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Article

Liver Function Test among Patients with Non-Alcoholic Fatty Liver Disease

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Abstract: NAFLD is one of the most common liver diseases worldwide, and is associated with obesity, type 2 diabetes, high blood pressure, and body fat imbalance. The disease is classified into two main types: simple fatty liver, which does not usually lead to liver damage, and non-alcoholic steatohepatitis (NASH), which can progress to scarring (fibrosis) of the liver or even liver failure. The effect of non-alcoholic fatty liver disease on liver function levels and the relationship between them. The study included 75 patients with NAFLD, 34 males and 41 females, 18-75 years of age, they are diagnosed by ultrasound at GIT & hepatobiliary, and other group as control group included 81 apparently healthy subjucts 46 males and 35 females, 18-75 years of age. Physiological measurements include waist circumference, height, weight, BP, Approximately 5 ml of venous blood samples were obtained from both control individuals and patients with NAFLD after 10-12 hour overnight fasting by using sterile disposable syringes for the assessment of total serum bilirubin, unconjugated and conjugated bilirubin, Aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The results shows a significant increase in the levels of AST and ALT ($P<0.01$) in patients group, while there is non-significant change ($P>0.05$) in each of total TSB, direct bilirubin, indirect bilirubin, total protein, albumin and globulin. The results were based on the determination of unconjugated and conjugated bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT). The serum of people who have non-alcoholic fatty liver disease (NAFLD) may contain elevated levels of AST and ALT. Because bilirubin, albumin, globulin, and total protein do not have any effect on non-alcoholic fatty liver disease (NAFLD), it is reasonable to assume that their levels do not change in people with NAFLD.

Keywords: Non-alcoholic fatty liver disease, aspartate aminotransferase, Alanine transaminase, Total Serum Bilirubin

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1. Introduction

In cases when there are no other known factors that contribute to the buildup of secondary fat in the liver, hepatic steatosis, also known as ectopic fat deposition in the liver, is classified as nonalcoholic fatty liver disease (NAFLD). The accumulation of fat in healthy livers is considered abnormal when it exceeds 5% of hepatocytes, despite the fact that fat can accumulate in healthy livers. Nonalcoholic steatohepatitis (NASH), which is a kind of nonalcoholic fatty liver disease (NAFLD), is characterized by inflammation and cell death in fatty liver tissue [1].

NAFLD is increasing in prevalence in Western nations and affects 25% of the world's population. Nonalcoholic fatty liver disease (NAFLD) is a chronic liver illness that is on

the rise in Western industrialized countries. It is more common in those with central obesity, type 2 diabetes, dyslipidemia, and metabolic syndrome [2].

Even experienced doctors may find it challenging to recognize abnormal liver enzyme levels in seemingly healthy patients. NAFLD commonly leads to abnormal liver test results in blood donors. After excluding other possible causes of liver disease, it identifies increased levels of alanine aminotransferase (ALT) and aspirate aminotransferase (AST) that do not produce symptoms in up to 90% of cases [3], [4].

The development and advancement of NAFLD are influenced by both hereditary and environmental factors. Compared to the general population, those with first-degree relatives had a greater risk of developing NAFLD. Proteins that attach to histones at their amino termini, like CREBH and SIRT1, help regulate gene expression and maintain the integrity of the histone structure. According to genetic research, SIRT1 activation contributes to NAFLD progression. The key component that activates NAFLD's cancer-inducing capacity is aberrant DNA methylation [5].

Multiple variables produce the second impact, which in turn causes liver damage and the start of NASH. The vulnerability of the liver to injury is enhanced as fatty acid accumulation occurs. Hypothesized culprits in the damage include cytochrome P450 fatty acid metabolism, peroxisomal fatty acid oxidation, reactive oxygen species (ROS) generation from the mitochondrial respiratory chain, and hepatic metabolism of alcohol obtained from the gut. Leptin, tumor necrosis factor (TNF)-alpha, and interleukin (IL)-6 are inflammatory chemicals released from adipose tissue that injure hepatocytes; obesity plays a role in this second strike. Hepatocytes go through swelling, cytoskeletal aggregation, cell death, and cell death [6], [7].

However, this concept has changed as more information has emerged about the direct impact of FFA on causing liver damage. Fatty acid flow to the liver is enhanced with obesity and insulin resistance. Hepatic fat buildup occurs when glycerol is mixed with free fatty acids to produce triglycerides or when they are oxidized through β-oxidation. It is well-known that FFA produce toxicity due to their ability to directly trigger inflammatory pathways and enhance oxidative stress, as shown in recent research [8].

A subtype of non-alcoholic fatty liver disease (NAFL) is defined by the presence of liver fat without significant inflammation or damage. It is uncommon for non-alcoholic fatty liver disease (NAFL) to progress to the point where it causes liver damage or complications. The non-alcoholic fatty liver disease (NAFL) symptom of an enlarged liver is pain. One symptom of NASH is liver inflammation. Fatty accumulation, inflammation, and liver damage characterize a subtype of non-alcoholic steatohepatitis (NASH). The development of hepatic fibrosis is a possible outcome of the inflammation and liver damage induced by NASH. Cirrhosis, often called nonalcoholic steatohepatitis (NASH), is a chronic liver disease that damages and scars the liver. Cirrhosis increases a person's risk of developing liver cancer [9], [10].

Patients with non-alcoholic fatty liver disease (NAFLD) had a higher prevalence of peripheral vascular disease, coronary disease, and cerebrovascular disease compared to those without type 2 diabetes, as indicated by a study involving individuals with type 2 diabetes. Even after accounting for common cardiovascular disease risk factors, medication usage, and variables associated to diabetes, this remained true [11].

Cirrhosis, an important component of Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH), is characterized by widespread liver scarring. Liver damage, such as the inflammation seen in NASH, results in the progression to cirrhosis. The liver forms fibrosis scars as a defense mechanism to combat inflammation. As inflammation persists, fibrosis progressively occupies more liver tissue. Possible risks of untreated cirrhosis progression include ascites, ruptured esophageal varices leading to

hemorrhage, hepatic encephalopathy, liver malignancy, and end-stage liver failure indicating complete liver functioning. Cirrhosis occurs in around 5-12% of individuals with NASH [12], [13].

In most cases, imaging investigations or abnormal liver biochemistry tests that show hepatic steatosis or hepatomegaly unintentionally indicate NAFLD/NASH. Sonography, computed tomography, and magnetic resonance imaging are all forms of imaging [14].

Due to its low cost and ease of accessibility, ultrasonography has found extensive use. For NAFLD to be diagnosed, there must be substantial alcohol consumption, no history of viral or autoimmune hepatitis, congenital liver disease, or drug-induced liver disease, and the patient must not have any other liver disorders. Both ALT and AST levels are frequently moderately increased, ranging from two to five times the upper limit of normal; however, ALT levels are usually two times more than AST levels [15], [16].

2. Materials and Methods

The study included 75 patients with NAFLD, 34 males and 41 females, 18-75 years of age, they are diagnosed by ultrasound at GIT & hepatobiliary, and other group as control group included 81 apparently healthy subjects 46 males and 35 females, 18-75 years of age, Physiological measurements include waist circumference, height, weight, BP, Approximately 5 ml of venous blood samples were obtained from both control individuals and patients with NAFLD after 10-12 hour overnight fasting by using sterile disposable syringes for the assessment of total serum bilirubin, unconjugated and conjugated bilirubin, Aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

3. Results

3.1. Liver function test among patients with NAFLD and control subjects

Table 1 regard liver function test among patients with NAFLD and control subjects, its shows a significant increase in the levels of AST and ALT (P<0.01) in patients group, while there is non-significant change (P>0.05) in each of total TSB, direct bilirubin, indirect bilirubin, total protein, albumin and globulin.

Table 1. Liver function test among patients with NAFLD and control subjects

Parameters	Patients group (n=75)	Control group (n=81)	P. value
	Mean ± SD	Mean ± SD	
AST (U/I)	25.01 ± 10.18	20.67 ± 4.71	<0.01
ALT (U/I)	22.18 ± 9.43	17.94 ± 6.01	<0.01
TSB (mg/dl)	0.56 ± 0.24	0.54 ± 0.16	NS
Direct Bilirubin (mg/dl)	0.26 ± 0.12	0.22 ± 0.13	NS
Indirect Bilirubin (mg/dl)	0.30 ± 0.11	0.32 ± 0.06	NS
Total Protein (g/dl)	6.34 ± 0.55	6.48 ± 0.63	NS
Albumin (g/dl)	2.26 ± 1.28	2.52 ± 1.27	NS
Globulin(g/dl)	3.90 ± 1.62	3.95 ± 1.37	NS

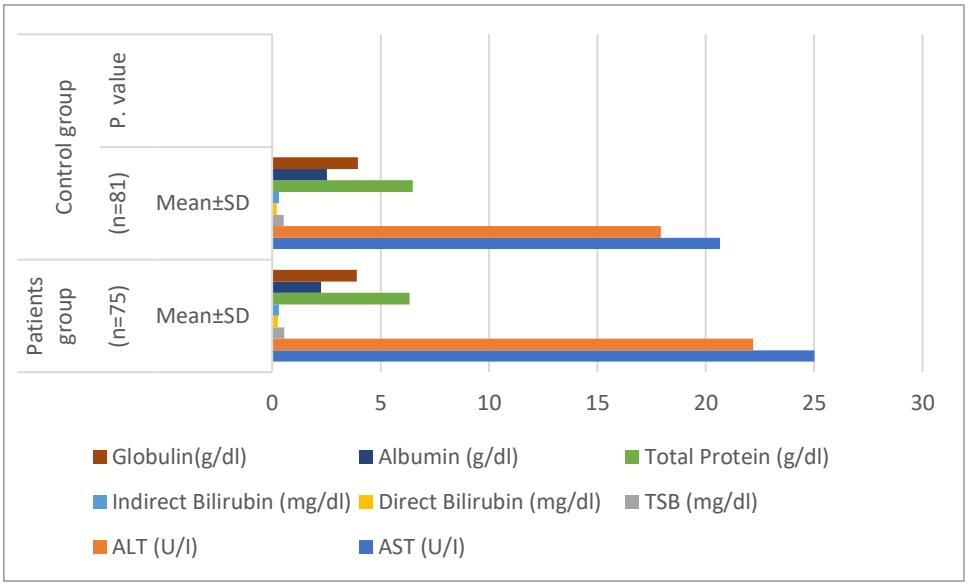

Figure 1. Liver function test among patients with NAFLD and control subjects

3.2. Liver function test among male patients with NAFLD and control subjects

As comparison of liver function test among male patients with NAFLD and control subjects, there was a significant increase in the levels of ALT in patients group (P<0.05), while there is significant decrease in total protein (P<0.01) and non-significant changes (P>0.05) in each of AST, TSB, direct bilirubin, indirect bilirubin, albumin and globulin as shown in Table 2.

Table 2. Liver function test among male patients with NAFLD and control subjects

Parameters	Patients group (n=34)	Control group (n=46)	P. value
	Mean ± SD	Mean ± SD	
AST (U/I)	23.05 ± 10.08	20.49 ± 4.80	NS
ALT (U/I)	23.42 ± 10.41	18.88 ± 6.33	<0.05
TSB (mg/dl)	0.63 ± 0.30	0.58 ± 0.15	NS
Direct Bilirubin (mg/dl)	0.27 ± 0.11	0.22 ± 0.10	NS
Indirect Bilirubin (mg/dl)	0.36 ± 0.15	0.36 ± 0.07	NS
Total Protein (g/dl)	6.17 ± 0.65	6.51 ± 0.61	<0.01
Albumin (g/dl)	2.18 ± 1.25	2.43 ± 1.26	NS
Globulin(g/dl)	3.16 ± 1.78	4.80 ± 1.36	NS

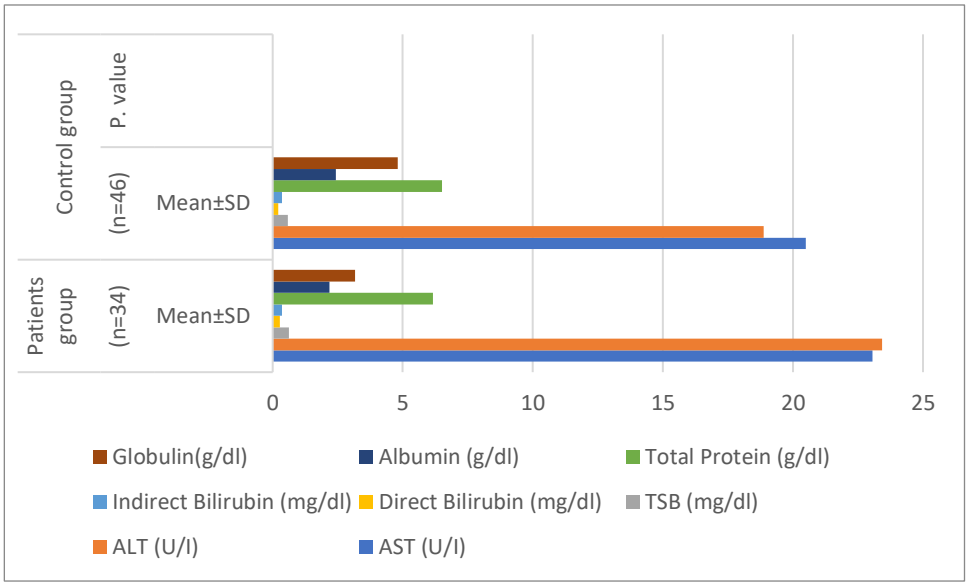

Figure 2. Liver function test among male patients with NAFLD

3.3. Liver function test among female patients with NAFLD and control subjects

Table 3 shows a significant increase in the levels of AST and ALT (P<0.01, P<0.05 respectively) in female patients group, while there is non-significant change (P>0.05) in each of TSB, total protein, albumin, globulin, direct and indirect bilirubin, and bilirubin.

Table 3. Liver function test among female patients with NAFLD and control subjects

Parameters	Patients group (n=41) Mean ± SD	Control group (n=35) Mean ± SD	P. value
AST (U/I)	26.63 ± 10.09	20.89 ± 4.65	<0.01
ALT (U/I)	21.15 ± 8.52	16.64 ± 5.37	<0.05
TSB (mg/dl)	0.52 ± 0.17	0.48 ± 0.16	NS
Direct Bilirubin (mg/dl)	0.25 ± 0.12	0.21 ± 0.15	NS
Indirect Bilirubin (mg/dl)	0.27 ± 0.07	0.27 ± 0.6	NS
Total Protein (g/dl)	6.49 ± 0.41	6.44 ± 0.66	NS
Albumin (g/dl)	2.33 ± 1.32	2.64 ± 1.28	NS
Globulin(g/dl)	4.14±1.46	3.79±1.39	NS

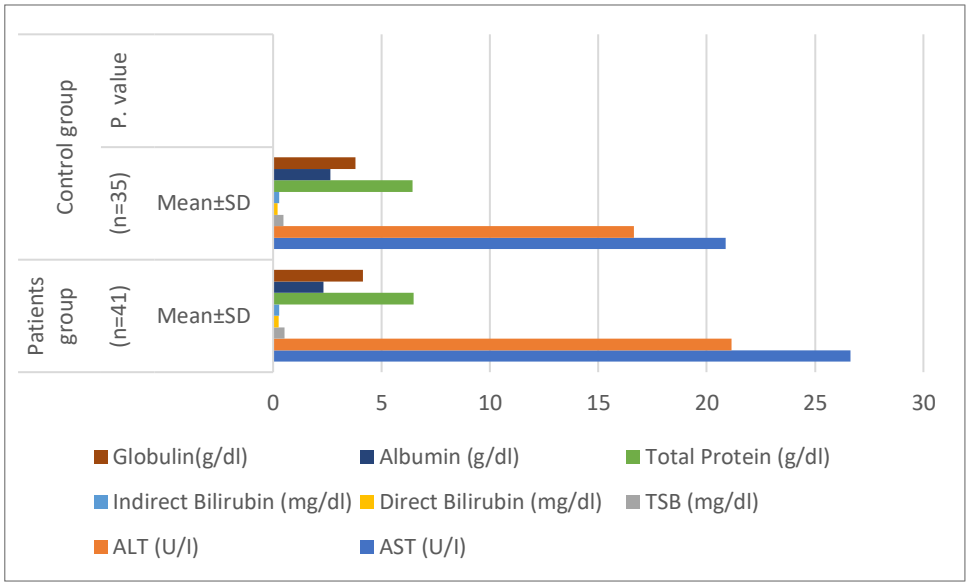

Figure 3. Liver function test among female patients with NAFLD

4. Discussion

The current study showed a significant increase in the levels of AST and ALT (P<0.01) in patients group. The results align with Thong and Quynh's study, which likewise reported increased AST levels in patients with NAFLD. When the amount of aspartate aminotransferase (AST) is twice the upper limit of normal, it is considered a specific biomarker of the severity of liver fibrosis in Asian studies [17], [18]. It's also in agreement with the results of a study by Nath et al. which show significant elevation (P<0.001) of AST in NAFLD 47.0 (U/L), Median (IQR) vs. 39.0 (30.0-56.0) for non-NAFLD [19].

In this study, the mean levels of hepatic enzymes were higher in the NAFLD group. In other cases, a significant relationship was observed with NAFLD. In the study of Novakovic et al. Liver enzymes (especially ALT and GGT) were discovered to have a strong association with NAFLD. A strong association between NAFLD and AST, ALT, and ALP has been shown in previous studies. According to Zakeri and Karmarat-Panah, dyslipidemia and ALT may contribute to the development and worsening of NAFLD [20], [21].

Elevated AST levels are indicative of liver inflammation and injury, which are common features of NAFLD. The diagnosis and evaluation of NAFLD severity rely heavily on AST in conjunction with other imaging studies and liver function testing. Medical practitioners should keep an eye on AST levels in NAFLD patients so they can track the disease's development and make informed treatment choices [22].

The current results show a significant increase in serum ALT in patients group compared to control subjects. These results in agreement with Dyson et al. who revealed mildly raised ALT in NAFLD patients [23]. An analysis of the probability of developing NAFLD in persons with normal serum ALT levels (below 35 U/L) found that an elevation in serum ALT levels, even if still within the normal range, was a clear indicator of NAFLD start. The study found a 2.7-fold rise in the occurrence of nonalcoholic fatty liver in diabetes individuals with high-normal ALT levels [24], [25].

Elevated ALT levels are a common finding in NAFLD patients, signifying hepatocellular damage and inflammation. Monitoring ALT levels is crucial in the diagnosis and management of NAFLD, as elevated ALT often prompts further evaluation, including imaging studies and liver biopsies, to assess the degree of liver damage and fibrosis. ALT levels can also serve as an important marker of treatment response and disease progression in clinical practice [26]. As the understanding of NAFLD continues to evolve, ALT remains a central player in its diagnosis and management, offering valuable insights into the liver's health and functioning.

The results of this study revealed a non-significant change (P>0.05) in each of total TSB, direct bilirubin, indirect bilirubin as showed in Table 2 and 3. These results agree with a study by Nath et al. who showed a non-significant difference in the levels of TSB between NAFLD and non-NAFLD [27].

In NAFLD, bilirubin levels have emerged as an essential parameter for evaluating disease severity and prognosis. Elevated serum bilirubin concentrations can serve as an indicator of liver dysfunction, inflammation, and oxidative stress, which are hallmark features of NAFLD. This protective effect of bilirubin is attributed to its potent antioxidant properties, as it can scavenge free radicals and attenuate oxidative damage within the liver. Moreover, bilirubin's potential to modulate inflammatory pathways further contributes to its significance in NAFLD [28].

The current study results showed a non-significant change (P>0.05) in the serum levels of albumin among patients and control group. A previous observational study by Penglei et al. on 193 patients with early NAFLD, viral hepatitis, and cirrhosis found no differences in albumin levels between healthy controls and patients with NAFLD and hepatitis, therefore our findings are in line with that [27].

The relationship between serum albumin and NAFLD has been explored in multiple studies, including the one by Özgönenel et al. Patients with NAFLD had lower blood albumin levels than controls, although this difference was not statistically significant. According to the findings, serum albumin might not be a good way to find NAFLD [29].

The recent study showed a non-significant difference in the serum levels of globulin among patients and control. However, numerous studies have shown that there is often no significant difference in globulin levels between individuals with NAFLD and those without the disease. Research carried out by Hafez et al. demonstrated that globulin levels were not significantly different between NAFLD patients and controls. This study emphasized that while liver function tests, which include globulin levels, can provide valuable information about liver health, they are not always indicative of NAFLD or its severity. These findings suggest that globulin alone may not be a reliable biomarker for diagnosing or differentiating NAFLD [30].

The lack of significant differences in globulin levels between NAFLD patients and controls can be attributed to several factors. NAFLD is a complex metabolic disorder that involves a spectrum of liver abnormalities. While liver function tests assess the liver's overall health, they may not specifically capture the nuances of NAFLD, which is closely associated with insulin resistance, lipid metabolism, and inflammation.

Several studies have investigated the relationship between total protein levels and NAFLD, with mixed results. For instance, a study by Targher et al. found no significant differences in total protein levels between NAFLD patients and control subjects. Similarly, another study by Alaaeldin et al. reported similar findings, where total protein levels did not distinguish between NAFLD patients and those without liver disease [30], [31].

The study discovered that insulin, HbA1c, and FBG levels are notably elevated in NAFLD patients compared to control individuals. Research by Jain et al., Novakovic et al., and Pardhe et al. further supported this result. Increased fasting glucose levels are significantly associated with NAFLD, according to univariate research [31].

In this investigation, patients with NAFLD had significantly elevated levels of insulin, HbA1c, and FBG compared to the control group. Verified by research from Jain et al., Novakovic et al., and Pardhe et al., this finding was deemed credible. According to the individual studies, non-alcoholic fatty liver disease is strongly associated with elevated fasting glucose levels [31], [32].

5. Conclusion

The serum of people with non-alcoholic fatty liver disease (NAFLD) may contain elevated levels of AST and ALT. Because bilirubin, albumin, globulin, and total protein have no effect on NAFLD, it is reasonable to assume that their levels do not change in

people with NAFLD. There is no relationship between NAFLD and albumin and globulin levels. The functional disorder that occurs in the liver is limited in the first stage to liver enzymes, so significant differences were observed between them.

6. Ethical Approval

All patients involved in this work were informed and the agreement was obtained verbally from each one before the process of sample collection. This study was approved by the Committee on Publication Ethics at the Department of Clinical Chemistry, College of Medicine, University of Basra.

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