RESEARCH ARTICLE

Significance of serum ferritin and De Ritis ratio in non-alcoholic fatty liver disease as diagnostic markers

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Abstract

Objective: To ascertain the significance of serum ferritin and De Ritis ratio as diagnostic markers in patients of non-alcoholic fatty liver disease with and without type 2 diabetes mellitus.

Methods: The comparative cross-sectional study was conducted from February to October 2022 at the Radiology Department of Combined Military Hospital, Rawalpindi, Pakistan, and comprised individuals aged 30-65 who were divided into 3 groups. Healthy controls formed group I, non-alcoholic fatty liver disease patients without type 2 diabetes mellitus formed group II and non-alcoholic fatty liver disease patients with type 2 diabetes mellitus were in group III. Blood 5ml was withdrawn and assessed for alkaline phosphatase, aspartate transaminase, alanine transaminase and ferritin. De Ritis ratio was calculated and subjected to intergroup comparison. Data was analysed using SPSS 22.

Results: Of the 210 subjects, 110(52.4%) were males and 100(47.6%) were females, with 70(33.3%) in each of the three groups. Group I had 38(54.3%) females and 32(45.7%) males with mean age 37.50±4.513. In group II, there were 27(38.6%) females and 43(61.4%) males with mean age 45.86±9.646, while in group III there were 35(50%) females and 35(50%) males with mean age 54.01±9.243 years. Serum ferritin levels were significantly increased in patient groups II and III compared to control group I ($p<0.05$). De Ritis ratio was markedly raised in groups II and III compared to group I ($p<0.05$). Ferritin was significantly correlated to age, weight, height, fasting blood glucose, haemoglobin, alkaline phosphatase, aspartate aminotransferase, alanine transaminase and bilirubin ($p<0.05$). De Ritis ratio had a significant correlation with body mass index and fasting blood glucose ($p<0.05$).

Conclusion: Serum ferritin and De Ritis ratio were found to be useful diagnostic indicators for non-alcoholic fatty liver disease, highlighting their importance in improving disease screening.

Key Words: NAFLD, Non-alcoholic steatohepatitis, Ferritin, SGPT, SGOT.

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Introduction

The term non-alcoholic fatty liver disease (NAFLD), coined by Ludwig in 1980, refers to a continuum of liver conditions caused by the presence of steatosis in >5% of hepatocytes without the use of alcohol[1]. NAFLD has a wide range of severity and progression, from mild steatosis to the more inflammatory condition called non-alcoholic steatohepatitis (NASH), which eventually leads to the formation of cirrhosis[2]. Along with age, ethnicity and gender, other factors like nutrition, metabolic status, genetic susceptibility and epigenetic variables are also involved in the complex etiopathogenesis of NAFLD [3]. NAFLD is now considered a epidemic with recent estimate of 24% worldwide prevalence [4]. NAFLD is a multisystem disease acting as a substantial risk factor for the development and progression of type 2 diabetes mellitus (T2DM)[5]. T2DM and NAFLD have a bidirectional relationship in disease progression [6]. Despite tremendous research on NAFLD, accurate early-stage diagnosis is still lacking since it is based on history with evidence of hepatic steatosis by imaging [7] rather than on the basis of certain pathology-related biomarkers. Puncture liver biopsy is the gold standard for determining if a patient has NASH or liver fibrosis,[8] but it is an invasive and expensive procedure with inherent surgical risks. The significance of many indirect biochemical indicators of NAFLD, which are molecules released into the circulation as a result of a diseased process in the liver but are similarly capable of reflecting the existence and activity of inflammation has been reported to include aminotransferases, like alanine transaminase (ALT) and aspartate transaminase (AST), substances produced by the liver in hepatocytes, such as ferritin, haptoglobin, alkaline phosphatase (ALP) and coagulation factors[9]. Recent studies have shown that iron levels may be a good indicator of the severity of liver disease [10]. Although iron is essential for life, excess cellular iron is toxic as labile iron

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can lead to oxidative stress (OS) and insulin resistance (IR), which are key features of NAFLD pathogenesis. Hence, the cell has evolved an intricate system to balance cellular iron import, export and storage in order to regulate iron homeostasis [11]. The chief protein for storing iron within the cytoplasm of nucleated cells, particularly in the liver and muscles, is ferritin. A high amount of ferritin in the blood suggests an excess of iron in the body. Since ferritin is referred to as an acute-phase protein, and serum concentrations rise in inflammatory situations, it can be used as a marker for such disorders [12]. A definite correlation is present between ALT and AST, markers of hepatocellular damage, and serum ferritin [13]. The AST/ALT ratio, commonly known as the De Ritis ratio (DRR), is a helpful indication for the aetiology of liver[14]. DR holds a promise as a diagnostic tool for assessing NAFLD, but its practical implementation has been limited in Pakistan due to paucity of data. The current study was planned to investigate the efficacy of ferritin as a surrogate biomarker for screening NAFLD status when combined with DRR.

Materials and Methods

The comparative cross-sectional study was conducted from February to October 2022 at the Radiology Department of Combined Military Hospital (CMH), Rawalpindi, Pakistan. After approval from institutional ethics review committee, the sample size was calculated using OpenEpi calculator[15] taking into account NAFLD prevalence in Pakistan of 14%. The sample was raised using non-probability purposive sampling technique. Those included were diagnosed NAFLD patients of either gender aged 30-65 years with and without T2DM. Individuals suffering from NAFLD due to alcohol consumption along with those suffering from chronic illnesses other than hypertension taking medication for >6 months were excluded. Healthy controls matched for age and gender were also included. After taking written informed consent, the subjects were divided into 3 groups; controls in group I, NAFLD patients without T2DM in group II, and NAFLD patients with T2DM in group III.

A structured proforma was used to record general information, such as name, gender and age, smoking/addiction, personal history, and any other medical history of each study participant. Height and weight were recorded, and body mass index (BMI) was calculated[2].

After fasting for 6-8 hours, hepatic assessment of the patients was carried out using real-time sonography. On the basis of parenchymal brightness, liver-to-kidney contrast, vascular blurring of portal or hepatic vein and gallbladder wall definition, fatty liver was determined by consultant radiologists. Qualitative grades were labelled mild, moderate or severe, or grade 0-3, with 0 being normal. Blood 5ml was drawn under aseptic conditions from all the subjects' anti-cubital vein after 10 hours of fasting. The serum was isolated from the blood after the blood had been centrifuged at 3,000 rpm for 20 minutes. The serum was kept at -20 degrees C till the biochemical tests were carried out at the institutional laboratory. Using an automatic biochemical analyser, fasting blood sugar (FBS), ALT, AST and bilirubin were measured using commercial kits. Using an enzyme-linked immunoassay (ELISA) analyser and kit, fasting serum ferritin was quantified.

Data was analysed using SPSS 22. Categorical data was expressed as frequencies and percentages, while quantitative data was expressed as means ± standaed deviation. One-way analysis of variance (ANOVA) was used to make intergroup comparisons, followed by a post-hoc Tukey test. The association of DRR and ferritin with other biochemical markers was assessed using Pearson's r coefficient. P<0.05 was considered significant.

Results

Of the 210 subjects, 110(52.4%) were males and 100(47.6%) were females, with 70(33.3%) in each of the three groups. Group I had 38(54.3%) females and 32(45.7%) males with mean age 37.50±4.513. In group II, there were 27(38.6%) females and 43(61.4%) males with mean age 45.86±9.646, while in group III there were

Table-1: One-way analysis of variance (ANOVA) of clinical parameters related to the study groups.

Parameters	Group I (controls) Mean ± SD	Group II (NAFLD without T2DM) Mean ± SD	Group III (NAFLD with T2DM) Mean ± SD	Sign (*p*-value)
ALT(U/L)	29.01 ± 6.942	37.07 ± 15.217	33.11± 16.00	0.002*
AST(U/L)	23.00 ± 3.897	34.74 ± 14.703	34.74± 14.703	0.001*
Deritis ratio	0.8133 ± 0.128	0.94815±0.1424	1.0126 ±0.1409	0.001*
ALP(U/L)	96.39 ± 28.723	109.86 ± 29.424	116.06 ± 39.542	0.002*
Ferritin(ng/ml)	58.70± 26.025	114.42± 84.846	114.46± 114.64	0.001*
Age (years)	37.50±4.513	45.86±9.646	54.01±9.243	0.001*
Height(feet)	5.62±0.2717	5.540±0.343	5.476±0.264	0.012*
Weight(kilograms)	71.16±8.895	84.59±12.96	81.61±8.776	0.001*
BMI	24.25±3.0657	29.809±4.921	29.44±3.793	0.001*
FBG(mg/dl)	84.26±6.46	84.26±6.46	152.82±50.418	0.001*
Haemoglobin	13.864±1.6444	13.978±1.967	13.429±1.7582	0.164
Albumin (g/L)	45.73±3.938	44.67±5.426	45.20±3.069	0.341
Bilirubin (umol/L)	7.59±2.197	11.46±5.230	9.47±4.842	0.001*

*Shows significant results (≤0.05). NAFLD: Non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus, SD: Standard deviation, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, BMI: Body mass index, FBG: Fasting blood glucose

35(50%) females and 35(50%) males with mean age 54.01±9.243. Except haemglobin (Hb) (p>0.05), all variables, including ferritin and DRR, were significantly different across the groups (Table 1). Among the 140(66.6%) patients in groups II and III, 83(59.3%) had NAFLD grade 1, 44(31.4%) grade 2 and 13(9.3%) grade 3. Mean ALT, AST, ALP, DRR and ferritin had no significant relation with NAFLD grades (p>0.05) except for ferritin which increased in relation to grades (p<0.05).

Table-2: Correlation between serum ferritin with other study parameters.

Correlation of serum ferritin with	Pearson's correlation (r-value)	p-value
Age	0.186	0.007*
Weight	0.196	0.004*
Height	0.158	0.022*
BMI	0.064	0.357
FBG	0.202	0.003*
Haemoglobin	0.208	0.002*
ALT	0.399	0.001*
AST	0.400	0.001*
Deritis ratio	0.032	0.645
ALP	0.230	0.001*
Albumin	0.010	0.885
Bilirubin	0.351	0.001*

*Shows significant results (≤0.05)

• Correlation is significant at the 0.01 level (2-tailed). ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, BMI: Body mass index, FBG: Fasting blood glucose.

Ferritin was significantly correlated to age, weight, height, FBG, Hb, ALP, AST, ALT and bilirubin (p<0.05) (Table 2). DRR had a significant correlation with BMI and FBG (p<0.05) (Figure).

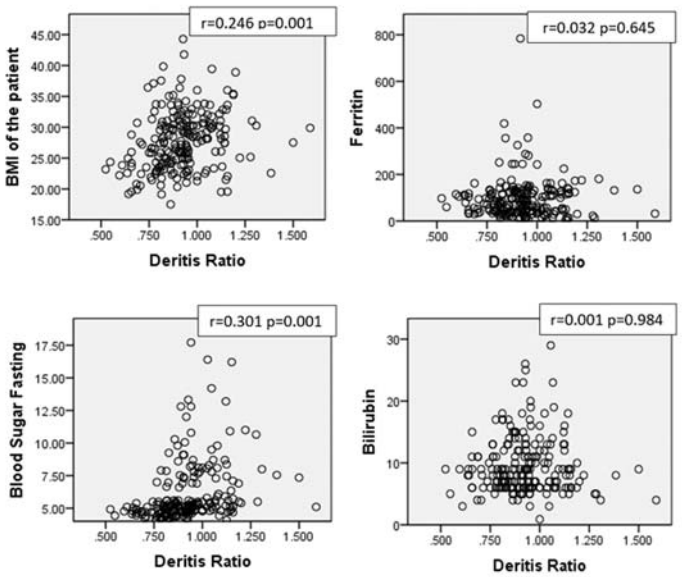

Figure: Correlation of Deritis ratio with body mass index (BMI), ferritin, fasting blood glucose (FBG) and bilirubin.

Discussion

Early non-invasive diagnosis of fatty liver disease is crucial for therapeutic purposes because it can prevent serious problems from developing as the illness progresses to more advanced stages. The DRR, sometimes referred to as the AST/ALT ratio, is frequently used to assess NAFLD and liver function deterioration[16]. DRR was significantly increased (p<0.05) in NAFLD cases (1.2204±0.17954) in a study done in Nepal [17] which is in congruence with the current results. Sanju et al. found a correlation of AST, ALT and ALP with both alcoholic fatty liver disease (AFLD) and NAFLD. However, AST and ALP had a stronger correlation than ALT with both AFLD and NAFLD (p=0.003). As such, the researchers concluded that DRR was not significantly linked to FLDs[18]. This was in contrast to the current study where ANOVA showed significant relationship of DRR with the two NAFLD groups (p=0.001). A study with 85 subjects concluded that women (65.88%) had a higher incidence of NAFLD than men (34.12%). Only ALT revealed a significant variation across grade 1 and grade 2 NAFLD (p=0.027)[19]. However, the current study had no significant variation acorss the grades, but significant difference was seen between the controls and the NAFLD grades.

The connection between liver enzymes and diabetes has been examined in various studies, with one study stating that the occurrence of diabetes was correlated with liver damage as seen by raised ALT and gamma gamma glutamyl transferase (GGT) values [20]. In the current study, a marked increase in liver enzymes was observed in the patient groups although no significant difference was seen, implying that deranged liver enzymes could be a marker for NAFLD and diabetes that have a bidirectional aetiological relationship. Additionally, the DRR was said to be closely connected to metabolic syndrome and, hence, T2DM[21]. The current study assessed DRR as an NAFLD predictive marker, and found it to be markedly raised in NAFLD with and without T2DM compared to the controls. Hence, it must be given due importance in NAFLD diagnosis.

Iron is an essential element for normal body functioning, but has the potential to be hazardous when in excess. It can alter lipid metabolism, oxidative stress, and insulin signalling, which may exacerbate the onset of NAFLD[22,23]. Previous studies have shown that the level of serum ferritin, which is the main storage form of

iron in the body, is correlated with T2DM and NAFLD[24]. Amin et al. reported that serum ferritin levels were increased in T2DM patients with NAFLD [2]. In the present study, compared to the controls (58.70±26.025), NAFLD patients with T2DM (114.46±114.641) and without T2DM (114.42±84.846) had significantly increased serum ferritin levels. A recent study led to the identification of ferritin as a potential non-invasive predictive biomarker of NAFLD with significantly higher value of serum ferritin in NAFLD ($p<0.001$) compared to controls, whose surrogate value increased when combined with other routine biochemical measurements[26].

The current study has several limitations. A larger cohort could have improved generalizability of the findings. A wider age range could provide a more thorough picture of the condition because the study's focus was on people aged 30-65 years, who may not accurately represent the complete age spectrum of NAFLD patients. Longitudinal studies might give a more reliable result in finding out whether ferritin and DRR are accurate predictors of the progression of the disease, or if they are only the outcome of a liver function change. Besides, the study had a cross-sectional design which only provides a snapshot of an ongoing process.

Conclusion

DRR and serum ferritin had a strong correlation with anthropometric measures in NAFLD patients with and without T2DM. DRR and serum ferritin can be used as diagnostic markers of liver disorder for screening liver diseases.

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Author's Contributions

KM and **AR:** Study design, data review and final approval of the manuscript .

AM and **MII:** Acquisition of data

All authors performed: Data collection, analysis, interpretation and writing.