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CORRELATION BETWEEN FETUIN-A LEVELS AND NAFLD FIBROSIS SCORE (NFS) IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) PATIENTS

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Abstract. Fetuin-A is a plasma glycoprotein expressed in the liver. Fatty liver accumulation because changing fetuin-A expression leads to nonalcoholic fatty liver disease (NAFLD). The benefits of serum fetuin-A as a predictor of fibrosis biomarkers in NAFLD patients remain unclear. Non-invasive scoring system predicting fibrosis among patients with NAFLD is the NAFLD fibrosis score (NFS). **Objective.** This study aims to find the correlation between circulating fetuin-A levels and NFS in NAFLD patients. **Methods.** Eighty subjects (male, 54; female, 26) followed this cross-sectional study. NAFLD is diagnosed with an abdominal ultrasound. The serum levels of fetuin-A measured by ELISA. NFS is used to identify the probability of fibrosis in subjects. The correlation between Fetuin-A levels and NFS was determined using Pearson correlation tests. A p-value < 0.05 was significant. **Results.** There was significant difference in fetuin-A levels ($p < 0.001$) between NAFLD degree I (421.71 pg/mL), NAFLD degree II (494.88 pg/mL), and NAFLD degree III (692 pg/mL). The NFS low probability score in 46 subjects and NFS indeterminate score in 34 subjects. Serum level of fetuin-A correlate significantly with NFS ($r = 0.478$, $p < 0.001$). **Conclusion.** There is a positive correlation between serum levels of fetuin-A and NFS in NAFLD patients.

Keyword: Fetuin-A, NAFLD, NAFLD Fibrosis Score

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1 Introduction

Non Alcoholic Fatty Liver Disease (NAFLD) is a condition that covers the entire clinical spectrum of fatty liver or simple steatosis to steatohepatitis and cirrhosis of the liver without a significant amount of alcohol consumption. The main problem of NAFLD is its progressiveness, progressing to liver cirrhosis, liver failure, and hepatocellular carcinoma. [1][2][3][4][5][6]

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The gold standard in diagnosis and prognosis in NAFLD is liver biopsy. The procedure is invasive, expensive, and has high errors in sampling and the risk of complications, i.e., illness, bleeding, and even in some cases, death. The NAFLD Fibrosis Score (NFS) is a scoring system to identify the severity of liver fibrosis without liver biopsy among NAFLD patients.[7][8][9][10]

A potential biomarker in NAFLD is fetuin-A. Fetuin-A has adipogenic properties, reducing the expression of adipokine adiponectin atheroprotective and worsening the absorption and storage of free fatty acids in adipocytes. In human serum, levels of Fetuin-A are positively correlated with liver fat as measured by standard resonance spectroscopy.[11][12][13]

The study aimed to determine whether there was a significant correlation of Fetuin-A levels with the NAFLD Fibrosis Score. This study can be helpful in strategies for achieving a diagnosis, and determining the prognosis of the disease to minimize invasive action in patients.

2 Methods

This research is a cross-sectional study conducted at the Adam Malik Hajj Center General Hospital Medan, Indonesia, with consecutive sampling methods between July and September 2021. In this study, 80 patients were diagnosed with NAFLD. The exclusion criteria in the study were Hepatitis B or hepatitis C virus infection, malignancy or autoimmune patients, pregnant women, and patients who consumed alcohol >30 grams/day in men and >20 grams/day in women.

The study subjects with metabolic syndrome were >18 years old and diagnosed with NAFLD based on anamnesis, physical examination, abdominal ultrasound, and laboratory examination. Anamnesis also includes the history of previous diseases and the history of consuming alcohol. We establish the diagnosis of NAFLD using abdominal ultrasound.

Serum fetuin-A is taken by venous blood draw using a serum separator tube and stored at ≤20^{o}C—Fetuin-A quantitative measurement using ELISA method with ChemWell 2910 Automated EIA with Sandwich Immunoassay technique. The fetuin-A unit is pg/mL. All NAFLD patients are classified based on NFS criteria.

Data analysis is created in statistical data software and presented in the form of tables and figures. The correlation between Fetuin-A levels and NFS was determined using Pearson correlation tests. We conducted Statistical analysis using Statistical Package for Social Sciences (SPSS) version 25. A p-value < 0.05 was considered significant.

3 Results

Table 1. Basic Characteristics of NAFLD Patients

Demographic Characteristics	**n = 80**
Gender, n (%)	
Man	54 (67,5%) [a]
Woman	26 (32,5%)
Age, year	54,81 ± 8,66 [b]
Long Suffering from DM, years	6,84 ± 2,72 [b]
Body Mass Index, kg/cm^2	23,13 ± 3,13 [b]
Obesity, n (%)	
Yes	42 (52,5%) [a]
No	38 (47,5%)
NFS	-1.15 ± 0.98 [b]
NFS group	
Low Probability Score	46 (57.5%) [a]
Indeterminate Score	34 (42.5%)
NAFLD degree, n (%)	
Degree I	32 (40%) [a]
Degree II	29 (36,2%)
Degree III	19 (23,8%)
Albumin, mg/dL	2.41 ± 0,46 [b]
HbA1c, %	10,07 ± 2,04 [b]
Platelet 10^9/L	298,76 ± 88,83 [b]
AST, IU/L	23,63 ± 4,69 [b]
ALT, IU/L	46,5 ± 27,97 [b]
Fetuin A, pg/mL	512,55 ± 202,6 [b]

Data in table 1 shows that subjects with NAFLD degree I amounted to 32 patients (40%), degree II amounted to 29 patients (36,2%), and degree III amounted to 19 patients (23,8%). The NFS has an average of -1,15. NAFLD patients had an average fetuin-A level of 512,55.

Table 2. NFS Relationship with NAFLD Degrees

NAFLD Degree	n	NFS	p*
		Median (Min – Max)	
Degree I	32	-1,84 (-2,67 s/d -0,85)	<0,001
Degree II	29	-1,46 (-1,96 s/d 0,38)	
Degree III	19	0,39 (-1,63 s/d 0,67)	

*Kruskal Wallis

We can see in table 2 that there is a significant relationship between NFS values and NAFLD degrees (p-value <0,001) using the Kruskal Wallis test. The highest NFS average in NAFLD degree III is -0,43.

Table 3. Relationship of Fetuin-A Levels with NAFLD Degrees

NAFLD Degree	n	Fetuin-A, pg/mL	p*
		Average (SD)	
Degree I	32	421,71 ± 124,16	<0,001
Degree II	29	494,88 ± 168,52	
Degree III	19	692,53 ± 246,06	

*Kruskal Wallis

Table 3 shows a significant difference in average fetuin-A levels between each group of NAFLD degrees (p<0,05) using the Mann-Whitney test. NAFLD degree III has a higher intermediate level than degrees I and II with 692,53 pg / mL.

Table 4. Correlation of Fetuin-A Levels with NFS in NAFLD patients

Variable	r	P value
NFS	0,478	< 0,001

*Pearson

Table 4 shows the results of the Pearson correlation between fetuin-A levels and NFS values. The test obtained an r = 0,478 with p < 0.001, positively correlating Fetuin-A levels and NFS in NAFLD patients.

4 Discussion

We obtained that the subjects were primarily male (67,5%) compared to women (32,5%). In Monem et al (2018), men suffered more from NAFLD at 68% than women at 32%. In Indonesia, Rahmatullah et al. found that men dominated NAFLD sufferers aged between 55 ± 10 years.[14]

However, another study conducted by Roberts et al (2021) in Australia showed different results where 56% were female and 44% male. The controversy about NAFLD prevalence in both men and women was not evident. A meta-analysis showed that women of NAFLD had a lower prevalence but a higher risk of progression to NASH and cirrhosis.[15][16]

Differences in NAFLD by gender are associated with the nature of the hormone estrogen. Gambineri et al. found an inverse relationship between estrogen and insulin resistance. Sex differences in hormone and lipid levels can be an independent protective factor for NAFLD. Middle-aged and older men with NAFLD had higher BMI and waist circumference than women.[17]

The average age of the subjects was 54,81 years, with the youngest age 30 years and the oldest aged 72 years. The study conducted by Roberts et al. (2021) in Australia also showed not many different results where the average age of subjects with NAFLD was 61,2 years. Likewise, in Brazil, a study conducted by Silva et al.(2018) got the average age of older NAFLD patients at 47,7 and 52,87 years.[15][18]

Some studies found a faster progression of NAFLD in women during menopause. The study showed that the prevalence of NAFLD in men (41,1%) was twice as high as in women (20,3%). The majority of women were 1,5 times higher at age ≥ 50 (25,8%) than < 50 years (17,0%). Reduce choline synthesis and liver export of very-low-density lipoproteins (VLDL) because of declined estrogen levels in postmenopausal women, leading to the accumulation of ectopic fats, as observed in hepatosteatosis.[19]

In this study, we found the average BMI at 23,13 kg/m^2, which belongs to the overweight category, with the lowest BMI of 14.5 kg/cm^2 and the highest BMI of 31 kg/cm^2. In this study, 42 subjects (52,5%) were obese. We found the same thing in a study by Rahmatullah et al. (2021) in Semarang, with overweight subjects 89,1%. Obesity is also the most comorbid in research conducted by Darmadi (2021) in Medan. This study is in line with Gavril et al. in Romania, where 94,5% belonged to the obese group. Research undertaken by Haukeland (2012) in Norway shows the average BMI of study subjects was obesity category II with a BMI of 30,5 kg/m^2; wherein the study, negative a correlation was found between fetuin-A and BMI with r =-0,21 and p = 0,026.[14][20][21][22]

Being overweight is the most common comorbid because, in an inflammatory state, the accumulation of fat in the abdomen will stimulate the release of proinflammatory cytokines that contribute to the pathophysiology of NAFLD. In obese conditions, the prevalence of NAFLD increases to 50-75% because of the relationship between intrahepatocellular triacylglycerol (IHTAG) and total adipocytes. This relationship makes NAFLD be referred to as the "liver manifestation of metabolic syndrome".[14][23][24]

The patients involved in the study had suffered from DM on average for 6,84 years. A similar result was found by Hossain et al. (2021) in Bangladesh, with an average long-suffering from DM is 5,1±3,8 years. Insulin resistance (IR) may cause the higher prevalence of NAFLD in T2DM because of the similarity in pathogenesis. Insulin resistance in the liver and extra liver tissues such as adipose tissue and skeletal muscle act synergistically, leading to systemic inflammation and

the release of proatherogenic and nephrotoxic factors. Increased entry of free fatty acids into ectopic tissue due to excessive lipolysis leads to the onset of insulin resistance and apoptosis in the muscles and liver. The lipotoxic state in NASH results in hepatocyte necroinflammation. Thus DM and insulin resistance increase the chance of developing into NAFLD and increase the progression of the disease to NASH and cirrhosis, thereby increasing mortality and morbidity.[16][25]

In this study, subjects who suffered from NAFLD based on abdominal ultrasound as many as 80 patients, where the most obtained were patients with NAFLD degree I, namely as many as 32 patients (40%), then followed by NAFLD degree II as many as 29 patients (36,3%) and degree III as many as 19 patients (23,8%). Almost all studies on NAFLD use biopsy modalities as confirmation of diagnosis. The study conducted by Droz et al. in 2021 involved 143 NAFLD patients and having liver biopsies as confirmation.[26]

However, in this study, we determined the NAFLD degree based on the abdominal ultrasound results conducted by experienced experts. Ethnic background, lifestyle, genetics, and diagnostic criteria or techniques can vary the prevalence and severity of NAFLD. A biopsy is a gold standard for diagnosing NAFLD and NASH, but ultrasound is a first-line diagnostic technique. Im et al. stated that the prevalence of NAFLD varied according to five diagnostic procedures: biopsy (51,4%), ultrasound (30,3%), computed tomography (22,9%), Fatty Liver Index (FLI, 20,0%), and fibroscan (52,5%).[19]

We can clinically use NFS to identify NAFLD patients with a higher likelihood of bridging fibrosis (stage 3) or cirrhosis (stage 4). NFS has the advantage that no unique test item is included.Transient elastography is another method for the non-invasive assessment of fibrosis stages. However, this method has a high failure rate of up to 41% in patients with obesity.[26][27]

Many previous studies have assessed the relationship between Fetuin-A levels and NAFLD. The studies generally compared groups with NAFLD with controls. In this study, researchers compared fetuin-A levels against each degree of NAFLD. Liu et al., in meta-analysis 2021, stated that fetuin-A levels in NAFLD sufferers were higher than in control (SMD = 0,43, 95% CI: 0,22–0,63, $p<0,001$), but no difference between fetuin-A levels in NAFLD and NASH patients. In addition, the relationship of fetuin-A levels with fibrosis remains unclear. Haukeland et al. investigated 111 NAFLD subjects with levels of 324±98 mg / L and 131 healthy controls with levels of 225±75 mg / L, and there was an increase in Fetuin-A levels in NAFLD patients ($p< 0,001$).[20][28]

This study showed a difference in Fetuin A levels between the group of grade I subjects and the second and third-degree subject groups. Similarly, for the grade I subjects with degree III, there was a significant difference in average Fetuin A levels ($p<0,05$). Fetuin-A is involved in developing insulin resistance in animal and human research that contributes to NAFLD

development. The reduction of Fetuin-A levels in the blood was found in lifestyle modifications and metformin administration for 12 weeks based on Lindarto's 2014 research. In animal studies, Fetuin-A promotes lipid-induced inflammation by binding free fatty acids to toll like receptors-4 (TLR-4), most likely contributing further to NAFLD development. In human studies, evidenced by biopsy results, both stool-A circulation levels and fetuin-A liver expression were higher in NAFLD than in healthy controls regardless of histological state and BMI class. Fetuin-A levels in NAFLD patients do not vary even with different diagnostic methods.[28][29][30]

The lowest average NFS value in the NAFLD group degree I was -1,87 (-2,67 to -0,85). Then the NFS average at NAFLD degree II was -0,85 (-1,96 to 0,38). The highest NFS in NAFLD degree III is -0,43 (-1,63 to 0,67). Using the Kruskal Wallis test, there is a significant relationship with the p-value < 0,001. Furthermore, the researchers analyzed the differences in Fetuin A levels with the NFS group and found a meaningful difference between the NFS groups with p = 0,008. The average fetuin-A was higher in subjects with NFS indeterminate score with a value of 582 ± 180,01 pg/mL and the group of subjects with NFS low probability score with a value of 461,22 ± 180,01 pg / mL. There was no NFS group with an advanced fibrosis score in this study.

In the study, There was a positive correlation between Fetuin A levels and NFS in NAFLD patients (r = 0,478, p<0,001). This result is in line with those stated by Mondal et al. in 2018 and Huang et al. (2015). There was a positive correlation between Fetuin A levels and fibrosis conditions in NAFLD but based on fatty liver index (FLI) parameters. Unlike the study conducted by Sato et al. in Japan in 2014, 295 subjects with NFS univariate analysis had a negative correlation with Fetuin-A levels (r =-0.2; p<0.01). [1][31]

The different results from previous studies could be due to metabolic factors such as obesity, T2DM, and hypertension. These metabolic factors can easily affect fetuin-A levels. Age and drugs to treat these comorbidities, such as metformin and pioglitazone, also play a role in influencing fetuin-A levels. In addition, the technique used to diagnose NAFLD also affects the relationship between fetuin-a and liver inflammation. Measuring fetuin-A concentration (such as different ELISA kits) is also the reason for the differences in various studies.

There are some limitations to this study. First, this study is cross-sectional, so the causal relationship between NAFLD and fetuin-A levels is unclear. Apart from collecting and adjusting possible confounding, unmeasured and undefined factors indicate the possible residual effect, such as NAFLD duration. Second, researchers did not perform liver biopsies, the gold standard for NAFLD diagnosis. The absence of subjects with advanced fibrosis due to sampling limitations was also a limitation in the study.

5 Conclusion

There was a positive correlation between fetuin A levels and NFS in NAFLD patients.

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