Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Index Copernicus value = 85.10

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Incidence of Liver Steatosis in Different Genotypes of Chronic Hepatitis C within the Uzbek Population

Shokhista Rustamovna BAKIEVA, Aziza Saidullaevna KHIKMATULLAEVA, Muazzam Alievna ABDUKADYROVA

Research Institute of Virology of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan

ABSTRACT:
According to literature, fatty liver is observed in 50% of patients with chronic viral hepatitis C. In this group of patients, steatosis is detected 2.5 times more often than in the general population. Hepatitis C virus can induce liver steatosis. The presence of fatty degeneration of the liver provokes accelerated fibrogenesis and a decrease in the effectiveness of antiviral therapy. The basis for the treatment of liver steatosis caused by hepatitis C virus is antiviral and pathogenetic therapy.
Key words: chronic viral hepatitis C, fatty liver, liver fibrosis

Received: 4 January 2021 Accepted: 8 February, 2021

Corresponding author: Shokhista R. BAKIEVA med.author@yandex.ru

This article may be cited as: Bakieva SR, Khikmatullaeva AS, Abdukadyrova MA. Incidence of Liver Steatosis in Different Genotypes of Chronic Hepatitis C within the Uzbek Population. J Adv Med Dent Scie Res 2021;9(4):57-62.

INTRODUCTION

Non-alcoholic fatty liver disease is a hepatic manifestation of metabolic syndrome and is defined as the accumulation of fat in the liver in patients who do not consume excessive amounts of alcohol.

The prevalence of non-alcoholic fatty liver disease among adults is 20–30% and higher in industrialized countries [2]. Non-alcoholic fatty liver disease is asymptomatic in the most affected patients and is associated with obesity and features of metabolic syndrome, namely arterial hypertension, dyslipidemia, central obesity, and insulin resistance or diabetes [15, 23]. The term non-alcoholic fatty liver disease encompasses a wide range of conditions, from simple fat accumulation ("fatty liver" or steatosis) to non-alcoholic steatohepatitis, fibrosis and cirrhosis of the liver with its clinical consequences [21]. Thus, hepatic steatosis against the background of chronic viral hepatitis C is an urgent problem of modern infectious diseases due to the high prevalence, severe consequences and negative impact on the effectiveness of antiviral therapy.

Despite its high prevalence, only a small proportion of patients with non-alcoholic fatty liver disease have non-alcoholic steatohepatitis with a consequent increased risk of liver fibrosis, cirrhosis, and hepatocellular carcinoma. While patients with simple fatty liver disease live about the same life expectancy for the general population, people with non-alcoholic steatohepatitis have impaired survival, primarily due to cardiovascular and liver-related causes. This concept has recently been challenged by two long-term follow-up studies that showed that severe fibrosis, but not the presence of non-alcoholic steatohepatitis (according to the NAS diagnosis) predicted overall mortality in patients with non-alcoholic fatty liver disease [3, 12].

This could be due to deficiencies in the NAS assessment, since the presence and degree of steatosis is disproportionate to the impact compared to lobular inflammation and abdominal distention, while portal inflammation is not included in the assessment [6].

On the other hand, lobular inflammation and abdominal distention may be epiphenomenon similar to simple steatosis, not associated with activation of fibrogenic pathways.

Liver steatosis is characterized by the accumulation of lipids in hepatocytes and is usually associated with metabolic factors, causing primary liver damage. In the past, the presence of hepatic steatosis was considered a benign condition. However, steatosis in synergy with another damaging agent can lead to the

development of oxidative stress and increase the damage to hepatocytes [9, 20]. At present, the interests of researchers are focused on the pathophysiological mechanisms of the onset of steatosis in patients with chronic viral hepatitis C, and the existing data reflect the participation of viral and host factors in this process [14].

Fat accumulates in the liver in the form of triglycerides, and this occurs simultaneously with increased lipotoxicity due to high levels of free fatty acids, free cholesterol and other lipid metabolites: as a consequence, mitochondrial dysfunction with oxidative stress and production of reactive oxygen species and stress-related mechanisms endoplasmic reticulum are activated [10].

In addition, altered intestinal flora leads to further production of fatty acids in the intestine, increased permeability of the small intestine and thus increased absorption of fatty acids and increased levels of circulating molecules that promote the activation of inflammatory pathways and the release of pro-inflammatory cytokines such as IL-6 and TNF-α [17].

In subjects predisposed to genetic factors or epigenetic modifications, all these factors affect the fat content in hepatocytes and the inflammatory environment of the liver, which leads to a state of chronic inflammation of the liver (Fig. 2) through heterogeneous pathways of hepatocellular damage with possible progression to hepatocellular death (for both direct toxicity and mechanisms of activation of apoptosis), activation of hepatic stellate cells and deposition of the fibrous matrix.

The diagnosis of non-alcoholic fatty liver disease remains one of the exceptions, and liver biopsy is still the gold standard for differentiating fatty liver from non-alcoholic steatohepatitis and determining the stage of fibrosis, although several non-invasive markers have recently been introduced for the latter [8]. Long-term observations of patients infected with the hepatitis C virus make it possible to state a high incidence of liver cirrhosis and hepatocellular carcinoma in them. Over the past decades, significant advances have been made in the diagnosis and treatment of chronic liver diseases. The emergence of direct-acting antiviral drugs revolutionized the treatment of viral hepatitis C. The effectiveness of treatment with direct-acting drugs was 95-98%. Despite the success in the treatment of viral hepatitis C, many questions remain unresolved. One of these issues is the study of the influence of metabolic changes and hepatic steatosis as factors influencing the effectiveness of antiviral therapy and disease outcomes, especially considering that hepatic steatosis is often detected in patients with viral hepatitis C [1].

According to the results of various studies, fatty degeneration of hepatocytes is observed in almost 50% of patients infected with the hepatitis C virus [4, 5, 25]. In patients infected with HCV, steatosis is diagnosed 2.5 times more often than in the general population [13]. To understand the causes of liver steatosis and to develop ways of influencing it in patients with chronic viral hepatitis C, two forms of steatosis should be differentiated: metabolic and HCV-induced. The possibility of metabolic steatosis is not directly related to HCV infection, however, as noted above, the combination of this form of steatosis and chronic viral hepatitis C may be associated with faster progression of fibrosis [7]. In this regard, patients with liver disease (steatosis or type 2 diabetes) should be periodically carefully examined clinically [11].

Another form of steatosis detected in patients with chronic viral hepatitis C is fatty infiltration caused directly by HCV [18, 19, 24]. Although the exact mechanism of HCV action on liver cells is not fully understood, the role of virus-induced steatosis as the only pathway of direct cytopathic action of the virus is recognized [16, 19]. According to many researchers, steatosis is one of the factors that accelerate the progression of the disease to the stage of cirrhosis, and also reduce the likelihood of success of antiviral therapy [22, 24].

PURPOSE OF THE STUDY

The objectives of this study were formulated as follows:

- to determine the frequency and severity of hepatic steatosis in patients with chronic HCV infection with different HCV genotypes in the Uzbek population.
- to determine the association of steatosis with virological and metabolic factors.

MATERIAL AND METHODS

The study was conducted at the Research Institute of Virology of the Ministry of Health of the Republic of Uzbekistan for the period 2019-2020. 62 patients with chronic viral hepatitis C were examined, including 28 men and 34 women. All patients were over 18 years of age.

The median age of the patients was 47 years with an interquantile range (22–78 years). In terms of gender, the groups did not differ significantly.

The exclusion criteria for patients were: 1) the presence of concomitant liver pathology (liver cirrhosis, autoimmune hepatitis, alpha-1 antitrypsin deficiency, viral hepatitis B, primary biliary cirrhosis, Wilson-Konovalov disease, Budd-Chiari syndrome, hemochromatosis) or any liver disease in the stage decompensation; 2) the presence of diabetes mellitus and / or obvious clinical and laboratory manifestations of metabolic syndrome, incl. insulin resistance; 3) HIV co-infection; 4) regular alcohol consumption of more than 50 g / day for men and 25 g / day for women over the past two years; 5) the presence of a previous direct-acting antiviral treatment for chronic viral hepatitis C. Biochemical parameters (bilirubin level, serum activity of alanine aminotransferase, aspartate aminotransferase, cholesterol) were measured on a Mindray BA-88A analyzer, Germany,

using Human Diagnostics Worldwide reagents, Germany.

In addition to general clinical examination methods, all patients underwent a complete serological examination for markers of viral hepatitis B, C, D (for surface antigen HBSAg, anti-HDV, anti-HCV) by enzyme-linked immunosorbent assay (ELISA) on microparticles (DS, Nizhny Novgorod). All patients were positive for anti-HCV in the third generation ELISA test, as well as positive for HCV RNA in the blood. To measure the HCV RNA level, we used a real-time polymerase chain reaction with a PCR amplifier on Rotor-Gene Q (Corbett Research, Australia) for 36 wells, using AmpliSens-HCV-FL reagents for detecting hepatitis C virus RNA (lower threshold of sensitivity 50 IU / ml), AmpliSens-HCV genotype-FL for the detection and determination of hepatitis C genotypes (Russia), FBUN Central Research Institute of Epidemiology of Rospotrebnadzor, manufacturer of AmpliSens® reagent kits. Today FBSI Central Research Institute of Epidemiology of Rospotrebnadzor is the largest high-tech import-substituting biotechnological production of modern diagnostic drugs in Russia. Each patient was tested for HCV genotype and blood HCV RNA quantification.

All patients underwent ultrasound examination of the abdominal organs in the morning on an empty stomach after at least 10 hours of fasting, and also fibroelastometry of the liver tissue was performed. The diagnosis of hepatic steatosis and the degree of hepatic steatosis were made on the basis of the results of ultrasound examination (Philips clear Veu 350 device, with a convex multi-frequency transducer with 5-2 MHz) and transient elastography (FibroScan FS-502 device Echosens, France) of the liver using the attenuation parameter ultrasonic wave (Controlled Attenuation Parameter - CAP) in decibels per meter (dB / m), which correlates with the degree of steatosis:

Level of steatosis	***Activity of steatosis***	***Liver damage, in (%)***	***In dB/m***
S0	no steatosis	-	≤228
S1	mild steatosis	≤ 5% of hepatocytes with steatosis	229-268
S2	moderate steatosis	6–32% of hepatocytes with steatosis	269-301
S3	manifest steatosis	33–60% of hepatocytes with steatosis	302-346
S4	severe steatosis or cirrhosis	61-100% of hepatocytes with steatosis	347≤ and upper

The diagnostic criteria were also changed biochemical parameters: increased activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, cholesterol.

RESULTS AND DISCUSSION

It is very difficult to determine fatty hepatosis at the initial stage, when it may be asymptomatic, which makes it difficult to diagnose the disease. In most cases, hepatic steatosis is not accompanied by any clinical symptoms [1]. Hepatic parameters are usually within the normal range. The diagnosis is made when the inflammatory process intensifies in the liver tissues and fibrotic changes appear. Then symptoms such as heaviness, pain in the right hypochondrium, bitterness in the mouth, flatulence, etc. appear [2]. However, these are not specific symptoms and can accompany other liver diseases. Fatigue, malaise, abdominal discomfort, enlarged liver and spleen are common. Currently, one of the urgent problems is the identification of patients who require careful monitoring and differentiated treatment before, during and after antiviral therapy. This group includes patients with regression of hepatic fibrosis in chronic hepatitis after a sustained virological response, patients with liver cirrhosis, at risk of developing hepatocellular carcinoma, and patients with hepatic steatosis. Later, signs characteristic of liver failure may appear: lack of appetite, astheno-vegetative manifestations. Usually, steatosis is detected by chance on ultrasound or when the biochemical parameters of the blood change. If you suspect liver steatosis, it is necessary to conduct laboratory and instrumental studies.

In the present study, an attempt was made to identify the factors associated with steatosis in patients with chronic viral hepatitis C. In this study, out of 62 patients with chronic viral hepatitis C, steatosis was detected in 41 (66.13%) people (Table 1). In the same group, 15 (36.59%) people were found to be obese, an increased level of cholesterol in the blood (34.15%), which corresponds to the signs of metabolic syndrome.

Patients with steatosis were predominantly female, significantly older, were more likely to suffer from obesity and hypertension, and had a larger waist.

As in most studies around the world [1, 2, 3], according to our data, 1 HCV genotype prevailed in patients. The frequency of occurrence of genotype 1 HCV was 69.35% (20/23), genotype 3 - 22.58% (6/8), and genotype 2 - 8.07% (2/3). Other genotypes were not found in this study.

Liver steatosis was significantly more frequent in patients with HCV genotype 1 - in 72.09% of patients (15/16), while in HCV genotype 3 it was observed only in 42.86% of patients (3/3), and in 2 HCV genotype - in 80.0% of patients (2/2).

According to the first fibroscan (Table 1), liver steatosis was observed in 66.13% (20/21) of patients with chronic viral hepatitis C, 33.87% (8/13) had no steatosis - S0.

Table 1. Characteristics of patients depending on the presence and severity of hepatic steatosis prior to the initiation of direct-acting antiviral drugs

	Without steatosis (S0) n = 21	With the presence of steatosis (S1 + S2 + S3 + S4) n = 41			
		Mild steatosis (S1) n = 19	Moderate steatosis (S2) n = 7	Mainfest steatosis (S3) n=10	Severe steatosis (S4) n = 5
n=28/32 (male / female)	8/13	11/8	4/3	3/7	2/3
Age, in year	40,48±17,4	52,89±17,89	46,71±16,71	57,3±27,3	43,0±11,0
Genotype 1, n=43	12	16	4	7	4
Genotype 3, n=14	8	3	1	2	0
Genotype 2, n=5	1	0	2	1	1

Note: n is the number of observations; S-steatosis.

Of the identified steatosis, 46.34% (11/8) had mild steatosis - S1, 17.07% (4/3) - moderate steatosis - S2, and 24.39% (3/7) had severe steatosis. - S3, and 12.20% (2/3) had steatosis with liver damage more than 70% of the total field - S4 and this was not liver cirrhosis.

Taking into account that chronic viral hepatitis C proceeds without cytolitolytic manifestations, biochemical blood tests were prescribed to 41 patients with fatty hepatosis on Fibroscan. In the blood, the level of bilirubin increased in 10 out of 41 (24.39%), in 18 (43.9%) ALaT, and in 14 (34.15%) ASaT. There was a slight increase in cholesterol content in 15 (36.59%) patients, however, the increase in triglycerides remained within the normal range, only in 6 out of 15 patients it was higher than normal.

It should be noted that the increase in the parameters of the lipid spectrum and liver enzymes does not always depend on the severity of steatosis. In Table 2, it can be seen that biochemical parameters were within normal limits or slightly increased with severe steatosis than steatosis with moderate or minimal activity.

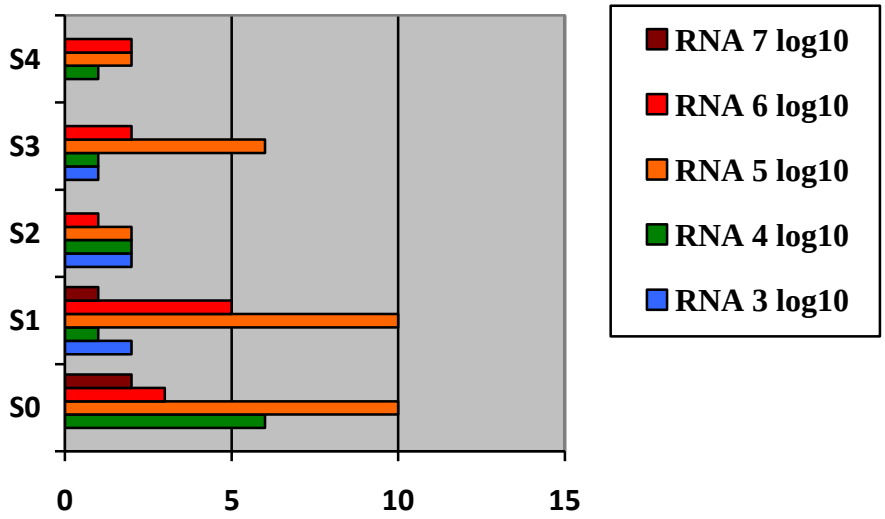
Table 2. Laboratory characteristics of steatosis in patients prior to treatment with direct-acting antiviral drugs

	Without steatosis (S0) n = 21	With the presence of steatosis (S1 + S2 + S3 + S4) n = 41			
		Mild steatosis (S1)	Moderate steatosis (S2)	Mainfest steatosis (S3)	Severe steatosis (S4)
n=62	21	19	7	10	5
ALT	47,45±17,44	60,29±42,22	132,84±95,84	41,57±22,55	64,21±28,12
AST	47,5±32,1	40,33±29,86	98,37±69,37	39,17±22,07	46,89±26,18
Bilirubin	20,64±10,44	15,68±6,87	19,31±12,98	20,6±8,1	16,49±5,38
Cholesterol	4,57±2,27	4,68±1,57	4,88±1,58	4,4±1,0	4,69±0,89

Note: n - is the number of observations; S-steatosis; ALT - alanine aminotransferase; AST - aspartate aminotransferase (N - but normal values; men - ≤40 U / l, women - ≤35 U / l); RNA HCV - hepatitis C virus ribonucleic acid;

Also, the amount of viral load did not give a clear picture depending on the severity of steatosis (Figure 1). The incidence of severe and total steatosis with RNA HCV 10^6 and 10^7 IU / ml was observed less frequently than with RNA HCV 10^5 IU / ml.

Figure 1. The incidence of the degree of hepatic steatosis under different viral loads



Note: RNA HCV - hepatitis C virus ribonucleic acid; S-steatosis;

Severe to moderate steatosis was found in patients with a viral load of 10^3 IU / ml. In fact, the results obtained indicate the absence of a relationship between the factors of the virus and steatosis and reflect the conjugation of metabolic parameters with the formation of steatosis in chronic viral hepatitis C.

CONCLUSION

1. Chronic viral hepatitis C can induce hepatic steatosis through a variety of mechanisms.
2. In chronic HCV infection caused by 1 genotype of the virus, hepatic steatosis occurs significantly more often than with 3 HCV genotype (almost 2 times).
3. HCV RNA viral load was not associated with severity of hepatic steatosis.

REFERENCES:

1. Целиковский А.В. и др. Влияние стеатоза печени на эффективность комбинированной противовирусной терапии хронического гепатита С// Современные проблемы науки и образования. – 2012. – № 6.;
2. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346:1221–31.
3. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2015; 149:389–97 [e10].
4. Bjornsson E, Angulo P. Hepatitis C and steatosis. Arch. Med Res, 2007.- Vol.38.-№6.- 621-627.
5. Bondini S., Younossi Z.M. Non-alcoholic fatty liver disease and hepatitis C infection. Minerva Gastroenterology Dietol., 2006.-Vol. 52.-№2.-P.135-143.
6. Brunt EM. Histopathology of non-alcoholic fatty liver disease. Clin Liver Dis 2009; 13:533–44.
7. Bugianesi E., Marchesini G., Gentilcore E. et al. Fibrosis in genotype 3 chronic hepatitis C and nonalcoholic fatty liver disease: role of insulin resistance and hepatic steatosis/ Hepatology, 2006.- Vol.44.- №6- P.648-655.
8. Buzzetti E, Lombardi R, De Luca L, et al. Noninvasive assessment of fibrosis in patients with nonalcoholic fatty liver disease. Int J Endocrinol 2015; 2015:343828.
9. Castera L. Steatosis, insulin resistance and fibrosis progression in chronic hepatitis // Minerva Gastroenterol. Dietol. — 2006. — Vol. 52. — P. 125–134.
10. Cusi K. Role of insulin resistance and lipotoxicity in nonalcoholic steatohepatitis. Clin Liver Dis 2009; 13:545–63.
11. EASL Clinical Practice Guidelines 2016.
12. Ekstedt M, Franzen LE, Mathiesen UL, et al. Low clinical relevance of the nonalcoholic fatty liver disease activity score (NAS) in predicting fibrosis progression. Scand J Gastroenterol 2012; 47:108–15.
13. Eugene J., Yoon and Ke-Qin Hu Hepatitis C virus (HCV) Infection and Hepatic Steatosis. IntJ.Med Sci., 2006.- Vol.3- №2.- P.53-56.
14. Fartoux L., Poujol-Robert A., Guechot J. et al. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C // Gut. — 2005. — Vol. 54. — P. 1003–1008.
15. Hassan K, Bhalla V, El Regal ME, et al. Nonalcoholic fatty liver disease: a comprehensive review of a growing epidemic. World J Gastroenterol 2014; 20:12082–101.
16. Kim K.H., Hong S.P., Kim K., Park M.J. et al. HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARgamma. BiochemBiophys Res Commun, 2007.- Vol.20.- №355.- P.883-888.
17. Kirpich IA, Marsano LS, McClain CJ. Gut-liver axis, nutrition, and non-alcoholic fatty liver disease. Clin Biochem 2015; 48(13-14):923–30.
18. Moriishi K., Mochizuki R., Moriya K., et al. Critical role of PA28gamma in hepatitis C, virus – associated

steatogenesis and hepatocarcinogenesis. – AcadSci USA, 2007.- Vol. 30.- №5.- P. 1661-1666.
19. Negro F. Mechanisms and significance of liver steatosis in hepatitis C, virus infection. – World Journal Gastroenterology, 2006.- Vol.14 - №42.- P. 6756-6761.
20. Powell E.F., Jonsson J.R., Clouston A.D. Steatosis: co-factor in other liver diseases // Hepatology. — 2005. — Vol. 42. — P. 5–13.
21. Ratziu V, Bellentani S, Cortez-Pinto H, et al. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol 2010; 53:372–84.
22. Szanto P., Grigorescu M., Dumitru I., Serban A. Steatosis hepatitis C, virus infection. Response to anti-viral therapy/ Journal Gastrointestin Liver Dis., 2006.- Vol.15.- №2.- P. 117-124.
23. Tsochatzis E, Papatheodoridis GV, Manesis EK, et al. Metabolic syndrome is associated with severe fibrosis in chronic viral hepatitis and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2008; 27:80–9.
24. Walsh M.J., Jonsson J.R., Richardson M.M., et al. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signaling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1.- Gut., 2006.- Vol 55.- №4.- P. 529-535.
25. Waris G., Felmlee D.J., Negro F., Siddiqui A. Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation via oxidative stress. Journal Virology, 2007.- Vol. 81.- №15.- P.8122-8130.