COMPARISON OF SERUM FERRITIN LEVELS IN NAFLD PATIENTS AND HEALTHY CONTROLS

Mehwish Qamar, Syeda Abeer Fatima, Haseeb Ahmed Khan, Qudsia Umaira Khan, Amna Nadeem, Hina Sikandar*

Combined Military Hospital Lahore/National University of Medical Sciences (NUMS) Pakistan, *Shalimar Memorial College of Medicine & Dentistry, Lahore Pakistan

ABSTRACT

Objective: To determine and compare serum ferritin levels in non-alcoholic fatty liver disease (NAFLD) patients of our ethnicity.

Study Design: Cross sectional comparative study.

Place and Duration of Study: The study was conducted at Radiology department of Shalamar Hospital, Medical OPD of Combined Military Hospital, Lahore and Medical OPD of Omer Hospital, Lahore, from Jul 2015 to Sep 2015.

Methodology: A total number of 43 non-alcoholic fatty liver disease patients diagnosed after ultrasonography were included in the study. An equal number of age, gender and body mass index (BMI) matched healthy controls were selected by non-probability purposive sampling. Fasting serum ferritin levels, body mass index and waist circumference were measured in both the cases and controls. Data were entered and analyzed on SPSS 21. For quantitative data median IQR was calculated. Mann Whitney U-test was used for group comparison. To find an association, Spearman's Ranked correlation was applied. A *p*-value of ≤0.05 was taken as significant.

Results: The non-alcoholic fatty liver disease cases had significantly higher serum ferritin ($p<0.001$) compared to the controls and a significant correlation between ferritin with body mass index and waist circumference in the non-alcoholic fatty liver disease subjects was recorded ($p=0.04$ and $p=0.03$ respectively).

Conclusion: The non-alcoholic fatty liver disease patients have higher levels of serum ferritin as compared to the healthy controls in our ethnicity but within normal range.

Keywords: BMI, NAFLD, Serum ferritin, Waist circumference.

INTRODUCTION

Aggregation or accumulation of lipids in the hepatocytes, exceeding 5% weight of the liver with no history of significant ethanol intake (<20g /dl) and viral hepatic infections of B and C types is known to be non-alcoholic fatty liver disease (NAFLD)[1]. It consists of a wide variety of liver damage consisting of simple steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis along with hepatocellular carcinoma, all of which are included in NAFLD[2]. 1n 1980s Ludwig found similar lesions on liver biopsy, of alcoholic fatty liver in people with no history of alcohol intake and used the term NASH and NAFLD for the first time[3]. In the last two decades nonalcoholic fatty liver disease (NAFLD) has become a substantial health hazard in parallel with obesity and type 2 diabetes mellitus. NAFLD is now attributed to be the commonest chronic liver disease of the Western World. Its prevalence has increased drastically with increasing weight. About 60-95% of obese western adults are reported with NAFLD[4]. According to world gastroenterology organization the prevalence of NAFLD in Pakistan is 18% and that of obesity and diabetes mellitus is 10.3% and 10% respectively[5]. Many risk factors are attributed to play a prime role in causing fat accumulation in the liver, out of which the most pronounced ones are, increasing age, type 2 diabetes, insulin resistance and visceral fat.

With still an unclear pathogenesis a "multi hit theory" has been proposed in which the first hit is caused by insulin resistance which leads to a reversible fat accumulation in the hepatic cells. This makes the liver vulnerable to the next

Correspondence: Dr Mehwish Qamar, House No. 675, Askari 9, Zarar Shaheed Road, Lahore Cantt Pakistan

Received: 08 Apr 2019; revised received: 08 Sep 2020; accepted: 12 Oct 2020

hit which is due to oxidative stress attributed to cytokine induction, adipokines (leptin and adiponectin) and increased hepatic iron deposition all leading to inflammation of the liver[6]. In the last hit the genetic variation has a pivotal role in the progression of the disease.

Iron is an important mineral not only involved in hemoglobin synthesis but in many enzymatic reactions as well. When in an excess, the normal carbohydrate metabolism, functioning of adipose tissue and beta-cell are all affected[7]. In inflammation the hepcidin levels increase which causes iron retention in the liver. This iron through Fenton catalyst reaction and by increasing lipid peroxidation in fatty liver causes progression of the disease. Ferritin is an iron storage protein present in the cytoplasm of nucleated cells especially in the liver and muscles. An increased level of ferritin in the serum not only indicates iron over load in the body but inflammation also and hence can be used as their marker[8]. In inflammatory conditions like that of obesity and accumulation of visceral fat causes release of different cytokines and oxidative stress, which causes damage to the cells. Due to this damage, ferritin spills into the serum. A definite correlation is present between markers of hepatocellular damage (ALT and AST) and serum ferritin[9]. Keeping this association of ferritin with inflammation and iron overload the study was conducted to find its levels in our population. In our part of the world 'hikmat' has a strong influence as 70 percent of our population lives in the rural areas where few doctors are available and people prefer going to a 'hakeem'. The hakeems give iron supplementation to the NAFLD patients. A controversial role of dietary iron on NAFLD and its progression have been reported. The study was conducted with the aim to find out the levels of ferritin in the NAFLD patients in our population for the better understanding of its role in the disease.

METHODOLOGY

This cross-sectional comparative study was conducted from July 2015 to September 2015 in Radiology department of Shalamar Hospital and Medical OPD of Combined Military Hospital (CMH), Lahore and Omer Hospital Lahore. The study protocol was approved by Ethics Review Committee of Postgraduate Medical Institute Lahore. The sample size for the study was calculated by using the formula n=(Z 1α/2+Z 1-β)2 (σ 12+σ 22)/(μ1-μ2)2 , after replacing the values in the formula as following[10]:

Z 1α/2 = 1.96 (95% confidence interval)

Z 1-β = 1.28 (90% power of the test)

μ 1 = 22.9

μ 2 = 10.3

σ 1 = 23.3

σ 2 = 10.4

n = (1.96+1.28)2 (23.32 +10.42)/ (22.9-10.3)2

n= 43 for each group

With non-probability purposive sampling, 43 subjects with hyperechoic liver pattern on ultrasonography and deranged liver function tests (ALT >30 IU/ml for males and ALT >19 IU/ml for females) were labeled as cases and an equal number of age, gender and BMI matched subjects with normal liver echoic pattern on ultrasonography and normal liver function tests were labeled as controls. Upon history and hospital laboratory data, the patients with liver cirrhosis, other liver disease (viral hepatitis, autoimmune hepatitis, haemochromatosis, Wilson's disease, α1-antitrypsin deficiency), type 1 diabetes mellitus, endocrine disorders, renal failure, thrombotic disorders, cancer, pregnancy and use of any weight loosing drugs or OCPs in past 6 months were excluded from the study. A Written informed consent was taken from all the subjects. Personal history, past history, drug history, and socioeconomic history was recorded on a pre-formed performa and general physical examination was conducted on all the subjects. Body mass index (BMI) was calculated by the formula; body weight (kg)/ height (m^2). The body mass was measured on a platform scale without the shoes and light clothing. The height was measured with a stadiometer. The waist circumference was mea-

sured in centimeters of the subjects in a standing position with a flexible measuring tape (1mm accuracy), at the midpoint between the bottom edge of the last rib and the iliac crest without compression of tissues.

After 10 hours of fasting 5ml of blood was drawn from the anti-cubital vein of the subjects under aseptic measures. The blood was centrifuged in yellow capped test tube at 3,000 rpm for 15 minutes, serum was separated and stored at -20°C. Quantitative determination of fasting serum ferritin was done by using enzyme linked immunoassay test kit [Bio-Check, Inc. USA] and ELISA analyzer [Rayto RT 2100 C, USA]. Hospital data for liver function tests and complete blood count were used in the study. Data was entered and analyzed by using IBM-SPSS version 21 for Windows 10, x 64 bit. After applying the Shapiro-Wilk test the data was found to be non-normally distributed. The quantitative variables were presented in median (IQR) while categorical data was presented in frequency and percentage. For group comparison of categorical data Fisher Exact test was used while for the quantitative data, Mann Whitney U-test was used. Spearman's ranked correlation was applied to find out relationship between the quantitative variables and *p*-value ≤0.05 was considered significant.

RESULTS

The study was performed on a total number of 86 subjects out of which 43 (50%) subjects were labeled as cases and rest of the 43 (50%) as controls. Each group comprised of almost an equal number of both the genders with 22 males and 21 females (51% and 49% respectively). More than half of the cases were non diabetic and normotensive. Upon calculation of the BMI most of the subjects (72%) were found to be obese as prede-

Table-I: Frequency distribution of general characteristics among cases and controls.

Parameters		Cases (n=43) n (%)	Controls (n=43) n (%)
BMI	Normal (18-22.99)	4 (9)	4 (9)
	Overweight (23-24.99)	8 (19)	8 (19)
	Obese (>25)	31 (72)	31 (72)
Waist Circumference (WC)	Normal	3 (7)	12 (28)
	Raised (>90 for males and >80 for females)	40 (93)	31 (72)
Type-II Diabetes Mellitus	Absent	32 (74)	42 (98)
	Present	11 (26)	1 (2)
Blood pressure	Raised	15 (35)	6 (14)
	Normal	28 (65)	37 (86)

Table-II: Frequency distribution and comparison of serum ferritin levels in cases and controls using Fisher exact test.

	Serum Ferritin Levels			*p*-value
	Below Normal	Normal	Above Normal	
Cases (n=43), n (%)	-	40 (93)	3 (7)	<0.01*
Controls (n=43) , n (%)	2 (5)	40 (93)	1 (2)	<0.01*

**p-value <0.05 is considered as significant*

Table-III: Comparison of serum ferritin levels between cases and controls using Mann Whitney U test.

	Cases, Median (IQR)	Controls, Median (IQR)	*p*-value
Serum ferritin (ng/ml)	80.10 (56.50)	42.8 (35.2)	<0.01*

**p-value <0.05 was considered statistically significant.*

Table-IV: Correlation between serum ferritin with other parameters using Spearman's correlation (n=43).

Correlation of Serum Ferritin With	Spearman's Correlation r	*p*-value
Waist circumference	0.49	<0.01*
Body Mass Index (BMI)	0.35	0.02*
Alanine Aminotransferase (ALT)	0.60	<0.01*
Aspartate Aminotransferase (AST)	0.60	<0.01*

**p-value <0.05 was considered statistically significant.*

fined by WHO[11] with a median (IQR) age of 46.98 (12.61) years in the cases. The waist circumference of all the female cases was more than 80cm, which according to WHO is a cut off value for the development of increased risk of metabolic diseases in Asian ethnicity[12]. While in the male cases, more than 86% had waist circumference >90 cm. Statistically highly significant difference was noted between the waist circumferences of cases and controls (p<0.01) (table-I). Among the cases and controls, 40 (93%) subjects in each group had normal ferritin levels with highly significant (p<0.01) difference in the number of subjects between the categories (table-II). As shown in table-III the serum ferritin levels were higher in cases with a median (IQR) of 80.10 (56.50) ng/ml, compared to 42.8 (35.2) ng/ml in controls with a highly significant (p<0.01) difference between the two groups. Serum ferritin was significantly correlated to waist circumference, BMI, ALT and AST (p<0.01, p=0.02, p<0.01 and p<0.01) in the NAFLD subjects as shown in table-IV.

DISCUSSION

Serum ferritin was measured and compared using Mann Whitney U test between the cases of NAFLD and healthy controls. The cases and controls were age, gender and BMI matched. The patients with deranged LFTs and hyperechoic margins of the liver on ultra-sonography were labeled as cases in this study. More than 70 percent of the subjects in the study were obese (BMI >25 kg/m^2) and were middle aged, with the age range of 46.98 ± 12.61 years. In many previous studies an association between the prevalence of NAFLD to gender has been reported Some of these studies have reported male gender association[13,14]. While others have found a strong association of female gender to the prevalence of the disease[15,16]. This is contrary to our findings as we found no association of gender with NAFLD. This discrepancy is probably due to the non-purposive sampling technique and a smaller sample size, although the diagnostic modality used were same that is the abdominal ultrasonography. Similar to our findings, a study conducted in Taiwan on 607 NAFLD subjects aged 50-59 had no gender association[17]. The reason could be due to the hormonal changes in the late adulthood.

The cases had a significantly raised waist circumference as compared to the controls (p<0.001) which is inconsistent to a Brazilian study in which 247 obese adolescents, aged from 12 to 19 years were included[18]. The reason for this raise in the waist circumference in diseased compared to the healthy group is that the waist circumference is an independent predictor of visceral fat hence in NAFLD it will be raised.

Serum ferritin levels were found to be within a normal rage (<250 for males and <120 for females), although significantly higher in the NAFLD patients as compared to healthy subjects (p<0.001). Similar results have been reported by Ployzos[19]. While another study reported hyperferritinemia in NAFLD subjects. The most probable cause could be higher BMI ≥34 kg/m^2 and increased prevalence of NASH and cirrhotic subjects in that study population[20]. Manousou[21] reported hyperferritinemia only in the NASH patients while Parikh[22] reported a significant difference of serum ferritin levels among NAFLD and controls as well as between NAFL and NASH but all in the normal range. In chronic liver diseases like NAFLD, high ferritin levels reflect the increased release of it from the damaged cells. Its levels increase in insulin resistance and inflammations like NAFLD[23].

After applying Spearman's Ranked Correlation, serum ferritin was found to have a statistically significant positive correlation with BMI, waist circumference, ALT and AST in NAFLD subjects. Nakeeb *et al*[24] reported a significant correlation between serum ferritin of NAFLD subjects with that of waist circumference and BMI in an Egyptian population. Jeong *et al* reported no association of ferritin with BMI in female NAFLD patient though a significant association of ferritin with waist circumference and BMI in male NAFLD subjects is reported[25]. A pediatric study showed a significant relationship between ferritin and ALT/AST[9]. The reason of this association is

that all these of these are being markers of inflammation are hence correlated to each other.

RECOMMENDATIONS

- Serum iron levels should also be measured and correlated to serum ferritin levels.
- Similar study with a larger sample size should be conducted.
- Serum ferritin in the hepatocytes should also be measured.

ACKNOWLEDGEMENT

We acknowledge Professor Dr. Muniza Saeed for her continuous guidance and support. Without her this research would have never been possible.

CONCLUSION

In our ethnicity the patients of NAFLD have a normal serum ferritin level and serum ferritin has a strong correlation with anthropometric measures in NAFLD.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. World J Hepatol 2017; 9(16): 715–32.
2. El-Kader SMA and Ashmawy EMSED. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol 2015; 7(6): 846-58.
3. Brown GT, Kleiner DE. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Metabol 2016; 65(8): 1080-86.
4. Ashtari S, Pourhoseingholi MA, Zali MR. Non-alcohol fatty liver disease in Asia: Prevention and planning. World J Hepatol 2015; 7(13): 1788-96.
5. World Gastroenterology Organisation. Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. 2012.
6. Fang YL, Chen H, Wang CL, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model". World J Gastroenterol 2018; 24(27): 2974–83.
7. Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: A promising therapeutic target. J Hepatol 2011; 55(44): 920-32.
8. Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics 2014; 6(1): 748-73.
9. Dubern B, Girardet JP, Tounian P. Insulin resistance and ferritin as major determinants of abnormal serum aminotransferase in severely obese children. Int J Pediatr Obes 2006; 1(2): 77-82.
10. FerreiraVSG, Pernambuco RB, Lopes EP, Morais CN, Rodrigues MC, Arruda MJ, et al. Frequency and risk factors associated withnon-alcoholic fatty liver disease in patients with type 2 diabetes mellitus. Arq Bras Endocrinol Metab 2010; 54 (4): 362-68.
11. World Health Organization, Western Pacific Region. The Asia-Pacific perspective: redefining obesity and its treatment. 2000. Available At: www.diabetes.com.au/pdf/obesity_report.pdf.
12. World Health Organization. Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation Geneva, 8–11 December 2008. http://apps.who.int/iris/bitstream/handle/10665/44583/9789241501491_eng.pdf.
13. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Epidemiol 2013; 178(1): 38-45.
14. Wang Z, Xu M, Hu Z, Hultström M, Lai E. Sex-specific prevalence of fatty liver disease and associated metabolic factors in Wuhan, south central China. Eur J Gastroenterol Hepatol 2014; 26(9): 1015-21.
15. Wang Z, Xu M, Peng J, Jiang L, Hu Z, Wang H, et al. Prevalence and associated metabolic factors of fatty liver disease in the elderly. Exp Gerontol 2013; 48(8): 705-09.
16. Summart U, Thinkhamrop B, Chamadol N, Khuntikeo N, Songthamwat M. Gender differences in the prevalence of nonalcoholic fatty liver disease in the Northeast of Thailand: A population-based cross-sectional study. F1000 Res 2017; 6(1): 1630-35.
17. Cheng HY, Wang HY, Chang WH, Lin SC, Chu CH, Wang TE, et al. Nonalcoholic Fatty Liver Disease: Prevalence, Influence on Age and Sex, and Relationship with Metabolic Syndrome and Insulin Resistance. Int J Gerontol 2013; 7(4): 194-98.
18. Clemente AP, Netto BD, de Carvalho-Ferreira JP, da Silveira Campos RM, de Piano Ganen A, Tock L, et al. Waist circumference as marker for screening nonalcoholic fatty liver disease in obese adolescents. Rev Paul Pediatr 2016; 34(1): 47-55.
19. Polyzos SA, Kountouras J, Zavos C, Papatheodorou A, Katsiki E, Patsiaoura K, et al. Serum ferritin in patients with nonalcoholic fatty liver disease: evaluation of ferritin to adiponectin ratio and ferritin by homeostatic model of assessment insulin resistance product as non-invasive markers. Immuno-Gastroentrol 2012; 1(2): 119-25.
20. Chandok N, Minuk G, Wengiel M and Uhanova J. Serum ferritin levels do not predict the stage of underlying non-alcoholic fatty liver disease. J Gastrointestin Liver Dis 2012; 21(1): 53-58.
21. Manousou P, Kalamboki G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, et al. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. Liver Int 2011; 31(5): 730-39.
22. Parikh P, Patel J, Ingle M, Sawant P. Serum ferritin levels predict histological severity in patients with nonalcoholic fatty liver disease in India. Indian J Gastroenterol 2015; 34(3): 200-08.
23. Liu B, Xuan X, Liu J, Li F, Yin FZ. The Relationship between serum ferritin and insulin resistance in different glucose metabolism in nonobese han adults. Int J Endocrinol 2015; 2015: 642194.
24. El Nakeeb N, Saleh SA, Massoud YM, Hussein A, Hamed R. Serum ferritin as a non-invasive marker in the prediction of hepatic fibrosis among Egyptian patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2017; 1(3): 112-19.
25. Jeong DW, Lee HW, Cho YH, Yi DW, Lee SY, Son SM, et al. Comparison of serum ferritin and vitamin d in association with the severity of nonalcoholic Fatty liver disease in korean adults. Endocrinol Metab (Seoul) 2014; 29(4): 479-88.