Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/ztx2qg63

DIAGNOSTIC SIGNIFICANCE OF SERUM TISSUE NECROSIS FACTOR-ALPHA LEVEL IN NON- ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Background: Tissue necrosis factor (TNF-α) is a cytokine involved in systemic inflammation during acute-phase reactions. The current study was designed to investigate the levels of pro-inflammatory cytokine (TNF-α) during the progression of non-alcoholic fatty liver disease (NAFLD) from simple steatosis to non-alcoholic steatohepatitis (NASH) in patients and correlate the levels of cytokine with the progression of NAFLD.

Methods: The study included 60 diagnosed cases of NAFLD, including 31 patients with Fatty liver and 29 patients with NASH. Blood samples were collected at admission, and random blood glucose and serum liver function tests were measured. TNF-alpha levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA).

Results: Of the 60 NAFLD patients, TNF-α levels were elevated in both groups100% of Fatty Liver patients and 100% of NASH patients had elevated, higher in NASH patients (mean = 16.61 ± 2.70 pg/mL) than in those with fatty liver (mean = 11.15 ± 1.52 pg/mL), with the difference being statistically significant ($P < 0.001$). ROC curve analysis of TNF-α revealed good discriminatory role in differentiation between NASH and Fatty Liver, with an AUC of 0.961 and a P value <0.0001 optimum cut-off level obtained was <13.5pg/ml, with a sensitivity of 89.66% and a specificity of 100%.

Conclusion: Tnf-alpha levels can discriminate NASH from fatty liver in NAFLD, its role as a supporting biomarker for disease severity.

Keywords: Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis, Tissue Necrosis factor-alpha, receiver operating curve, and area under the curve.

INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is becoming increasingly prevalent due to the progressive increase in the occurrence of obesity and diabetes. It involves more than one-fourth of the adults globally, with 25% and the general Indian population prevalence rate is 9% to 32%.[1] NAFLD is one of the most common causes of chronic liver diseases, leading to complications like simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, and eventually hepatocellular carcinoma (HCC), which is the foremost indication for liver transplantation.[2] Hence, timely

diagnosis and disease progression are important to avoid life-threatening complications. For diagnosis, ultrasonography, computed tomography (CT) are helpful, but liver biopsy is considered the gold standard.[3]

Structurally, TNF-α is a homotrimer protein consisting of 157 amino acids, mainly generated by activated macrophages, T-lymphocytes, and natural killer cells.[4] It is functionally known to trigger a series of various inflammatory molecules, including other cytokines and chemokines. TNF-α affects various facets of NAFLD-related liver damage. Raising hepatic fatty acid production and serum triglyceride levels, and thereby lowering HDL-cholesterol, causes hepatic steatosis. Moreover, TNF-α causes insulin resistance, hence aggravating the metabolic abnormalities in NAFLD. By activating hepatic stellate cells and proliferating them as well as by inducing hepatocyte death, this cytokine also causes hepatic fibrosis.[5]

This study aims to assess whether non-invasive blood testing for TNF-α can help determine disease severity, potentially reducing the need for liver biopsy and aiding in early treatment planning.

MATERIALS AND METHODS: This was a Descriptive, cross-sectional observational study conducted on a total of 60 patients. Patients aged 18 to 60 years included according to the inclusion and exclusion criteria in the Gastroenterology department, SMS super specialty hospital, Jaipur, of confirmed NAFLD, were included. The diagnosis was made by gastroenterologists using liver USG, ALT, AST, fibro scan, and liver biopsy.

Exclusion criteria included patients with age below 18 and above 60 years, History of alcohol intake >30 gm/day in males and >20gm/day in females, Person refuses to give informed consent, person has experienced dieting or bariatric surgery and massive rapid weight loss, diabetic and any malignancy and patients with Chronic liver disease due to other causes like viral, autoimmune, metabolic, biliary or drug induced.

Blood Samples

Venous Blood samples(5ml) were collected aseptically into metal-free, anticoagulant-free tubes. After allowing the blood to clot for 20 minutes at room temperature, the samples were centrifuged for 15 minutes. The resulting serum was used for routine biochemical parameters were analysed using the Beckman Coulter AU 5100 system. Tissue necrosis factor-alpha was analyzed in a designated lab using the Diaclone ELISA kit.

ANALYSIS OF THE STATISTICS AND DATA ENTRY

Quantitative data were summarized as mean and SD, whereas qualitative data were presented as proportion (%). An independent sample t test was used for comparison of quantitative variables, and chi-square test and Fisher's exact test were used for analysis of qualitative variables. ROC analysis was done to find out the optimum cut-off value of special markers. P value <0.05 was taken as significant. SPSS 26.0 version software was used for all statistical calculations.

RESULTS:

The present study was conducted on 60 patients with NAFLD and classified according to their clinical diagnosis. Out of these 31 (51.67%) were diagnosed with fatty liver, and 29 (48.33%) with NASH. Demographic, clinical, and Laboratory Features of the patients are presented in Table 1

Table 1. Baseline Clinical, Biochemical characteristics of the study population across NAFLD

	Fatty liver (n=31)	NASH (n=29)		P
Clinical data				
Age, years (Mean)	42.74 ± 11.62	49.14 ± 9.67		0.025
Body mass index, kg/m^2 (Mean)	26.38 ± 2.05	27.10 ± 2.19		0.192
Hypertension (%)	7 (46.67%)	8 (53.33%)		0.769
Smoking n (%)	5 (33.33%)	10 (66.67%)		0.139
Biochemical data				
Random Blood Sugar (mg/dl)	94.88 ± 11.26	98.69 ± 9.48		0.164
TC (mg/dl	172.58 ± 39.40	181.55 ± 36.88		0.367
TG (mg/dl)	173.03 ± 47.77	162.20 ± 21.65		0.268
HDL (mg/dl)	48.02 ± 7.98	48.52 ± 10.71		0.838
LDL (mg/dl)	92.49 ± 41.35	102.12 ± 33.64		0.329
VLDL (mg/dl	34.61 ± 9.55	32.44 ± 4.33		0.268
TNF-alpha (pg/ml)	11.15 ± 1.52	16.61 ± 2.70		0.001

RESULTS :

The mean age of patients with Fatty Liver was (42.74 ± 11.62 years) and NASH (49.14 ± 9.67 years), which was significantly higher than that of fatty liver (p= 0.025). The mean BMI was higher in the NASH group (27.10 ± 2.19 kg/m^2) compared to the Fatty Liver group (26.38 ± 2.05 kg/m^2), but the difference was not statistically significant. (P= 0.192)

Among hypertensive, 46.67% were diagnosed with Fatty Liver and 53.33% with NASH; the difference was not statistically significant (p = 0.769).

Among smokers, 33.33% with Fatty Liver and 66.67% with NASH. The association between smoking and disease type did not reach statistical significance (p = 0.139). RBS levels were slightly higher in the NASH group (98.69 ± 9.48 mg/dL) than in the Fatty Liver group (94.88 ± 11.26 mg/dL), but the difference was not statistically significant (p = 0.164).

Total Cholesterol levels were slightly higher in NASH patients (181.55 ± 36.88 mg/dL) compared to Fatty Liver (172.58 ± 39.40 mg/dL), but the difference was not statistically significant (p = 0.367).

Triglyceride levels were lower in the NASH group (162.20 ± 21.65 mg/dL) than in the Fatty Liver group (173.03 ± 47.77 mg/dL), with no significant difference (p = 0.268).

High-Density Lipoprotein levels were nearly identical between the two groups (Fatty Liver: 48.02 ± 7.98 mg/dL; NASH: 48.52 ± 10.71 mg/dL. (p=0.838).

Low-Density Lipoprotein levels were higher in NASH patients (102.12 ± 33.64 mg/dL) than in Fatty Liver patients (92.49 ± 41.35 mg/dL), but the difference was not statistically significant (p = 0.329).

Very Low-Density Lipoprotein levels showed no significant difference between groups (Fatty Liver: 34.61 ± 9.55 mg/dL; NASH: 32.44 ± 4.33 mg/dL (p = 0.268).
TNF-α levels were notably higher in NASH patients (mean = 16.61 ± 2.70 pg/mL) than in those with fatty liver (mean = 11.15 ± 1.52 pg/mL), with the difference statistically significant (P < 0.001).

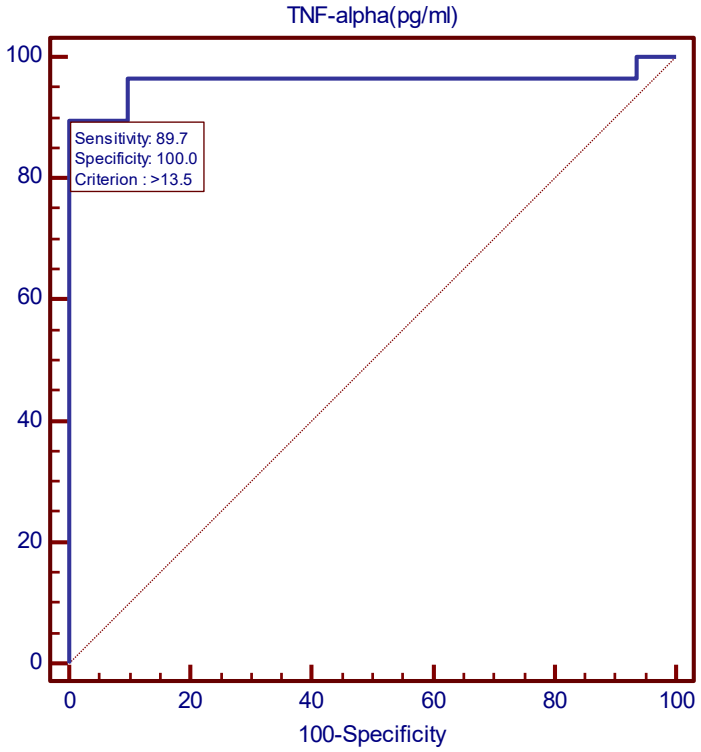

Figure 2: ROC curve analysis of serum TNF-α level to differentiate NASH and fatty liver

ROC curve analysis of TNF-α (Figure 2) showed an Area Under Curve (AUC) of 0.961 with a 95% confidence interval of 0.877 to 0.994, revealing a good discriminatory role and P value <0.0001. optimum cut off level obtained was <13.5pg/ml corresponding to the highest Youden index. At this cutoff off Tnf-alpha had a sensitivity of 89.66% and specificity of 100%.

DISCUSSION:

Tumor necrosis factor alpha (TNF-α) is a cytokine that has pleiotropic effects on various cell types. It has been identified as a major regulator of inflammatory responses and is known to be involved in the pathogenesis of some inflammatory and autoimmune diseases.[6]
In our study, the mean TNF-α levels were notably higher in NASH patients (mean = 16.61 ± 2.70 pg/mL) than in those with fatty liver (mean = 11.15 ± 1.52 pg/mL), with the difference statistically significant (P < 0.001). ROC curve analysis was done for finding the efficiency of serum TNF-α in discriminating NASH from Fatty liver, and it was found that TNF-α was found to be an outstanding marker in discriminating NASH patients from Fatty liver patients with AUC 0.961 and a model having a highly significant P value of <0.0001. The optimum cut-off level at a higher Youden index was obtained as 13.5pg/ml with a sensitivity of 89.66% and a specificity of 100%.
Khura et al.[7] in a similar study found that the mean value of TNF-α was 27.71±10.56, which was significantly higher (p<0.001) than in controls (0.54±0.51). Mostafa et al.[8] in their study concluded that endotoxemia and TNF alpha may contribute to the pathogenesis of NAFLD, especially in non-obese patients. Purnomo et al.[9] in a similar study found the AUC for TNF-α was found to be 0.778 with sensitivity 93% and specificity 48% for NASH prediction. The AUC is lower compared to our study, having an AUC of 0.961, a sensitivity of 89.66% and a specificity of 100% for NASH prediction.

STUDY LIMITATION:

This study is limited by its cross-sectional design and absence of follow-up data, which restricts the evaluation of the long-term diagnostic value of Tissue necrosis factor-alpha. It was a single-center, observational study with a relatively small sample size and uneven distribution across risk groups. As a hospital-based study, it is also subject to selection bias and may not be fully generalized to the broader NAFLD population.

CONCLUSION: The results presented here demonstrated the usefulness of Serum tissue necrosis factor-alpha in predicting the severity of NAFLD patients. In our cohorts, TNF-alpha is excellent marker to discriminate NASH and Fatty liver but multi-centric studies on larger sample size is recommended for further study.

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