8–8.5)
Serum Albumin (g/dl)	4.5±1.0 (3.5–5.5)	4.5±0.6 (3.2–6.0)
Globulin (g/dl)	1.6±0.5* (1.1–2.2)	2.9±0.69* (0.6–4.2)
A/G Ratio	1.65±0.55 (1.1–2.2)	1.29±0.39* (0.9–2.1)
Serum Creatinine (mg/dl)	0.7±0.2 (0.5–1.1)	0.8±0.2 (0.3–1.1)
Total bilirubin (mg/dl)	0.6±0.5 (0.1–1.2)	0.7±0.3 (0.5–1)
Direct bilirubin (mg/dl)	0.15±0.15* (0–0.3)	0.4±0.2*(0.2–1.2)
In direct bilirubin (mg/dl)	0.5±0.2 (0.3–0.7)	0.374±0.117(0.2–0.6)
Urea (mg/dl)	30±14.1(20–40)	25±8.3*(9–40)
SGPT or ALT (U/L)	25±14*(11–39)	74.1±34.2*(40–120)

Values are median (range)*different from HCV patients with healthy controls, p<0.05 (T test)

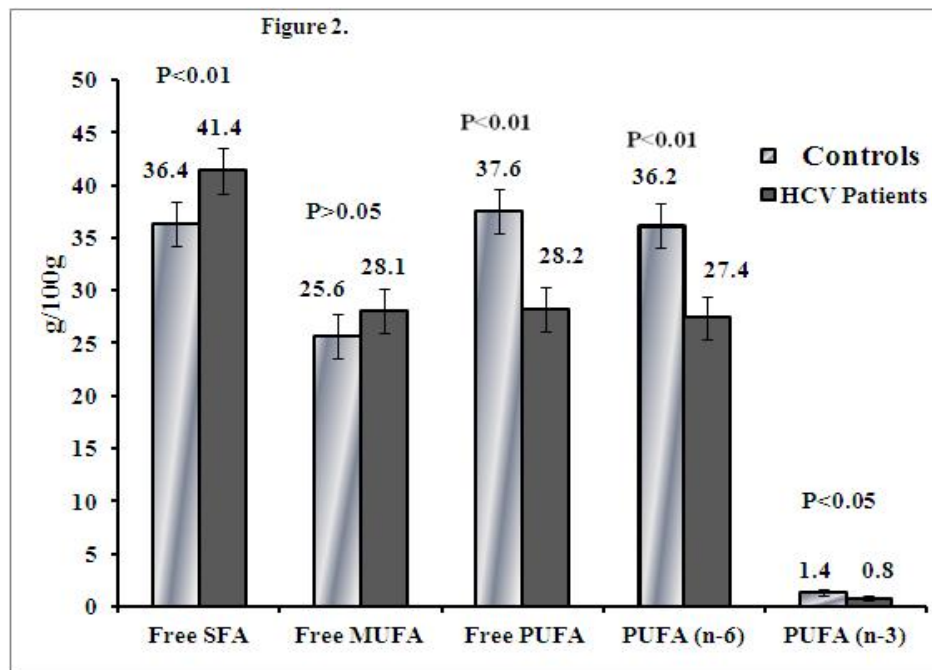


Fig. 2. Comparison of free SFA, MUFA, PUFA including n-3 and n-6 fatty acids in free FA composition of controls and HCV patients

Table 3. Lipid profile of HCV patients in comparison of controls subjects

Lipid profile	Control subjects	HCV patients
Cholesterol (mg/dl)	170.1±9.7	159.7±13.8*
LDL (mg/dl)	102.8±5.2	82.9±12.1*
HDL (mg/dl)	55.5±7.8	30.3±9.6*
Triglyceride (mg/dl)	126.5±12.1	114.0±1.3*
Total Lipid (mg/dl)	543.8±34.2	496.3±6.6*
Coronary risk (HDL/LDL Ratio)	2.4±0.86	5.1±2.39*

Values are mean ± standard deviation*different from HCV patients with healthy controls, p<0.05 (T test)

The fatty acids were extracted from serum to describe de novo fatty acid synthesis in acute HCV patients in comparison of healthy subjects. The present study gives the evidence that lipogenesis is increased, which produced higher levels of SFA and MUFA with reduced PUFA in acute HCV patients. The several studies reported that lipogenesis is up-regulated in HCV patients and tied to virus progression. Due to lipogenesis, SFA availability increases for plasma membranes and consequently PUFA content of cell membrane decreases. In cancer cells membrane lipid saturation is observed as well as hypothesized to give defense from chemotherapy

agents and cell death by plummeting peroxidation, owing the replacement of PUFA by SFA. The hepatic lipid droplets are produced by lipogenesis, while they are essential for HCV assembly [24-27].

Table 4. Total fatty acid composition of HCV patients in comparison of control subjects

Total fatty acids	Controls	HCV patients
C-14:0	1.1±3.9	2.4±0.65*
C-16:0	23.8±5.3	27.2±0.93*
C-18:0	14.7±5.5	13.9±2.2
C-20:0	0.4±1.1	0.3±0.1
C-14:1	0.5±0.98	0.3±0.02
C-16:1	2.6±2.2	3.5±0.4
C-18:1	19.8±4.6	20.3±0.95
C-22:1	1.9±1.4	2.5±0.33
C-24:1	0.35±0.73	ND*
C-18:2	25.0±5.4	21.3±0.5*
C-18:3 (n-3)	0.9±1.3	0.5±0.3*
C-20:4 (n-6)	5.9±2.2	5.2±0.2*
C-20:5 (n-3)	0.98±1.7	0.5±0.1
C-20:3 (n-6)	0.3±0.6	0.1±0.2
C-22:6	0.4±0.7	ND*

Values are mean ± standard deviation*different from HCV patients with healthy controls, p<0.05 (T test)

Table 5. Comparison free fatty acid composition between HCV patients and control subjects

Free fatty acids	Controls	HCV patients
C-14:0	1.5±0.6	2.05±0.02*
C-16:0	23.7±3.6	27.2±2.01*
C-18:0	10.8±4.1	12.03±2.97*
C-20:0	0.4±0.9	0.11±0.71
C-14:1	0.12±0.3	1.1±0.2
C-16:1	2.8±1.4	3.7±0.2*
C-18:1	20.6±2.9	20.7±1.21
C-22:1	2.1±1.3	2.5±0.4
C-18:2	29.9±4.7	22.5±1.9*
C-18:3 (n-3)	0.4±1.1	0.3±0.2
C-20:4 (n-6)	6.1±1.7	4.9±0.6*
C-20:5 (n-3)	1.0±1.6	0.2±0.3
C-20:3 (n-6)	0.2±0.7	0.3±0.2

Values are mean ± standard deviation*different from HCV patients with healthy controls, p<0.05 (T test)

The current study shows myristic, palmitic and oleic acid were elevated and nervonic, linoleic, α-linolenic, DHA, EPA and arachidonic acid were reduced in acute HCV patients. Leu et al. [28] has demonstrated in vitro studies that SFA including myristic, palmitic and oleic acid were enhanced in HCV replication while PUFA including arachidonic, linoleic, alpha-linolenic

DHA, and EPA have an ability to reduce the HCV replication.

In chronic liver diseases, increased oxidative stress has been reported, due to the release of reactive oxygen species from sequestered phagocytes and activated resident macrophages. The low level of EPA was observed in HCV patients. It is a highly unsaturated fatty acid and has a high susceptibility to oxidation; for prevention of oxidative damage antioxidant supplementation may be needed [29].

The concentration of myristic, myristoleic, palmitic, palmitoleic, stearic and oleic acid were increased in free form in HCV patients. The elevated FFA in the liver is a main cause of cell injury and death in nonalcoholic steatohepatitis (NASH). This is supported by the presence of elevated circulating FFA in NASH [27]. FFA directly induced apoptosis in hepatocytes. Saturated FFA's were substantially more toxic than monounsaturated FFA's despite causing a similar magnitude of cellular steatosis [30].

The HCV interferes with FA metabolism by altering the genes expression of metabolically important enzyme stearyl-CoA desaturase (SCD) in vitro and animal studies. Therefore, it is believable that changes occur in hepatic FA profile due to direct contribution of HCV. Variations take place in FADS1 (Δ5-desaturase) and FADS2 (Δ6-desaturase) or SCD genes may influence enzyme activity and FA composition [31]. Therefore, we acknowledged that currently no any study is available to investigate the fatty acid metabolism of HCV patients.

5. CONCLUSION

This study confirms that lipogenesis is increased in patients with acute HCV compared to healthy controls as apparent from low serum PUFA and higher SFA and MUFA. Further study is necessary to resolve if these aberrations are related to development of cirrhosis, steatosis, and viral replication.

6. LIMITATIONS

Our study has several limitations. First, the samples were collected from government hospitals of Sindh and may not be representative of all HCV patients in Pakistan. Second, we could not evaluate the effects of lipid profile discrepancy due to medication, diet, physical activity or additional aspects.

FUNDING

The work was completed with the support of departmental facilities at National Centre of Excellence in Analytical Chemistry and Institute of Biochemistry University of Sindh, Jamshoro, Pakistan. Moreover the staff of Taluka hospital TandoAdam district Sanghar and civil hospital Hyderabad (LMUHS) helped in collecting samples and taking consent from patients voluntary.

ACKNOWLEDGMENTS

We are thankful to the doctors and staff of Taluka hospital Tando Adam and civil hospital Hyderabad (LMUHS) for their cooperation in samples collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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