Original Research Article

Non Invasive Biomarkers, Serum Prolidase, Fibroscan, APRI Score in Non Alcoholic Fatty Liver Disease

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ABSTRACT

Background & objectives: NAFLD may progress from simple steatosis, non alcoholic steatohepatitis, cirrhosis & hepatocellular carcinoma. The fat content of liver is <5% & if it increases >5-10%, that shows steatosis. The spectrum is not linear, all steatosis patients may not develop NASH & cirrhosis but may show a backward direction. Two hit hypothesis is proposed for pathogenesis, where mitochondrial dysfunction with oxidative stress and activation of stellate cells lead to myofibroblast to secrete collagen. Liver biopsy is the gold standard procedure for diagnosis, due to limitations non invasive procedures have been developed, which are low cost and have easy accessibility. Serum Prolidase is used as direct marker to access the pathophysiology of fibrosis & reflect collagen turnover. Indirect markers of liver fibrosis are ratio of AST/ALT, APRI score & fibroscan. Fibroscan uses to measure tissue stiffness.
On this background the study aims at correlating non invasive markers S. Prolidase with liver function test & degree of fibrosis.
Materials & Methods: Fifty numbers of NAFLD cases & age & sex matched fifty numbers of healthy controls were chosen. BMI, FPG, lipid profile, liver function test, S. Prolidase, AST/ALT ratio, APRI score & fibroscan were measured in both groups.
Results: Serum Prolidase activity & fibroscan were significantly higher (p, 0.001) in NAFLD cases than control group. There was a significant positive correlation between S. Prolidase with fibroscan.
Conclusion: S. Prolidase may be considered as a cost effective marker for NAFLD.

Key Word: NASH, NAFLD, Prolidase, APRI, Fibroscan.

INTRODUCTION

Fatty liver disease is classified into alcoholic liver disease (ALD) and non alcoholic disease (NAFLD). The NAFLD may range from simple steatosis (SS) & non- alcoholic steatohepatitis (NASH) to cirrhosis & hepatocellular carcinoma. [1-3] The amount of alcohol intake is 20 gm/day to distinguish ALD from NAFLD. [4]

The prevalence of NAFLD in western adult & in India is 20-25% and 9-32% respectively. [5,6]

The fat content of liver by weight is <5% mostly triacylglycerol, the liver is said to be fatty when >5-10% of hepatocytes show steatosis histologically. [7] NAFLD categorises into non- alcoholic fatty liver (steatohepatitis with hepatocellular injury) and NASH (steatosis with hepatic damage, inflammation, scaring, & replacement with type-1 collagen). [8] The spectrum is not clear, not all patients of simple steatosis develop NASH & cirrhosis, may show a backward direction to SS to normal liver. [9]

"Two hit hypothesis" is proposed for aetiopathogenesis of fatty liver disease. In the first hit there occurs accumulation of triacylglycerol (steatosis) which initiates injury, accelerated by 'second hit' like inflammatory cytokines / adipokines, mitochondrial dysfunction & oxidative stress leads to steatohepatitis &/ or fibrosis. [10,11] Stellate cells in liver (fibrogenic cell) whose activation leads to proliferative, fibrogenic and contractile myofibroblasts to secrete large amount of collagen. [12] NAFLD can be responsible for transaminasemia, cryptogenic cirrhosis even hepatocellular carcinoma [2,13,14] Therefore, early recognition is required to avoid complications.

Invasive & non- invasive procedures are developed to diagnose fatty liver. Liver biopsy being an invasive procedure is old & gold standard procedure. But biopsy samples are extremely small i.e. about (1/50,000) parts of liver & has sampling errors is unavoidable. [15,16] This has prompted to envisage non- invasive procedures for diagnosis of NAFLD with or without fibrosis.

Serum biomarkers are divided into direct markers (access the pathophysiology of fibrosis) & indirect markers for liver damage. [17]

Estimation of serum Prolidase (a type of matrix metallo proteinases) is used to access matrix degeneration. [18] Serum Prolidase (E.C. 3.4.13.9) is an exopeptidase which cleaves aiminopeptides with c-terminal proline / hydroxyproline. [19,20] Prolidase activity is increased with fibrosis & collagen, thus increased level could reflect high collagen turnover. [21]

Indirect biomarkers such as ratio of aspartate transaminase to platelet index (APRI) may offer benefit besides liver function test. [22]

A shear wave in liver is produced by transmitting vibrations of mild amplitude & of low frequency. The tissue stiffness correlates with the speed of the wave. [23] Pulse echo ultrasonic acquisitions are performed to follow wave & the speed correlates to tissue stiffness (harder the tissue the shear propagates faster). Results are expressed in kilopascal (kPa). [24]

The present study is taken up to correlate non invasive biomarker, s. Prolidase with liver function test & degree of fibrosis.

MATERIALS & METHODS

A case control study was conducted in the Dept. of Biochemistry in collaboration with Dept. of Hepatology, SCB Medical College, Cuttack. The study was approved by institutional ethical committee & informed consent was obtained.

Fifty (50) numbers of patients of NAFLD with age group between 20-60 years attending OPD of Hepatology were taken as cases. Equal number of age & sex matched healthy volunteers were taken as control. The cases were selected with fibroscan score >8.

Subjects with alcohol habit >20 gm/day, Diabetes Mellitus, impaired biliary excretion, impaired renal function & drugs modifying liver function were excluded from the study.

Sample Processing

After overnight fast, 5 ml of blood was collected in sterile condition. 0.5 ml blood was transferred to fluoride tube for blood sugar, 0.5 ml blood transferred to EDTA tube for platelet count & 4 ml blood was transferred to plane tube for serum. Fluoride & plane tubes were centrifuged to separate plasma & serum. 1 ml serum was stored at -20^0C for Prolidase estimation.

Methods

The biochemical parameters, FPG, LFT, lipid profile, urea, creatinine were estimated by autoanalyser TOSHIBA 120 FR using commercial kits.

Assay of Serum Prolidase activity

Serum Prolidase activity was estimated using ELISA KIT (SANDWICH METHOD). Microtiter plate wells were

coated with Prolidase antibody. Serum containing Prolidase was added; it combined with the antibody coated on the microtiter wells. Then HRP labeled Prolidase antibody was added combines with serum Prolidase to form Antibody-Antigen- Enzyme antibody complex. After washing, TMB substrate was added. HRP enzyme- catalyses the reaction & a blue colour was formed. The reaction was terminated by adding sulphuric acid. The yellow colour developed was measured spectrophotometrically at 450 nm.

Fibroscan was done to find out fibrotic score.

Statistics

All results were expressed in mean ± SD. Test of significance was done by unpaired student 't' test. 'P' value <0.05 was taken as significance. Pearson coefficient of correlation used for correlation. All statistically analysis was done by SPSS version 24 software.

RESULTS

The age & sex distribution with BMI of study population is shown in table-1. The study was between the group of 20-60 yrs with mean age of 40.86±10.76 & 42.56± 0.47 in control & cases respectively. The ratio between male & female was 35:15 and 34:16 in control & case. Males outnumbered the females.

The BMI was statistically significant (p<0.001) higher in cases (28.21±3.5) than control (24.15±3.12)

Table-1: Age & sex distribution wth BMI in study population

	Control (n=50)	Cases (n=50)
Age	**40.86 ± 10.76**	**42.56 ± 0.47**
Sex		
Male	35	34
Female	15	16
BMI (kg/m²)	24.15 ± 3.12	28.21 ± 3.5 #

 # BMI in cases significantly high to control group (p<0.001)

The values of FPG, urea, creatinine, cholesterol, LDL, D. Bil, T. Bil show no statistical difference between control & cases.

Values of TG, VLDL, AST, ALT, & Alk. P show a statistical significant rise in cases (p<0.001) than control group. While HDL shows a statistical decrease in cases (p<0.05).

Table-2: comparison of biochemical parameters (FPG, lipid profile & LFT) in study population

Parameter	Control (n=50)	Cases (n=50)	'p' value
FPG (mg%)	110.40 ± 9.08	106.40 ± 14.6	NS
Urea (mg%)	22.52 ± 9.54	20.54 ± 6,42	NS
Creatinine (mg%)	0.63 ± o.20	0.60 ± 0.18	NS
Cholesterol (mg%)	17.98 ± 16.47	191.28 ±43.41	NS
TG (mg%)	137.22 ± 36.22	236.08 ± 80.7	0.001
HDL (mg%)	46.08 ± 8.04	37.62 ± 9.14	0.05
LDL (mg%)	103.50 ± 16.64	106.26 ± 39.93	NS
VLDL (mg%)	27.90 ± 7.72	47.64 ± 19.93	0.001
D.Bil (mg%)	0.40 ± 0.09	0.42 ± 0.11	NS
T. Bil (mg%)	0.96 ± 0.16	0.94 ±0.15	NS
AST (IU/L)	41.52 ± 18.07	65.44 ± 51.82	0.001
ALT (IU/L)	36.54 ± 18.23	55.16 ± 42.46	0.001
Alk.P (IU/L)	205.12 ± 85.20	266.12 ± 11.25	0.001

D. Bil= Direct bilirubin, T. Bil-=Total bilirubin, Alk.P= Alkaline phosphatase.

Table-3: comparison of special parameters among the study population

	Control (n=50)	Cases (n=50)	'p' value
Total platelet count (no/ml)	2.93 ± 0.31	2.87 ± 0.29	NS
Fibroscan (kPa)	0.88	15.00 ± 7.5	0.001
Prolidase (pg/dl)	1043.57 ± 396.89	2435.78 ± 1738.30	0.001
AST/ALT	1.35 ± 0.70	1.32 ± 0.75	NS
APRI	0.34 ± 0.17	0.59 ± 0.44	0.001

There is no significant difference in platelet count & AST/ALT ratio between cases & control groups. There is a statistical significant increase in fibroscan score, s. Prolidase APRI in cases in compare to control group (p<0.001).

Table-4: correlation of serum Prolidase with fibroscan & APRI

	S. Prolidase	
	'r' value	'p' value
Fibroscan	+ o.312	0.02
APRI score	+0.040	0.782

Table-5: correlation of serum Prolidase with LFT

	S. Prolidase	
	'r' value	'p' value
D. Bil (mg/dl)	+0.262	0.66
T. Bil (mg/dl)	+0.001	0.995
AST (IU/L)	+0.066	0.649
ALT (IU/L)	+0.174	0.226
Alk.P (IU/L)	+0.009	0.951

S. Prolidase activity show positive correlation with LFT.

There is a statistically significant (p<0.02) positive correlation between serum Prolidase with fibroscan, where as APRI score shows a positive correlation with Prolidase.

DISCUSSION

The fat content liver mostly triacylglycerol is <5% of weight, when this accounts >5-10% of weight in absence of excessive alcohol consumption, the term NAFLD is appropriate. [7] The range may vary from SS to NASH to cirrhosis to hepatocellular carcinoma. [1-3] The spectrum is not linear, but may show a backward direction. [9] "Two hit theory" suggests that in first hit, there occurs steatosis & injury followed by "second hit" leads to steatosis & fibrosis. [10,11] Activation of stellate cells causes fibrogenic & contractile myofibroblasts to secrete large amount of collagen. [12]

It has been recognised, NAFLD is an important cause of transaminasemia, cryptogenic cirrhosis even hepatocellular carcinoma. [2,13,14]

Therefore, early recognition is necessary to avoid complication.

Invasive & non- invasive procedures are adopted. Liver biopsy, though an invasive procedure (gold standard) has some limitations. [15,16] Non invasive such as serum & imaging techniques are being used. Due to cost effective alternative to liver biopsy serum markers can be used repetitively. [25]

Serum Prolidase activity is used to measured matrix degradation could act as direct marker. [17] Indirect markers like APRI offers benefit besides LFT. [22] Fibroscan is used to measure tissue stiffness. [23]

It was observed that BMI of cases was significantly raised in compared to control group (p<0.001) (table -1) which is in agreement with the study by Marchesini et al. [26]

Increase flux of fatty acids due to visceral adipocity is cause of NAFLD. In the Dionyoss Nutrition and liver study, Bedogni et al found BMI is an independent marker of NAFLD. [5]

We found that there was statistical increase in TG & VLDL levels when compared to control group (p<0.001) where as HDL value showed a statistical low value in cases (p<0.05) which is in conformation with study by Marchesini et al [26] and Ryan et al. [27]

In the present study, there was significant higher value of AST,ALT & Alk.P (p<0.001) in cases compared to control group. This observation is in agreement with the findings of Berasian et al. [28]

There was no statistical difference in platelet count & AST/ALT ratio between cases & control group. Fibroscan score showed a significant higher value in cases (p<0.001) compared to control group. Wong et al has demonstrated usefulness of fibroscan in cases of both whites & Asian origin. [29] Our study showed a significant higher APRI score in cases than control group (p<0.001). Lorez-a-del-Casttillo et al stated that APRI was capable of predicting significant fibrosis in NAFLD. [30]

There was significant increase in serum Prolidase activity in NAFLD cases than control group (p<0.001) an agreement with study by Kayadibi et al, they used it to differentiate between steatosis & NASH in NAFLD cases. They concluded the strong correlation between serum Prolidase activity & liver fibrosis. Thus, it can be used as a marker to differentiate NASH from steatosis. [31]

Serum Prolidase showed a positive correlation with fibroscan & APRI score. But it was significant between serum Prolidase & fibroscan (p<0.02).Similar positive correlation was found between serum Prolidase with biochemical parameters of liver function test. Kayadibi et al also found the [positive correlation of Prolidase with AST, ALT in NASH patients. [31]

CONCLUSION

The inflammation caused by fatty infiltration, there was high values of liver enzymes. Serum Prolidase showed a significant positive correlation with established parameters of hepatic fibrosis as fibroscan & APRI, The present study reveals that serum Prolidase could be used non invasive marker of hepatic fibrosis. However multicentric & larger series studies could confirm & would beneficial as measurement of serum Prolidase could be cost effective & simple.

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