Original Article:

Survey of the Relationship between Fatty Liver Disease in Men by Ultrasound and Serum Ferritin Level

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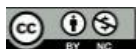
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Abstract

Introduction: The most common hepatic disease is fatty liver. Raised serum ferritin levels are often described as an indicator of liver function. The present study aimed to evaluate the correlation between fatty liver in men by ultrasound and serum ferritin levels in patients referred to Imam Khomeini Hospital in Jiroft in 2020.

Materials and Methods: In this case series study, there is both a descriptive and an analytical component. The study population consisted of all male patients with fatty liver. Patients were included in the study after being visited by a specialist and thus diagnoses their fatty liver were mades by ultrasound. After clinical examination and diagnosis of fatty liver, patients were prepared on a fasting basis for Paraclinical tests (serum levels of iron, and ferritin). After collecting data from patient surveys based on a researcher-made checklist, the data were entered into SPSS V23 (22).

Results: The mean age of patients was 46.8 ±16.7. 27. There was a statistically significant correlation between ferritin level and disease severity based on the ultrasound view (P-value = 0.000). Also, there was a significant correlation between serum iron level and disease severity based on the ultrasound view (P-value = 0.005).

Conclusion: The present study findings indicate a correlation between the severity of fatty liver disease and the level of serum ferritin and iron. Therefore, we need to pay special attention to the level of these indicators in patients with fatty liver.

Keywords: Fatty liver, Ferritin, Serum

1. Introduction

Liver is one of the most important and essential organs in the human body that performs vital metabolic functions [1].

Detoxification of drugs, excretion of waste products due to destruction and renewal of red blood cells in the form of bile, production of blood coagulation factors, storage of sugar in the form of glycogen, and regulation of sugar and fat metabolism are the most

important roles of the liver. It also plays an important role in absorbing fat and defending against microbes and toxins absorbed through food [2].

Liver disease is inherently different in its etiologies, chronicity, and likelihood of recovery [3]. On the other hand, the incidence and prevalence of liver disease have not been well established even in population-based studies. However, studies have shown that liver disease accounts for approximately 2 million deaths per year worldwide [4].

Fatty liver is a disease of human liver cells that has recently increased due to the increase in obesity in various communities, and approximately 1 billion individuals worldwide are affected [5]. The fatty liver disease seems to be one of the most common liver diseases in the world [6] and its prevalence varies from 2.8% to 24% in different communities [7, 8]. The importance of this disease is due to the destruction of liver cells and if not diagnosed early and treated properly, it can lead to an advanced and irreversible liver disease called liver cirrhosis [9].

Most cases of fatty liver disease can be diagnosed by simple liver tests in blood samples or by performing simple imaging methods such as liver ultrasonography [10]. The diagnostic susceptibility of fatty liver disease by laboratory tests depends on the definition of the normal level of liver enzymes in the serum [11]. In a study conducted in Iran by Jamali et al. on the general population, it was found that the normal level of these enzymes in serum is lower than the normal level provided by the manufacturers of laboratory measuring instruments and it seems that the normal level of liver enzymes should be determined and interpreted separately based on gender and weight [12]. The accuracy of diagnosis of fatty liver disease also varies based on the sensitivity of the imaging method used, so the prevalence of this disease will be different in various studies based on the imaging method used. In a study, the prevalence of fatty liver based on liver ultrasound findings was 68%, while in the same study, when (Magnetic resonance imagin) MRI was used to diagnose the disease, the prevalence of the fatty liver disease in the same population reached about 40% [13]. Due to the inconsistencies in the diagnosis of this disorder, it is necessary to conduct extensive studies in this field. Also, given to the recent studies and lack of sufficient information in Iran in this field, the present study was conducted to evaluate the correlation between fatty liver in men based on serum ferritin level and by abdominal ultrasonography view of patients referred to Imam Khomeini Hospital in Jiroft in 2020.

2. Materials and Methods

Procedure

Before the study, the necessary permits were obtained from the University Ethics Committee (Ethics Code = IR.JMU.REC.1399.045). The study was also carried out by the Helsinki Declaration Principles.

In this case series study, there is both a descriptive and an analytical component. The study population consisted of all male patients with fatty liver referred to the internal ward of Imam Khomeini Hospital in Jiroft in August and September, 2020.

Sampling

Patients were included in the study after being visited by a specialist and thus diagnoses their fatty liver were mades by ultrasound. After entering the study, patients became fully aware of the study, and informed consent was obtained to participate in the study. Patients were told that the information received from them would remain strictly confidential.

After clinical examination and diagnosis of fatty liver, patients were prepared on a fasting basis for Paraclinical tests (serum levels of iron, ferritin). Then, one of the project colleagues, after disinfecting the patient's elbows, took 10 ccs of venous blood from the patients with sterile and disposable syringes (with a 27-gauge needle) and the sample was collected inside two test tubes. Blood samples were then transferred to the laboratory within a maximum of 1 hour. In the laboratory, the samples were separated from the clot for 25 minutes at 25,000 rpm in a Biotech centrifuge for serum separation and transferred to 5 cc clear plastic tubes. To evaluate the serum level of ferritin, a Science antibody kit (Iran) by RIA method with the serial number (Fr 0108) was used.

Sample size

The participants were those who were referred to the internal ward of Imam Khomeini Hospital due to liver problems over the period of two months, from the beginning of August to the end of September in 2020.

Data Collection and Data analysis

Data collection scales included a researcher-made checklist in which patients' demographic information (including age, education level, disease severity, BMI, and residence) and serum ferritin and

iron levels, and liver ultrasound profile were recorded.

After collecting data from patient surveys based on a researcher-made checklist, the data were entered into SPSS (22). After descriptive analysis including frequency, mean and standard deviation, we used inferential statistics – Spearman's correlation coefficient, Chi-squared test, and Independent T-test.

3. Results

In this study, 72 male patients with fatty liver who were referred to the internal ward of Imam Khomeini Hospital in Jiroft were selected to participate in the study. The mean age of the patients was 46.8 ± 16.7 years. The minimum and maximum ages were 14 and 87, respectively.

Table 1 indicates that 27 patients have bachelor's degrees or higher, comprising 37.5% of the total patients. The results also indicate that most of the patients referred were from urban areas (49 patients and 68.1%).

The results of Table 1 indicate that concerning the history of chronic disease, 29 patients had hypertension (40.3%) and 3 patients had the rheumatic disease (4.2%). Also, 25 patients (34.7%) consumed alcohol. In addition, the results show that the order of symptoms from maximum to minimum frequency is equal to extreme weakness (22 patients), abdominal pain (17 patients), weight loss (16 patients), and anorexia (3 patients).

Table 2 indicates that the highest mean serum ferritin is related to grade 3 (184.8) and the highest mean serum iron level is related to grade 2 (68.3). Also, the highest standard deviation of ferritin level is related to grade 3, and the highest standard deviation of serum iron level is related to grade 2. There is also a statistically significant correlation between ferritin level and disease severity based on ultrasound (0.000). Finally, there is a significant correlation between serum iron level and disease severity based on ultrasound (0.005).

Table 3 indicates that the highest mean ages are observed in grade 1, grade 3, and then in grade 2 in the ultrasound view, respectively. Also, the highest age deviation based on ultrasound view is in grade 1, grade 2, and finally in grade 3, respectively. Finally, the results indicate that there is no statistically significant correlation between age and fatty liver (P-Value = 0.206).

Table 1. Frequency of variables studied by patients referred to the internal ward of Imam Khomeini Hospital in Jiroft in 2020

Variable	Number	Percentage	Variable	Number	Percentage
Diploma	19	26.4	Extreme weakness	22	30.6
Under-Diploma	11	15.3	Anorexia	3	4.2
Upper- Diploma	15	20.8	Weight loss	16	22.2
Bachelor's degree and higher	27	37.5	Abdominal pain	17	23.6
Urban	49	68.1	Decreased concentration	16	22.2
Rural	23	31.9	Cardiovascular disease	11	15.3
Drug consumption	47	65.3	Respiratory disease	8	11.1
Alcohol consumption	25	34.7	Hyperlipidemia	24	33.3
Family history of liver disease	32	44.4	Rheumatic disease	3	4.2
Diabetes	24	33.3	Dull skin	19	26.4
Hypertension	29	40.3			

Table 2. Relationship between serum ferritin and iron levels with disease severity based on ultrasound view

Disease severity	Serum ferritin levels (Micrograms per liter)				Serum Iron levels (Micrograms per deciliter)			
	Number	Mean	SD	P-value	Number	Mean	SD	P-value
Grade 1	14	66.6	59.9		14	44.5	18.2	
Grade 2	31	106.6	80.4	0.000	31	58	24.4	0.005
Grade 3	27	184.8	89.6		27	68.3	19.5	

Table 3. Correlation between fatty liver based on ultrasound view by age in patients

Grade	Mean	SD	P-value
Grade 1	53.07	21.5	
Grade 2	43.5	17.4	0.206
Grade 3	47.4	12.2	

Table 4. Correlation between fatty liver based on ultrasound view in terms of Body mass index (BMI) in patients

Grade	Mean	SD	P-value
Grade 1	23.07	2.9	
Grade 2	23.4	3.7	0.001
Grade 3	26.4	3.3	

Table 4 indicates that the highest mean BMI is in grade 3, grade 2, and finally in grade 1, respectively, and the highest BMI standard deviation is observed in grade 2, grade 3, and finally in grade 1, respectively. The results also indicate that there is a statistically significant correlation between fatty liver and BMI (P-Value = 0.001).

Table 5 indicates that in Grade 1, from the highest to the lowest frequency of education level is equal to undergraduate (7 people), bachelor and higher (4 people), diploma (3 people), and post-diploma (0 people), respectively.

In Grade 2, from the highest to the lowest frequency of education level is equal to undergraduate (10 people) and bachelor and higher (10 people), post-diploma (7 people), diploma (4 people), respectively.

Finally, in grade 3, from the highest to the lowest frequency of education level is equal to bachelor's degree and higher (13 people), post-diploma (8 people), diploma (4 people), under-diploma (2 people), respectively. There is also a statistically significant correlation between fatty liver and education level (0.004).

Table 5. Correlation of fatty liver based on ultrasound view according to the education level in patients

Rows	Grade 1		Grade 2		Grade 3		P-value
	Number	Percentage	Number	Percentage	Number	Percentage	
Diploma	7	9.7	10	13.8	2	2.7	
Under-Diploma	3	4.1	4	5.5	4	5.5	
Upper- Diploma	0	0	7	9.7	8	11.1	0.004
Bachelor's degree and higher	4	5.5	10	13.8	13	18	

4. Discussion

This study aimed to evaluate the association between fatty liver in men based on serum ferritin level and by abdominal ultrasonography view of patients referred to Imam Khomeini Hospital in Jiroft in 2020. Patients with fatty liver who were referred to the internal ward of Imam Khomeini Hospital in Jiroft were selected. The mean age of patients was 46.8 ±16.7. Also in this regard, a study by Matsuura et al. in Japan showed that the age of onset of fatty liver disease was 45 to 49 years and was more common in men [14].

The results indicated that there was also a statistically significant correlation between ferritin levels and the severity of the fatty liver disease. Finally, there is a significant correlation between serum iron and the severity of the fatty liver disease. In a study by Angulo et al., on 144 patients with fatty liver, serum ferritin levels after control for age, obesity, diabetes, and AST/ALT ratio as confounders did not indicate a significant correlation with the severity of fatty liver [15]; this is in accordance with the findings of Lonardo et al. [16] and Younossi et al. [17]. While Kowdley et al. showed in their study in patients with fatty liver that serum ferritin levels increased approximately 1.5 times their normal level and has been suggested as an independent predictor of advanced liver fibrosis [18], which is in accordance with this study.

A possible mechanism for analyzing the correlation between ferritin and the severity of fatty liver disease follows lipid peroxidation, which promotes hepatic steatosis, increased oxidative stress, changes in fatty acid metabolism, and stellar cell activation. In response to oxidative damage caused by lipid peroxidation, hepatic endothelial cells increase the expression of the antioxidant molecule Oxygenase I and ferritin [19]. The reaction between iron and oxygenase and porphyrin produces redox material as a

result of liver damage. Ferritin, as a proinflammatory cytokine, activates stellate cells and induces phosphoinositol 3 kinase, a mitogen-activating protein, and increases NFKB expression and fibrogenesis [20]. Therefore, ferritin is involved in the process associated with the pathogenesis of NASH including inflammation, apoptosis, and oxidative stress [18]. Therefore, considering the role of ferritin, it seems that the Ferritin/Alt ratio, as mentioned in the Ozawa study, is a quick and simple indicator for the diagnosis of acute liver damage [21].

The results of this study indicated that most of the patients had a history of hypertension, hyperlipidemia, and diabetes; statistically, there is a significant correlation between fatty liver and hyperlipidemia. There is also a significant correlation between fatty liver and BMI. The results are consistent with the study of Ludwig et al. whose results indicated that 90% of patients were obese, 35% of patients had hyperlipidemia and 25% were suffering from diabetes [22].

According to studies conducted in Eastern countries, obesity, inactivity, and switching to high-calorie diets have increased compared to Western societies. It seems that in our country we are witnessing this upward trend of increasing obesity and its adverse effects such as hyperlipidemia, hypertension, diabetes, and finally fatty liver disease [23, 24]. Marchesini et al. showed that metabolic syndrome is a group of disorders with hypertension, hyperlipidemia, obesity, and diabetes and that recent studies have shown that the severity of the fatty liver disease increases with the number of diseases that constitute this syndrome [25].

The findings of this study indicated that most patients had symptoms of extreme weakness, abdominal pain, and weight loss. Lu et al. showed in their study that most patients complain about vague pain in the upper and right abdomen region or a feeling of premature fatigue [26].

There is also a statistically significant correlation between fatty liver and education level. Alavian et al. examined the frequency of fatty liver and some of its risk factors in asymptomatic carriers of hepatitis B virus referred to the hepatitis clinic of Tehran Blood Transfusion Organization. Their results showed that there is no significant correlation between fatty liver disease and the patient's education level [27], that contradicts the findings of this study. There is no statistically significant relationship between fatty liver and alcohol consumption. Alavian et al. showed that the history of alcohol consumption has no significant relationship with fatty liver [27]. Although the exact amount of alcohol consumed daily to hurt the liver has not yet been determined, there is evidence that daily consumption of more than 30 grams per day for men and 20 grams per day for women is required to hurt the liver [28]. One possible reason for justifying this finding is the unreliability of the history of alcohol consumption. Another possible reason is the low alcohol consumption in our country because in some cases it has been stated that low alcohol consumption does not increase the prevalence of fatty liver [29].

5. Conclusion

According to the present study and the results obtained, it can be said that there is a significant correlation between serum ferritin and serum iron levels with the severity of fatty liver disease in the studied patients. Therefore, health professionals must pay special attention to the above-mentioned factors to prevent the occurrence of problems related to fatty liver and to deal with such cases with high awareness.

Ethical Considerations

Compliance with ethical guidelines

Funding

Author's contributions

The authors equally contributed to preparing this article.

Conflict of interest

The Authors declare that there is no conflict of interest.

Acknowledgments

References

[1] Guillot A, Tacke F. Liver macrophages: old dogmas and new insights. Hepatol Commun. 2019; 3(6):730-43. [DOI:10.1002/hep4.1356]

[2] Wang S, Zhang J, Zhu L, Song L, Meng Z, Jia Q, et al. Association between liver function and metabolic

syndrome in Chinese men and women. Sci Rep. 2017; 7:1-9. [DOI: 10.1038/srep44844]

[3] Koyama Y, Brenner DA. Liver inflammation and fibrosis. J Clin Invest. 2017; 127(1):55-64. [DOI: 10.1172/JCI88881] [PMID] [PMCID]

[4] Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019; 70(1):151-71. [DOI: 10.1016/j.jhep.2018.09.014] [PMID]

[5] Eslam M, Sanyal AJ, George J, Panel IC. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology. 2020; 158(7):1999-2014. [DOI:10.1053/j.gastro.2019.11.312] [PMID]

[6] Kuchay MS, Choudhary N, Mishra SK, Misra A. Nonalcoholic fatty liver disease should be considered for treatment allocation in standard management algorithms for type 2 diabetes. Diabetes Metab Syndr. 2020; 14(6):2233-9. [DOI:10.1016/j.dsx.2020.11.015] [PMID]

[7] Kamran SM, Ahmad M, Ayaz SB, Ahmad I. Frequency of non-alcoholic fatty liver disease in newly diagnosed cases of hepatitis c virus infection and its correlation with genotypes in normal weight patients. Pak Armed Forces Med J. 2018; 68(5):1444-48. https://www.pafmj.org/index.php/PAFMJ/article/view/2344

[8] Hyysalo JE. Prevalence and Genetics of Non-Alcoholic Fatty Liver Disease. Helsinki; 2017. https://helda.helsinki.fi/handle/10138/228381

[9] Li B, Zhang C, Zhan YT. Nonalcoholic fatty liver disease cirrhosis: a review of its epidemiology, risk factors, clinical presentation, diagnosis, management, and prognosis. Can J Gastroenterol Hepatol. 2018; 2018:1-8. [DOI:10.1155/2018/2784537] [PMID] [PMCID]

[10] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018; 67(1):328-57. [DOI:10.1002/hep.29367] [PMID]

[11] Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. Hepatology. 2017; 66(5):1486-501. [DOI:10.1002/hep.29302] [PMID]

[12] Jamali R, Pourshams A, AMINI S, Deyhim MR, Rezvan H, Malekzadeh R. The upper normal limit of serum alanine aminotransferase in Golestan Province, northeast Iran. Arch Iran Med. 2008; 11(6):602-7. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=122055

[13] Pacifico L, Celestre M, Anania C, Paolantonio P, Chiesa C, Laghi A. MRI and ultrasound for hepatic fat quantification: relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease. Acta paediatrica. 2007; 96(4):542-7. [DOI:10.1111/j.1651-2227.2007.00186.x] [PMID]

[14] Matsuura K, Tobe K, Tsuji T. Fatty liver and obesity in university students. Nihon Shokakibyo Gakkai Zasshi. 1995; 92(10):1743-51. [PMID]

[15] Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. 1999; 30(6):1356-62. [DOI:10.1002/hep.510300604] [PMID]

[16] Lonardo A, Loria P, Leonardi F, Borsatti A, Neri P, Pulvirenti M, et al. Fasting insulin and uric acid levels but not indices of iron metabolism are independent predictors of non-alcoholic fatty liver disease. A case-control study. Dig Liver Dis. 2002; 34(3):204-11. [DOI:10.1016/s1590-8658(02)80194-3] [PMID]

[17] Younossi ZM, Gramlich T, Bacon BR, Matteoni CA, Boparai N, O'Neill R, et al. Hepatic iron and nonalcoholic fatty liver disease. Hepatology. 1999; 30(4):847-50. [DOI:10.1002/hep.510300407] [PMID]

[18] Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology. 2012; 55(1):77-85. [DOI:10.1002/hep.24706] [PMID] [PMCID]

[19] Malaguarnera L, Madeddu R, Palio E, Arena N, Malaguarnera M. Heme oxygenase-1 levels and oxidative stress-related parameters in non-alcoholic fatty liver disease patients. J Hepatol. 2005; 42(4):585-91. [DOI:10.1016/j.jhep.2004.11.040] [PMID]

[20] Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB–regulated signaling in rat hepatic stellate cells. Hepatology. 2009; 49(3):887-900. [DOI:10.1002/hep.22716] [PMID] [PMCID]

[21] Ozawa E, Abiru S, Nagaoka S, Yano K, Komori A, Migita K, et al. Ferritin/alanine aminotransferase ratio as a possible marker for predicting the prognosis of acute liver injury. J Gastroenterol Hepatol. 2011; 26(8):1326-32. [DOI:10.1111/j.1440-1746.2011.06743.x] [PMID]

[22] Ludwig J, Viggiano TR, Mcgill DB, Oh B. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc; 1980; 55(7):434-8. [PMID]

[23] Chitturi S, Farrell GC, George J. Non-alcoholic steatohepatitis in the Asia–Pacific region: Future shock? Gastroenterol. 2004; 19(4):368-74. [DOI:10.1111/j.1440-1746.2003.03252.x]

[24] Merat SH, Rezvan H, Nouraei M, Abou Alghasemi H,

Jamali R, Amini Kafiabad S, et al. Seroprevalence and risk factors of hepatitis A virus infection in Iran: a population based study. Arch Iran Med. 2010; 13(2):99-104. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=168237

[25] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology. 2003; 37(4):917-23. [DOI:10.1053/jhep.2003.50161] [PMID]

[26] Lu H, Zeng L, Liang B, Shu X, Xie D. High prevalence of coronary heart disease in type 2 diabetic patients with non-alcoholic fatty liver disease. Arch Med Res. 2009; 40(7):571-5. [DOI:10.1016/j.arcmed.2009.07.009] [PMID]

[27] Alavian S, Ramezani M, Bazzaz A, Azuzabadi Farahani M, Behnava B, Keshvari M. Frequency of fatty liver and some of its risk factors in asymptomatic carriers of HBV attending the Tehran blood transfusion organization hepatitis clinic. Iran J Endocrinol Metab. 2008; 10(2):99-106. https://www.sid.ir/en/journal/ViewPaper.aspx?ID=117702

[28] Naveau S, Giraud V, Borotto E, Aubert A, Capron F, Chaput J. Excess weight risk factor for alcoholic liver disease. Hepatology. 1997; 25(1):108-11. [DOI: 10.1002/hep.510250120] [PMID]

[29] Thomopoulos KC, Arvaniti V, Tsamantas AC, Dimitropoulou D, Gogos CA, Siagris D, et al. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. Eur J Gastroenterol Hepatol. 2006; 18(3):233-7. [DOI: 10.1097/00042737-200603000-00002] [PMID]