International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article.

Evaluation of Serum Ferritin as an Independent Predictor of Non-alcoholic Steatohepatitis (NASH)

Md. Zia Hayder Bosunia[1*], Md. Lutful Kabir[2], Abu Said Md Rahenur Mondol[3], Syed Abul Foez[4], Md. Shafiqus Saleheen[5], Arfin Akter[6]

[1*]Assistant Professor, Department of Hepatology, Rangpur Medical College, Rangpur, Bangladesh.
[2]Assistant Registrar, Department of Endocrinology, Rangpur Medical College Hospital, Rangpur, Bangladesh.
[3]Junior Consultant Medicine, Rangpur Medical College Hospital, Rangpur, Bangladesh.
[4]Assistant Professor, Department of Hepatology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh.
[5]Assistant Registrar, Department of Neurology, Rangpur Medical College Hospital Rangpur, Bangladesh.
[6]Pathologist, Department of Microbiology, Rangpur Medical College, Rangpur, Bangladesh.

ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury. The spectrum of NAFLD ranging from simple fatty liver which in general follows a benign non-progressive clinical course to non-alcoholic steatohepatitis (NASH), a more serious form of NAFLD that may progress to cirrhosis and end-stage liver disease. Differentiation between simple steatosis and nonalcoholic steatohepatitis (NASH) is important as NASH may progress to cirrhosis. Till now liver biopsy is gold standard to diagnose NASH. No other specific laboratory or/imaging technique is available to confirm the diagnosis of NASH.

Aims: To examine the relationship between serum ferritin and NAFLD severity.

Materials & Methods: Demographic, clinical, histologic laboratory and anthropometric data were analyzed in 52 adult patients with biopsy proven NAFLD at a tertiary care center over a 2 year period. We evaluated serum ferritin with respect to histological inflammation and fibrosis in NAFLD patients. The Kleiner scoring system was used to classify NAFLD. Those with concurrent liver diseases and co-existing disease that would alter serum ferritin level were excluded. Patients were stratified into two groups based on their histologic stage of disease: Non NASH fatty liver (NNFL), Non-alcoholic steatohepatitis (NASH).

Results: Among 52 patients 25 were diagnosed as NNFL (48.1%) and 27 were diagnosed as NASH (51.9%). No fibrosis was found in 4 (7.7%) patients. Stage 1 fibrosis was found in 41(78.8%) patients, Stage 2 fibrosis was found in 3(5.8%) patients. Stage 3 fibrosis was found in 4 (7.7%) patients and cirrhosis was not found in any patients. On multiple regression analysis, serum ferritin levels had no significant difference in between NNFL and NASH group. Mean serum ferritin level of total 52 patients was 97.29 µg/dl. Among NNFL and NASH patients mean serum ferritin was 94.9 µg/dl and 99.51 µg/dl (P = 0.8). On multiple comparison of serum ferritin value among the different stage of fibrosis, it was also found that stage of fibrosis cannot be predicted by serum ferritin value.

Conclusion: Serum ferritin level could not predict the stage of underlying NAFLD disease.

Keywords: Serum Ferritin, Predictor, Non-alcoholic Steatohepatitis (NASH).

***Correspondence to:**

Dr. Md. Zia Hayder Bosunia,
Assistant Professor,
Department of Hepatology,
Rangpur Medical College, Rangpur, Bangladesh.

Article History:

Received: 12-08-2018, **Revised:** 07-09-2018, **Accepted:** 25-09-2018

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2018.4.5.030	

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a wide spectrum of conditions ranging from simple fatty liver which in general follows a benign non-progressive clinical course to NASH, a more serious form of NAFLD that may progress to cirrhosis and end-stage liver disease.[1] Hepatic steatosis describes the accumulation of fat, mostly as triglyceride, cholesterol and phospholipids, in excess of 5-10% of liver weight. The term 'NAFLD' was introduced as a means of grouping all of the variants under one broad term. NASH remains, by definition, a clinical-pathological diagnosis requiring both exclusion of ethanol as a major contributor, and the presence of cell injury.[2] The accurate diagnosis of NASH remains dependent on specific histological parameters in a biopsy. The key parameters include steatosis (usually mixed macro - and microvesicular), cellular ballooning, inflammation and fibrosis which range from slight perisinusoidal fibrosis to bridging and cirrhosis.[3]

The major indicators of injury have been incorporated into a score commonly called the NAS (NAFLD Activity Score) and staging system of fibrosis, which currently is being used in most clinical trials .[4]

These features have also been used to divide NAFLD into four types: (1) simple steatosis, (2) steatosis with inflammation alone (3) steatosis with inflammation and ballooning, and (4) steatosis with inflammation and fibrosis (Kleiner et al. 2005). In general, the latter two types constitute NASH. The first two types can be grouped into non - NASH fatty liver (NNFL).[5] The division of NAFLD into NASH and NNFL is significant because of their relationship to prognosis and appropriate therapy.

As NASH is a histological diagnosis, liver biopsy is needed to definitively establish or rule it out. However, it is not practical to biopsy every patient with suspected NAFLD. Arguments against routine liver biopsy include the generally benign course of the disease in most cases, lack of established effective therapies and the risks of biopsy. Another important limitation of liver biopsy relates to the fact that histological analysis remains subjective, influenced by the skill and experience of the examining pathologist. Importantly, sampling error is well recognized, and longer cores are needed for accurate fibrosis staging.[6] In Bangladesh liver biopsy is not possible in all hospital due to lack of experts performing liver biopsy and management of facility in case of complications.

Therefore, noninvasive means are required for selecting only the patients at the highest risk for NASH and/or fibrosis to undergo biopsy are highly desired. Among non-invasive tests, the FibroTest used to estimate hepatic fibrosis includes serum α_2-macroglobulin, apolipoprotein A-1, haptoglobin, total bilirubin, and GGTP levels, and the necroinflammatory activity index combines the same five markers plus the serum ALT level. This FibroTest is highly sensitive and specific in diagnosing advanced fibrosis. But unfortunately, 33% of patients had a FibroTest score between 0.30 and 0.70, and in this range, the test is inaccurate for assessing the stage of fibrosis.

In Bangladesh, two study regarding noninvasive markers in diagnosis of NAFLD were performed in Dept. of Hepatology, BSMMU. Mir 2008 showed the correlation between APRI (AST to Platelet ratio index) and hepatic fibrosis in nonalcoholic steatohepatitis. In his study he found that APRI is not a useful guide to predict significant fibrosis in NASH patients. Mollick 2009 concluded in his study that ultrasonological grading and liver elasticity measured by fibroscan is sensitive noninvasive tool for detecting severity of steatosis and moderate to severe fibrosis in NAFLD.

Ferritin is a large protein shell molecular weight 450 KDa comprised of 24 subunits, covering an iron core containing up to 4000 atoms of iron. Ferritin acts as the soluble storage form of iron in tissue. It is found in most cell of the body, especially macrophages, hepatocytes and erythrocytes. Synthesis occurs in the liver and the rate correlates directly with the cellular iron content. Control of ferritin synthesis occurs post transcriptionally (at the mRNA level).

A number of in vitro and in vivo studies in hepatocytes and liver tissue suggest that inflammatory stimuli, particularly the proinflammatory cytokine, TNF-α, up-regulates ferritin.[7] Oxidative stress may also up-regulate ferritin, depending on the specific oxidant stimuli[8] at the level of transcription and translation.[9] Therefore, it is plausible that SF may reflect increased disease severity in NAFLD either because of increased ongoing hepatic or systemic inflammation or increased body iron stores or a combination of these factors.

There are several studies which evaluate the relation of serum ferritin with histologic severity in NAFLD patients. Kris et al. 2012 conclude a SF>1.5 x ULN is associated with hepatic iron deposition, a diagnosis of NASH, and worsened histologic activity and is an independent predictor of higher NAFLD activity score and advanced hepatic fibrosis among patients with NAFLD.[10] Manousou et al. 2011 found that diabetes, serum ferritin concentrations, body mass index (BMI) and AST were independently associated with NASH and conclude that serum ferritin concentrations and BMI are strongly associated with fibrosis, portal and lobular inflammation in NAFLD patients.[11] Yoneda et al. 2009 also found that serum ferritin concentration was significantly higher in the NASH patients than in the patients with simple steatosis.[12]

Serum ferritin is a non-invasive marker, relatively cheaper and affordable. Moreover, In Bangladesh there is no study to evaluate the relation between serum ferritin with NASH. So, this study will help to find out the relationship of serum ferritin (SF) to histological severity in adult patients with NAFLD and to identify whether specific threshold levels of serum ferritin would differentiate patients with more advanced disease.

MATERIALS AND METHODS

This is a cross sectional study, carried out during the period of January 2011 to December 2012 in the Department of Heparology, Bangabandhu Sheikh Mujib Medical University (BSMMU). A total number of 52 patients who fulfilled the inclusion criteria (1.Patients with ultrasonographic evidence of fatty liver. 2. Histopathological evidence of fatty change in liver.) and exclusion criteria (Co- infection with HBV or HCV, Hypothyroidism, Consumption of drugs causing fatty change in liver, patients with significant alcohol intake, presence of co-existing disease that would alter serum Ferritin level, co-morbid conditions) were selected from patients attending the department of Hepatology at Bangabandhu Sheikh Mujib Medical University (BSMMU) as indoor admitted patients.

All data were collected from structured questionnaire and were analyzed by SPSS. Qualitative data were analyzed by Chi-square test and Quantitative data were analyzed by student's t-test. P values below 0.05 were considered statistically significant. Ethical clearance for the study was taken from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University.

RESULTS AND OBSERVATIONS

A total of 52 patients who were diagnosed with fatty liver by USG with biopsy proven NAFLD (defined as >5 % steatosis). Table 1 and Table 2 summarizes the histologic characteristics of the patients.

Patients are divided into two groups: NNFL (simple fatty liver) group and NASH group.NAS score below 5 considered as NNFL and NAS score 5 or above consider as NASH. Among 52 NAFLD patients 25 patients were diagnosed with NNFL (48.1%) and 27 patients were diagnosed with NASH (51.9%)

Fibrosis stage: Among 52 patients no fibrosis found in 4 (7.7%) patients. 41(78.8%) having fibrosis stage 1. Stage 2 fibrosis had

found in 3(5.8%) patients. Stage 3 fibrosis had found in 4 (7.7%) patients. Cirrhosis had not found in any patients.

Serum ferritin level in between NNFL and NASH

Among NNFL and NASH patients mean serum ferritin was 94.9 µgm/l with SD ±70.14 and 99.51 µgm/l with SD ±63.6. P value was 0.8 which was not significant (Table 3). Multiple comparisons of serum ferritin value among the different stage of fibrosis were assessed by one way ANOVA test and found stage of fibrosis cannot be predicted by serum ferritin value.(Table : 4.)

In cut off value of serum ferritin 94 µgm/l sensitivity 44%, Specificity 44%. If this value raised to 129 µgm/l sensitivity will be 25% and specificity 28%

Table 1: Histopathology (Steatosis, lobular inflammation, hepatocellular ballooning)

	Score	Frequency	Percentage
Steatosis grade			
5-33	1	22	42.3
>33-66	2	26	50
>66	3	4	7.7
Lobular inflammation			
No foci	0	0	0
<2 foci/200 X	1	2	3.8
2-4 foci/200X	2	34	65.4
>4 foci/200 X	3	16	30.8
Hepatocellular ballooning			
None	0	2	3.8
Few	1	34	65.4
Many	2	16	30.8
Biopsy diagnosis			
NNFL		25	48.1
NASH		27	51.9

NNFL = Non NASH fatty liver; NASH =Non-alcoholic steatohepatitis

Table 2: Histopathology (Fibrosis) of NAFLD patients

Fibrosis stage	Score	Frequency	Percentage
None	0	4	7.7
Perisinusoidal, or portal/ periportal only	1	41	78.8
Perisinusoidal and portal/ periportal	2	3	5.8
Bridging	3	4	7.7
Cirrhosis	4	0	0
Total		**52**	**100**

Table 3: Comparisons of serum ferritin between NNFL and NASH groups

Normal values	Total	NNFL	NASH	P value
Male 30- 300 µgm/l	97.29(±66.22)	94.9(±70.14)	99.51(±63.6)	0.8
Female 22-120 µgm/l				

NNFL = Non NASH fatty liver; NASH =Non-alcoholic steatohepatitis.

Table: 4: Multiple comparison of serum ferritin value among the different Stage of fibrosis

Fibrosis stage	No of patients	Serum ferritin value(Mean ±SD)	Fibrosis stage	No of patients	Serum ferritin value(Mean ±SD)	P value
0	4	149.55± 69.6	1	41	86.79±86.79	0.4
0	4	149.55± 69.6	2	3	135.95±53.4	1.0
0	4	149.55± 69.6	3	4	123.69±123.6	1.0
1	41	86.79±86.79	2	3	135.95±53.4	1.0
1	41	86.79±86.79	3	4	123.69±123.6	1.0
2	3	135.95±53.4	3	4	123.69±123.6	1.0

Serum ferritin value expressed µgm/l;

One way ANOVA was done for comparisons between fibrosis stages;

P < 0.05 considered as significant.

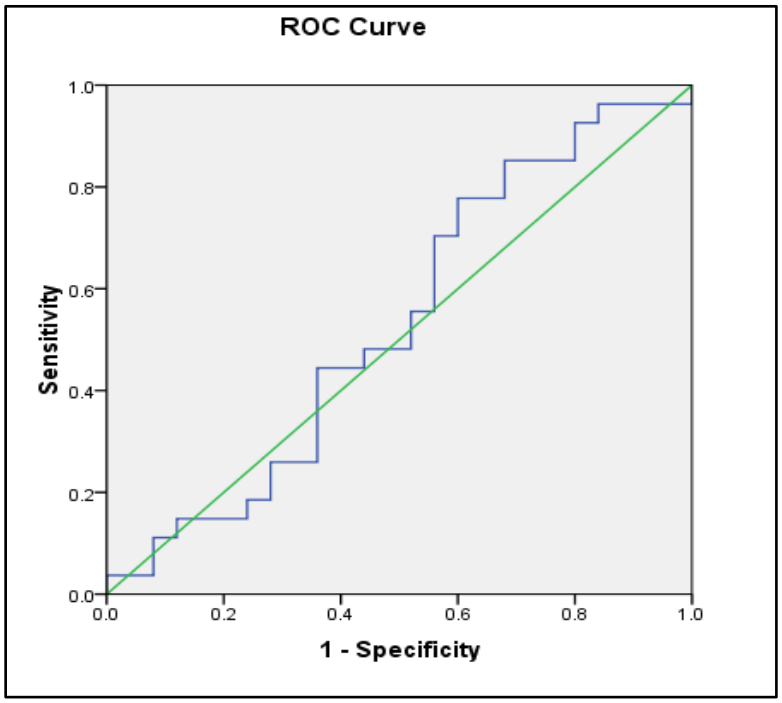

Fig 1: ROC Analysis of serum ferritin to predict NASH

DISCUSSION

In the present study, it is aimed to reveal serum ferritin as a reliable and feasible independent noninvasive parameter to predict nonalcoholic steatohepatitis (NASH). Kris et al said that a serum ferritin > 1.5 X ULN is associated with hepatic iron deposition, a diagnosis of NASH, and worsened histologic activity and is an independent predictor of advanced hepatic fibrosis among patients with NAFLD. Furthermore, elevated SF is independently associated with higher NAS, even among patients without hepatic iron deposition.[10] Manouso et al pointed out that serum ferritin and BMI are strongly associated with fibrosis, portal and lobular inflammation in NAFLD patients. They showed that increased concentrations of serum ferritin are an independent predictor of fibrosis (78 % sensitivity, 50% specificity) and inflammation, both portal and lobular (78% sensitivity, 60% specificity).[11] They used the cutoff of serum ferritin 240 ng/ml or more, and a BMI > 28.2, this combination identified patients at risk of having fibrosis with an 82% sensitivity and a 79% specificity .Yoneda et al found serum ferritin concentration was significantly higher in the NASH patients than in the patients with simple steatosis.[12] There is also evidence refuting the reliability of serum ferritin in predicting the stage of disease in NAFLD. Natasha et al revealed serum ferritin levels do not predict the stage of underlying NAFLD disease. They found Mean serum ferritin levels were similar in simple steatosis: 223.9 µgm/l; NASH: 240.7 µgm/l; cirrhosis: 271.3 µgm/l; p=0.84.[13] So, there is a conflicting evidence of the usefulness of serum ferritin as a prognosticator in studies of adults with NAFLD.

The present study demonstrated that serum ferritin was not elevated in NAFLD patients and there was no statistically significant correlation between serum ferritin level and stage of Non-alcoholic fatty liver disease. Here, mean serum ferritin among NNFL and NASH patients was 94.9 µgm/l (SD ±70.14) and 99.51 µgm/l (SD ±63.6) (*P* = 0.8).Though Natasha et al also showed increasing serum ferritin does not correlate with advancing stage of fatty liver disease but they found hyperferritinemia is common among patients with NAFLD. The reason of normal serum ferritin level in NAFLD patients in this study may be due to different ethnic group of study population with different religious and cultural background with other study population. Like other study population here no study subject was social drinker which may be a cause of normal serum ferritin level. Moreover, in this study there was no cirrhotic patient which may be another cause behind this normal serum ferritin level.

LIMITATIONS

Small sample size was a major limitation of this study. Moreover patients are not selected randomly and only selected those patients who attended OPD, so there may be selection bias.

CONCLUSION

This study indicates that serum ferritin levels do not predict the stage of Non-alcoholic fatty liver disease. Further research is required with a large sample size to determine whether serum ferritin is an independent non-invesive marker for NASH or not.

REFERENCES

1. Schaffner, F., Thaler, H., 1986.Nonalcoholic fatty liver disease. Prog Liver Dis. 8, 283-98.
2. Baumeister, S.E., Völzke, H., Marschall, P., 2008. Impact of fatty liver disease on health care utilization and costs in a general population: a 5-year observation. Gastroenterology. 134, 85 – 94.
3. Yeh, M.M., Brunt, E.M. 2007.Pathology of nonalcoholic fatty liver disease. Am. J. Clin. Pathol.128,837 –47.
4. Kleiner, D.E., Brunt, E.M, Van Natta, M.L. 2005. Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histologic scoring system for NAFLD. Hepatology.41,1313-21.
5. Marchesini. G., Bugianesi, E., Forlani, G., Cerrelli, F., Lenzi, M., Manini, R. et al. 2003. Nonalcoholic fatty liver, steatohepatitis and the metabolic syndrome. Hepatology. 37, 917-23.

6. Ratziu, V., Charlotte, F., Heurtier, A., 2005. LIDO Study Group. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology.125, 1898 – 1906.
7. Miller, L.L., Miller, S.C., Torti, S.V., Tsuji, Y., Torti, F.M., 1991.Iron-independent induction of ferritin H chain by tumor necrosis factor. Proc Natl Acad Sci, USA. 88, 4946-50.
8. Jang, M.K., Choi, M.S., Park, Y.B. 1999. Regulation of ferritin light chain gene expression by oxidized low-density lipoproteins in human monocytic THP-1 cells. Biochem Biophys Res Commun. 265, 577-83.
9. White, K., Munro, H.N. 1988.Induction of ferritin subunit synthesis by iron is regulated at both the transcriptional and translational levels. J Biol Chem. 263, 8938-42.
10. Kris, v.k., Patricia, B., Laura, A., Wilson, Matthew, M., Yeh, et al. 2012. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with Nonalcoholic fatty liver disease. Hepatology. 55, 77-85.
11. Manousou, P., Kalambokis, G., Grillo,F., Watkins, J., Xirouchakis, E., Pleguezuelo, M., et al. 2011. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. Liver international. 10,730-738.
12. Yoneda, M et al. 2010. Serum ferritin is a clinical biomarker in Japanese patients with nonalcoholic steatohepatitis (NASH) independent of HFE gene mutation. Dig Dis Sci. 55, 808-14.
13. Natasha, C.,Gerald, M., Matias, W., Julia,U,. et al. 2012. Serum ferritin levels do not predict the stage of underlying Non-alcoholic fatty liver disease. J gastrointestin Liver dis, 21, 53-58.

Source of Support: Nil. **Conflict of Interest:** None Declared.

Cite this article as: Md. Zia Hayder Bosunia, Md. Lutful Kabir, Abu Said Md Rahenur Mondol, Syed Abul Foez, Md. Shafiqus Saleheen, Arfin Akter. Evaluation of Serum Ferritin as an Independent Predictor of Non-alcoholic Steatohepatitis (NASH). Int J Med Res Prof. 2018 Sept; 4(5):128-32.
DOI:10.21276/ijmrp.2018.4.5.030