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ORIGINAL ARTICLE

Type IV Collagen: A Non Invasive Bio Marker To Detect Non - Alcoholic Steato Hepatitis (NASH), Among Non - Alcoholic Fatty Liver Disease (NAFLD) Patients

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ABSTRACT

Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) encompasses a disease spectrum ranging from simple hepatic steatosis to steatohepatitis (NASH), fibrosis and cirrhosis. It is becoming the leading cause for referral to liver clinics in most areas. The prevalence of NAFLD is most likely to continue to rise. Obesity, hyperglycaemia, type 2 diabetes mellitus and hypertriglyceridaemia are the most important risk factors. Genetic factors undoubtedly predispose to NAFLD. NAFLD has the potential to progress to hepatocellular carcinoma or liver failure, both being events that ultimately lead to early death.

Aim: To evaluate the Type-IV collagen – NASH test, a new bio marker for Non-Alcoholic Steato Hepatitis in patients with Non Alcoholic Fatty Liver Disease.

Materials and Methods: 69 patients with Non-Alcoholic Fatty Liver Disease (NAFLD) who were diagnosed by ultrasound scanning and age and sex matched 69 normal healthy individuals as controls, were selected for this study. The levels of serum Type-IV collagen, lipid profile and liver function test parameters were estimated in patients and were compared to controls.

Results: Type – IV collagen levels were significantly increased in patients with NASH among the NAFLD patients as compared to the controls. When compared to the liver function test parameters and the lipid profile levels, NASH was found to have a positive negative predictive value among the NAFLD patients.

Conclusion: In patients with NAFLD, the Type – IV collagen test was found to be a simple, non – invasive and reliable test to predict the presence or absence of NASH.

Key Words: Type – IV Collagen, hepatic fibrosis, Non-Alcoholic Fatty Liver Disease (NAFLD), Non-Alcoholic Steato Hepatitis (NASH).

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Introduction

Non Alcoholic Steatohepatitis (NASH) is an advanced form of Non Alcoholic Fatty Liver Disease (NAFLD) and has at least three components among the tetrad of steatosis, hepatocellular injury, focal mixed cell type inflammation and fibrosis [1]. Liver fibrosis is the common end-stage of most chronic liver diseases regardless of aetiology and its progression leads to cirrhosis and liver cancer [2].The prevalence of overweight persons is increasing in developed and developing countries [3]. Fatty metamorphosis in the liver is common in these obese subjects and non alcoholic fatty liver disease (NAFLD) has become wide spread with the increasing prevalence of obesity [4]. In India, the prevalence of NAFLD has been recently increasing [5], which may be attributable to the lifestyle changes including physical inactivity and an increase in daily fat consumption. It has been suggested that progression from

simple steatosis to steatohepatitis and then advanced fibrosis, is caused by two distinct events. Firstly, insulin resistance leads to the accumulation of fat within hepatocytes and secondly, mitochondrial reactive oxygen species cause lipid peroxidation, cytokine induction, and the induction of the Fas ligand. The lipid peroxidation products and some cytokines can start the inflammatory response in the liver, which either directly causes cellular damage or draws the inflammatory cells to the liver parenchyma [6]. Most patients with NAFLD have a benign clinical course, but some patients progress to advanced liver disease, liver cirrhosis or even hepato cellular carcinoma [7]. NASH is a severe form of NAFLD and is strongly associated with older age, obesity and type II diabetes mellitus [8]. The definitive diagnosis of NASH requires liver biopsy, but it is an invasive procedure that may cause undesirable complications. The clinical conditions for liver biopsy should be limited in patients with NAFLD who are likely to have NASH or significant fibrosis. Liver fibrotic change is an important pathological finding to stage NAFLD or to determine the prognosis of NAFLD [9]. NAFLD patients with severe fibrosis have poor prognosis [10]. Many clinical variables have been proposed as the predictors of severe fibrosis in patients with NAFLD, like old age, type II diabetes mellitus, obesity, serum transaminase levels, peripheral platelet counts, etc.

Biochemical markers of liver fibrosis are strongly associated with the degree of fibrosis in patients with chronic viral hepatitis [11]. However, very little information and only few data was available on the usefulness of the non invasive markers of liver fibrosis, in order to evaluate the degree of liver fibrosis in patients with NASH among the NAFLD patients.

The present study is an attempt to evaluate Type-IV collagen as a new bio marker for Non- Alcoholic Steato Hepatitis in patients with Non Alcoholic Fatty Liver Disease patients at an early stage. In our country, presently 10 – 28 % of the general population is suffering from Non Alcoholic Fatty Liver Diseases (NAFLD) out of which around 3 – 5 % suffering from Non Alcoholic Steato Hepatitis (NASH), which progresses to the end stage of liver disease. So, if we can be able to diagnose and treat the patients at the NASH stage, we can be able to save the life of these patients from end stage liver disease.

Materials and Methods

The study was conducted in the Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha University, Chennai, T.N, India and the Department of Biochemistry, Apollo Hospitals, Chennai, T.N, India. Sixty – nine subjects who were diagnosed with non alcoholic steato hepatitis were chosen as the study subjects. The diagnosis of nonalcoholic steatohepatitis was made according to the following criteria: 1) abnormal liver function tests at least for three months and the absence of previous liver disease history, 2) ultrasonographic diagnosis of liver steatosis, 3) histological diagnosis of fatty infiltration, lobular or portal inflammation and/or Mallory bodies, fibrosis or cirrhosis. In all patients, the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total Bilirubin, gamma-glutamyl transpeptidase (GGT), total cholesterol, triglycerides, HDL - cholesterol, LDL - cholesterol and the cholesterol / HDL ratio were measured. Equal number of healthy volunteers with no history of liver disease, drug usage, surgery or alcohol consumption, with biochemical liver function tests and sonographical examination of the liver within normal limits, were selected as the control group. Due permission was obtained from the ethical committee of the institution before the start of the study. Written consents were also taken from the patients prior to study and the objectives of the study were fully explained. The complete clinical and personal history of the subjects was recorded.

Inclusion Criteria

- Patients with an ultrasound diagnosis of liver steatosis and with a persistent increase of at least 1.5 times the upper limit of the normality of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and/or γ-glutamyl transpeptidase (γ-GT) levels for a minimum period of three months were studied.
- Patients with suspected NAFLD who were hospitalized and having steatosis at liver biopsy were included.

Exclusion Criteria

- The exclusion criteria included a daily alcohol consumption of at least 50 gm of pure ethanol equivalent for males and 30 gm for females during the preceding year, the incidence concomitant liver diseases (the presence of HCV antibody or HBs antigen, auto-immune hepatitis, hemochromatosis diagnosed by genetic markers, Wilson's disease, alpha anti-trypsin deficiency), the presence of HIV antibodies and immunosuppression and an interval greater than 3 months between serum sampling and liver biopsy.
- Patients with non insulin dependent diabetes mellitus (NIDDM), taking hepatotoxic drugs, with systemic diseases or infections, those presenting hepatitis B or C virus or other concomitant liver disease were excluded.
- Patients with clinical and/or ultrasound signs of liver failure and/ or portal hypertension were also excluded.

The controls and the study subjects were divided into two groups.

Group 1 (Controls): Sixty nine healthy volunteers with similar socio economic status were chosen as controls.

Group 2 (Study subjects): Sixty nine patients with clinically diagnosed non alcoholic Steato hepatitis were chosen as study subjects.

The venous blood samples obtained from these subjects in the morning after an overnight fasting were used for the analysis. Serum was separated by centrifugation at 1,000 g for 15 minutes at $+4^0$C. The separated serum was used for the estimation of Lipid profile, Liver Function Tests (LFT) and Type IV - Collagen. Lipid profile and LFT were estimated by using kit methods and a Hitachi – 912 autoanalyzer. Serum Type IV Collagen levels were estimated by the Latex Turbidometric Immunoassay (LTIA), by using two kinds of antibodies and the PANASSAY IV C kit for the determination of human Type IV collagen in serum. Necessary care was taken during sample collection, storage and assay.

Chemicals

All reagents that were used were of analytical reagent grade or the highest grade available and were obtained from Sigma Chemicals, St.Louis, MO, USA.

Statistical Analysis:

The statistical analysis between groups 1 (controls) and 2 (patients) was performed by the independent student t – test, by using the SPSS statistical package for Windows, Version 15. The data were expressed as mean $\pm$ SD. P values < 0.05 was considered as significant.

Results

There was a statistically significant increase in Type IV – Collagen levels in the serum of the patients with non alcoholic steato hepatitis as compared to controls. An adverse lipid profile i.e. significantly elevated serum cholesterol, triglycerides and LDL Cholesterol along with unaltered HDL Cholesterol levels were also observed in patients with non alcoholic steato hepatitis as compared to controls. Altered liver function test parameters were also observed in patients with non alcoholic steato hepatitis as compared to the controls.

The comparison of the serum lipid profile of the study group and the control group are shown in [Table/Fig 1].

(Table/Fig 1) Comparison of serum lipid profile of patients with non alcoholic Steato hepatitis compared to controls.

Parameter	Group 1 (controls) n = 69	Group 2 (Patients) n = 69
Cholesterol (mg/dl)	154 ± 13	195 ± 17 **
Triglycerides (mg/dl)	132.4 ± 12.2	162.8 ± 11.18 **
HDL Cholesterol (mg/dl)	48 ± 6	45 ± 5 NS
LDL Cholesterol (mg/dl)	95 ± 12	120 ± 19 *
Cholesterol / HDL Ratio	3.2 ± 0.4	4.3 ± 0.4 **
*P < 0.05 compared to controls * *P < 0.01 compared to controls NS – Not Significant		

The comparison of the serum Liver Function Test parameters of the study group and the control group are shown in [Table/Fig 2].

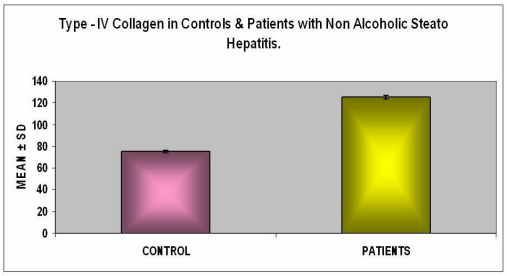
(Table/Fig 2) Comparison of serum Liver Function Test (LFT) parameters of patients with non alcoholic Steato hepatitis compared to controls.

Parameter	Group 1 (controls) n = 69	Group 2 (Patients) n = 69
Total Bilirubin (mg/dl)	0.8 ± 0.2	1.3 ± 0.2*
Direct Bilirubin(mg/dl)	0.3 ± 0.1	0.5 ± 0.1 NS
Indirect Bilirubin (mg/dl)	0.5 ± 0.1	0.8 ± 0.1*
ALT (U/L)	21.28 ± 0.14	73.26 ± 0.24**
AST (U/L)	24.52 ± 0.17	62.82 ± 0.47 **
GGT (U/L)	28.46 ± 1.82	100.24 ± 2.12 **
*P < 0.05 compared to controls * *P < 0.01 compared to controls NS – Not Significant		

The mean ± SD of Type IV - Collagen in the controls and patients with non alcoholic Steato hepatitis is described in [Table/Fig 3] and [Table/Fig 4].

(Table/Fig 3) The mean ± SD values of serum Type IV - Collagen in controls and patients with non alcoholic steato hepatitis.

Parameter	Type IV - Collagen (ng / ml)	95 % Confidence Interval (CI) for Mean
Group 1 (controls) n = 69	75.18 ± 1.4	74.84 – 75.51
Group 2 (Patients) n = 69	125.38 ± 1.8 ***	124.94 – 125.81
*** P < 0.001 compared to controls		



(Table/Fig 4) The mean ± SD values of serum Type IV - Collagen in controls and patients with non alcoholic steato hepatitis.

Discussion

In the present study, an adverse lipid profile i.e. significantly elevated serum cholesterol, triglycerides and LDL Cholesterol along with unaltered HDL Cholesterol levels were observed in patients with non alcoholic steato hepatitis as compared to controls. Altered liver function test parameters were also observed in patients with non alcoholic steato hepatitis as compared to controls. NAFLD is currently the most prevalent liver disease worldwide. Nonalcoholic steatohepatitis is an asymptomatic disease in a large proportion (48%-100%) of patients. The laboratory features of NASH are non diagnostic. Although mild to moderate elevations of serum aminotransferase levels are present in 70%-100% of the patients with NASH, there is no significant correlation between the degree of serum aminotransferase elevation and the histological features ([12],[13]. Elevated aminotransferase levels cannot be used to distinguish benign steatosis from NASH because steatosis alone can cause elevation, even without the evidence of overt cellular injury in the liver biopsy [14],[15].

Differentiation between these NAFLD forms can be safely performed only by liver biopsy [16]. However, due to the high prevalence of the disease, it would be necessary to find noninvasive parameters which would allow the selection of patients at a higher risk of developing cirrhosis to be submitted to a biopsy, despite the slight risk of mortality and morbidity involved.

In the present study, Type IV – Collagen levels were found to be significantly increased in patients with non alcoholic steato hepatitis when compared to controls. Chronic liver disease is a series of progressive disorders culminating in liver cirrhosis and those which are characterized by excessive amounts of deposited collagen. Type IV Collagen is the main collagen component of the basement membrane, being co-distributed with laminin in the perisinusoidal space [17], [18]. Studies on patients with chronic

liver disease have demonstrated a relationship between serum COL-IV values and the severity of liver fibrosis [19],[17].

Although various types of collagen like type I, III, IV, V and VI increase proportionally in the liver with the progression of fibrosis, type IV collagen, a constituent of the basement membrane, is particularly important and noteworthy for the following reasons: to relate the aggravation of hepatocellular damage and hepatocellular dysfunction, to play an important role in hepatocellular regeneration, to play an important role in hepatocellular regeneration and the rearrangement of the lobular architecture and for the earliest type of collagen to be synthesized by hepatocytes in experimental liver injuries. Similar reports of elevated Type IV Collagen levels in serum were reported by V.N.Dos santos et al [20].

Conclusion

Type – IV collagen levels were significantly increased in patients with NASH among NAFLD patients as compared to controls. When compared to liver function test parameters and lipid profile levels, NASH was found to have a positive negative predictive value among the NAFLD patients.

In patients with NAFLD, the Type – IV collagen test was found to be a simple, non – invasive and reliable test to predict the presence or absence of NASH.

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