Original Article

Can liver enzyme profile be a predictor of NAFLD in type-2 diabetes mellitus (T2DM) patients?

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ABSTRACT

Population-based studies reported that the prevalence of NAFLD in T2DM patients, ranging from 30% - 70%. This study aims to determine differences in liver enzyme profiles in NAFLD patients with and without DM and to make elevated liver enzyme levels a predictor or early screening for NAFLD in T2DM patients. This research is an observational analytic study with a *cross sectional approach*. Research data collection was carried out in October 2022 at Tugurejo Hospital, Semarang, Indonesia. The research was conducted on the medical records of patients diagnosed with NAFLD from 2020-2021 using the total sampling method. In addition to liver enzyme levels, confounding variables were also taken which are also risk factors for NAFLD, including: age, sex, BMI, lipid profile and blood sugar levels. Prognostic factors were analyzed by bivariate analysis test and multivariate analysis using logistic regression analysis to obtain relative risk values (RR). The results showed that AST (p, OR, CI95% = 0.216, 0.998, (0.995-1.001)) and ALT (p, OR, CI95% = 0.870, 1.000, (0.994-1.005)). are not significant as a predictor of NAFLD in T2DM patients. Other variables that were studied showed that Body Mass Index (p, OR, CI95% = 0.023, 0.874, (0.779-0.982)) and triglyceride levels ((p, OR, CI95% = 0.006, 0.992, (0.986-0.998)) can be used as a predictor of NAFLD in T2DM patients.

Keywords: NAFLD, NAFLD predictor, T2DM, Transaminase enzyme, Liver enzyme

Introduction

NAFLD is a continuation of the process of insulin resistance [1]. The diagnosis of NAFLD is based on the results of anamnesis and clinical examination, liver function tests, radiological image and histopathological examination. Abnormal liver function tests are often the main reason why NAFLD patients are referred to a hepatologist. Elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most common laboratory abnormalities. In the early phase of steatosis, ALT levels are usually higher than AST. Elevated bilirubin values, decreased albumin and prolonged plasma prothrombin time indicate advanced liver disease and liver failure has occurred [2, 3].

Liver function tests (LFT) are often used in clinical practice to screen for liver disease, monitor the progress of previously known diseases, and monitor the effects of drugs that have the potential to be hepatotoxic. Common liver function tests include: ALT, AST, alkaline phosphatase, bilirubin, albumin and prothrombin time. ALT and AST provide an overview of the concentration of intracellular liver enzymes that enter the circulation and act as markers of liver cell/hepatocyte injury. Alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and bilirubin are markers of biliary system function and cholestasis [4]. Albumin and prothrombin time are reflection of liver synthesis function. ALT and AST are normally < 30-40 units/L. An increase in aminotransferases of more than 8 times the upper limit of normal indicates acute hepatitis, ischemic hepatitis, or drug or toxin-related liver injury. Meanwhile, transaminases that increase slightly but over a long time are often found in patients with diabetes mellitus [4, 5].

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The theory behind the improvement in liver function test results in diabetic patients is that the liver plays a role in maintaining normal blood glucose levels during fasting and after meals. Loss of insulin effect in the liver can lead to glycogenolysis and increased hepatic glucose production. Abnormalities in triglyceride stores and lipolysis in insulin-sensitive tissues such as the liver are early manifestations of conditions associated with insulin resistance, and they appear earlier than fasting hyperglycemia [6]. However, the precise role of genetic, environmental and metabolic factors and the sequence of events that lead to insulin resistance remains unclear [6].

In experiments conducted on animals, it was found that chronic hyperinsulinemia is a predisposing factor to the occurrence of insulin resistance in the liver. It is characterized by the failure of insulin to signal an increase in insulin 2nd receptor substrate. There is also an upregulation of SREBP-1c (sterol regulatory element binding-protein-1c) which causes lipogenesis. Lipogenesis occurs mainly in the liver causing an increase in intracellular triglycerides and leading to fatty liver. It can also increase the synthesis of VLDL secretion. Therefore it can be concluded that the condition of hyperinsulinemia can be directly correlated with insulin resistance in the liver and this is related to fatty liver [7].

The condition of excess free fatty acids found in insulin resistance is known to have a toxic effect on hepatocytes. Possible mechanisms for this are cell membrane damage at high concentrations of free fatty acids, mitochondrial dysfunction, toxin formation, and activation and inhibition of important metabolic regulatory steps [8]. Another possibility that can lead to increased levels of transaminases in insulin resistance states includes oxidative stress caused by reactive lipid peroxidation and inflammatory cells which have recently been involved [9]. The state of insulin resistance is also characterized by the emergence of proinflammatory cytokines such as TNF-α (tumor necrosis factor-alpha) which can also play a role in the hepatocellular injury. In a preeliminary study it was found that an increase in the TNF-α promoter was found in patients with NASH (nonalcoholic steatohepatitis) [10]. The above theories are theories that link the elevation of transaminitis with hepatocyte injury. A hypothesis can also be drawn that states that an increase in ALT, which is a gluconeogenic enzyme whose gene transcription can be suppressed by insulin, may indicate a disturbance in insulin signaling, not just hepatocellular injury [11].

Materials and Methods

Study design

This study was an observational analytic study with a cross-sectional approach. Data collection was carried out in October 2022 at Tugurejo Hospital, Semarang City, Central Java Province, Indonesia. The study was conducted on the medical records of patients diagnosed with NAFLD from 2020 -2021 using the total sampling method. The research data is secondary data obtained from the patient's medical records including age, sex, body mass index, presence or absence of DM comorbidities, random blood sugar laboratory results, fasting blood sugar, lipid profile, liver enzyme profile including ALT and AST.

Statistical analysis

Data analysis was carried out by looking at parameters that differed significantly in groups of patients with type 2 diabetes mellitus and non-diabetes using the T-Independent test if the data were normally distributed and the MannWhitney test if the data were not normally distributed. Prognostic factors were analyzed using univariate analysis using the kai-square test (X2 test) and multivariate analysis using logistic regression analysis to obtain relative risk values (RR).

Ethical considerations

The study protocol was approved by the Clinical Research Ethics Committee of Medical Faculty, University of Muhammadiyah Semarang (approval date: 23.11.2022, approval number: 066/EC/KEPK-FK/UNIMUS/2022) The study was conducted in accordance with the 1975 Declaration of Helsinki, as revised in 2013.

Results and Discussion

Table 1. Baseline Characteristics of the participants

Characteristics	N	%	Mean ± SD	Median (min-max)
Age			51.59 ± 12.30	53 (8 – 82)
Gender				
Man	41	38,3		
Woman	66	61.7		
BMI (Body Mass Index)			23.79 ± 4.15	23.3 (14.0 – 44.0)
Blood Sugar Level			176.66 ± 135.15	129 (57 – 665)
Fasting Blood Sugar			158.40 ± 112.79	102 (50 – 570)
Total Cholesterol			152.84 ± 49.47	132 (60 – 310)
TG (Triglycerides)			225.89 ± 90.30	215 (86 – 624)
Cholesterol-LDL			129.99 ± 99.56	104 (25 – 990)

Cholesterol-HDL	83.09 ± 85.63	57 (9 – 657)
AST (Aspartate Aminotransferase)	94.44 ± 149.89	40 (7 – 824)
ALT (Alanine Aminotransferase)	57.34 ± 92.35	37 (7 – 895)

Abbreviations: SD: Standart Deviation; N:number of participants

This study included 107 male and female participants. Their characteristics are presented in **(Table 1)**. Subject gender was divided into 41 male subjects and 66 female subjects, with an average age of 51.6 years old. The research data was taken from patient medical records including BMI, Blood Sugar Level, Fasting Blood Sugar, Total Cholesterol, Triglyceride, Cholesterol-LDL, Cholesterol-HDL, AST and ALT.

Table 2. Results of the Chi Square test for the relationship between the incidence of DM and NAFLD

DM Incidence	NAFLD Incidence		p	OR (95% CI)
	NAFLD	Non NAFLD		
DM	30 (53,6%)	26 (46,4%)	0,632	1,30 (0,61 – 2,78)
Non DM	24 (47,1%)	27 (52,9%)		

Abbreviations : OR: Odd Ratio; CI: Confidence Interval

* Significant (p <0.05)

Table 2 shows that participants were grouped into 54 patients who had NAFLD and 53 patients who were Non-NAFLD. For the chi-square test analysis, subjects were regrouped into 30 DM patients with NAFLD and 26 DM patients without NAFLD (total patients with DM = 56 patients). Non-DM patients with NAFLD were 24 patients, Non-DM and Non-NAFLD patients were 27 patients (total Non-DM patients = 52 patients. The results of the chi square test using Continuity Correction obtained a p-value = 0.632, because the p-value > 0.05, it can be concluded that there is no significant relationship between the incidence of T2DM and NAFLD.

Table 3. Relationship of Independent Variables to NAFLD

Variable	NAFLD		p
	NAFLD	Non-NAFLD	
Age	51 (8 – 81)	56 (27 – 82)	0.131 ‡
Gender			
Man	23 (56.1%)	18 (43.9%)	0.472 ¥
Woman	31 (47%)	35 (53%)	
BMI (Body Mass Index)	25 (16.2 – 36.6)	22.2 (14 – 44)	**0.001 ‡ ***
Blood Sugar Level	150 (57 – 280)	114 (67 – 665)	0.697 ‡
Fasting Blood Sugar	101 (68 – 449)	103 (50 – 570)	0.282 ‡
Total Cholesterol	131 (90 – 261)	132 (60 – 310)	0.511 ‡
TG (Triglycerides)	223 (86 – 624)	189 (99 – 403)	**0.003 ‡ ***
Cholesterol-LDL	106.5 (55 – 990)	93 (25 – 221)	0.234 ‡
Cholesterol-HDL	39.5 (9 – 312)	67 (20 – 657)	0.489 ‡
AST (Aspartate Aminotransferase)	46 (12 – 690)	35 (7 – 824)	0.067 ‡
ALT (Alanine Aminotransferase)	39.5 (8 – 283)	32 (7 – 895)	0.111 ‡

* Significant (p <0.05); ¥ Continuity Corrections; ‡ Mann Whitney

From the analysis test results it is known that only BMI and TG have significant differences based on NAFLD as shown in **Table 3**. The test results also showed that increased levels of the transaminase enzyme did not have a significant effect on the occurrence of NAFLD. To find out the factors that have a strong influence, from the results of the bivariate test with a p-value <0.25, a logistic regression multivariate test was performed.

Table 4. Logistic Regression Test Results for NAFLD

Variable	p	RR	95% CI
Age	0.071	1.034	0.997 – 1.072
BMI (Body Mass Index)	**0.023***	0.874	0.779 – 0.982
TG (Triglycerides)	**0.006***	0.992	0.986 – 0.998

Cholesterol-LDL	0.511	0.999	0.995 – 1.003
AST (Aspartate Aminotransferase)	0.216	0.998	0.995 – 1.001
ALT (Alanine Aminotransferase)	0.870	1,000	0.994 – 1.005

Abbreviations : RR: Relative Risk; CI: Confidence Interval
* Significant ($p < 0.05$)

From the results of the logistic regression test, it was found that BMI and TG had a p value <0.05, so it can be concluded that BMI and TG are factors that have a strong influence on NAFLD.

The results of the multivariate logistic regression analysis are listed in **Table 4**. This analysis includes all factors that are thought to influence the occurrence of NAFLD. The results showed that BMI and TG have a strong influence on the occurrence of NAFLD.

The prevalence of NAFLD is increasing rapidly worldwide and is comparable to the increasing incidence of obesity and type 2 diabetes (T2DM). The prevalence of NAFLD is increased in conditions associated with insulin resistance, such as T2DM, obesity, dyslipidemia and metabolic syndrome [12]. Several population-based studies report that the prevalence of NAFLD is greater in patients with T2DM, ranging from 30% to 70%. A recent systematic review and meta-analysis showed that the overall prevalence of NAFLD was 55.5% among T2DM patients. In addition, T2DM patients are at higher risk for possible NASH and liver-related complications, including cirrhosis-related deaths, and facing NAFLD-related deaths 3-5 times higher than individuals without DM [12]. The theory behind the improvement in liver function test results in diabetic patients is that the liver plays a role in maintaining normal blood glucose levels during fasting and after meals. Loss of insulin effect in the liver can lead to glycogenolysis and increased hepatic glucose production. Abnormalities in triglyceride stores and lipolysis in insulin-sensitive tissues such as the liver are early manifestations of conditions associated with insulin resistance, and they appear earlier than fasting hyperglycemia [6]. However, the precise role of genetic, environment and metabolic factors and the sequence of events that lead to insulin resistance remains unclear. In this study, the increase in transaminase enzyme levels was not statistically a predictor of the occurrence of NAFLD in T2DM patients. It could be because the number of patients was too small. In the future, it may be necessary to carry out further research with a larger sample size. Apart from that, patients are not treated with restrictions on how long each subject has experienced diabetes, so this variable needs to be added to further studies. In this study it was found that BMI had a strong influence on the occurrence of NAFLD, this is in line with research conducted by Li *et al*. that obese individuals have more than three times the risk of NAFLD occurring. Increased awareness of the high risk of NAFLD in obese individuals may help prevent or delay the development of NAFLD [13]. Apart from BMI, this study found that triglycerides play an important role in the occurrence of NAFLD in DM patients. This was described in a cohort study by Xing Jie *et al*. that looked for the relationship between triglycerides, BMI and NAFLD, the results of the study found that this study could be used to determine the power of TG in mediating the relationship between BMI and the incidence of NAFLD. The conclusion of the study states that BMI is an independent risk factor for NAFLD while TG is associated with NAFLD in the high BMI group (BMI ≥24); TG contributes 26.050% of the relationship between BMI and NAFLD in people with obesity (BMI ≥24) [14].

Conclusion

T2DM patients accompanied by increased BMI and triglyceride levels can be used as a predictor of NAFLD compared to changes in AST and ALT levels. Suggestions for further research are to use a larger number of subjects and use cohort research methods to obtain more accurate data.

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Conflict of interest: None

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Ethics statement: The study involved an informed consent from where participants received information about the study. Participants gave their consent in an electronic form before taking part in the study.

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