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Research Article

RELATIONSHIP BETWEEN SERUM VITAMIN B12, HYPERHOMOCYSTEINEMIA IN NONALCOHOLIC FATTY LIVER DISEASE

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Abstract: Nonalcoholic fatty liver disease (NAFLD) represents a wide spectrum of liver damage and the prevalence is increasing worldwide. Hyper homocysteinemia is implicated as a causative factor of endothelial dysfunction, hepatic steatosis and cardio vascular disease (CVD). Since Vitamin B12 deficiency is one of the most important causative factors of hyper homocysteinemia. This study aims to elucidate serum levels of homocysteine and Vitamin B12 in south indian NAFLD patients. Patient diagnosed to have NAFLD by standard clinical, radiological and biochemical investigations formed the study group. Healthy volunteers formed the control group. Fasting blood samples were collected at Star medical diagnostics and were subjected to biochemical analysis. Vitamin B12 and homocysteine were assayed using chemiluminiscence commercial kits. Liver function tests (LFT), lipid profile and HOMA IR were assayed using auto analyzer. Vitamin B 12 deficiency (<223 picograms/ml), was observed in 43% of NAFLD patient and 72% of these Vitamin B12 deficiency patients had hyper homocysteinemia (>17.24 micro moles). Elevated ALT was found to be correlated with 67% of Vitamin B12 and 70% of hyper homocysteinemic NAFLD patients respectively. Our results indicate that hyper homocysteinemia may be one of the factors responsible for hepatic steatosis in NAFLD patients.

Keywords: Nonalcoholic Fatty Liver Disease (NAFLD), Vitamin B12, Hepatic Steatosis

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a wide spectrum of liver damage ranging from simple steatosis to steatohepatitis (NASH), and may progress to end-stage liver disease (ESLD) with cirrhosis and hepatocellular carcinoma (1, 2). NAFLD is the leading cause of chronic liver disease in India and western countries (3, 4). Epidemiological studies demonstrate around 9% to 32% of NAFLD prevalence in an Indian general population. Higher prevalence is seen in overweight or obesity and those with diabetes or prediabetes (5). NAFLD is scrupulously associated with metabolic syndrome including abdominal obesity, dyslipidemia, hypertension, insulin resistance and impaired glucose tolerance (6, 7). Almost90% of patients with NAFLD have at least one of the features of metabolic syndrome (MetS), and about 33% meet the complete (7, 8, 9) diagnosis. The relationship of NAFLD with features of MetS in adults has been reported (7, 10, 11). However, the etiology and pathogenesis remains uncertain (12).

Homocysteine is a sulphur-containing amino acid, which is an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine. However, studies reports that Hyperhomocysteinemia (HHcy) alters intracellular lipid metabolism(13). Earlier, prevalence of hyper-homocysteinemia has been reported in young Asian Indians [14], adolescent population [15], western Indians [16], in low socio-economic states of north India [17]. Further, studies reports vitamin B12 deficiency and hyperhomocysteinemia in rural and urban areas of India [18], diabetes retinopathy [19], insulin resistance [20], low dietary intake due to vegetarian food habits [20] and other factor may be impaire Vitamin B12 absorption [21]. Thus the data support the view that increased serum levels of homocysteine (Hcy) and lower levels of vitamin B12 may be associated with NAFLD.

The present study was designed to evaluate the utility of Body Mass Index (BMI), Fasting Blood sugar (FBS), Triglycerides (TGL), Total cholesterol (CHOL), Total bilirubin (TB), Alanine Transaminase (ALT), Aspartate Transaminase (AST), in the characterization and diagnosis of Nonalcoholic fatty liver disease.

MATERIAL AND METHODS

All the subjects were of South Indian origin and patients with ethanol consumption, viral hepatitis, autoimmune hepatitis, drug induced liver disease, cirrhosis, hemochromatosis, Wilson's disease, renal failure, cancer and addiction to any drugs are excluded for both NAFLD patients and controls. Further, Patients with NAFLD and controls were recruited on an outpatient basis. Determination of eligibility was based on medical history, physical examination and standard testes and procedures performed during the screening visit. All participants provided an informed consent.

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This study involved seventy nine NAFLD identified cases. The controls consist of twenty one genders matched healthy adults. For measurement of weight, subjects were instructed to stand still in the platform, with the body weight evenly distributed between both the feet. After removing heavy clothing weight was measured to the nearest of 0.1 kg. Height was measured using stadiometer with head held in Frankfort plane to the nearest of 0.1 cm. Body mass index (BMI) was calculated by the following formula; weight (kg)/height (cm). Waist circumference (WC) was measured midway between iliac crest and lowermost margin of the ribs, in quiet breathing. Hip circumference (HC) was measured at the maximum protruding part of buttocks at the level of the greater trochanter with the patient wearing minimal clothing and with feet together. Mid-thigh circumference was taken at the point in anterior midline of the thigh, midway between the inguinal ligament and base of patella to the nearest of 0.1 mm.

All blood samples were collected in the morning after an overnight fast. Blood samples were drawn in vacutainer blood collecting tubes (Becton-Dickinson, Franklin Lakes, NJ) according to standard guidelines for venipuncture and sample collection. Specimens were placed on ice and all specimens were processed within 30 minutes of collection. Serum was obtained after centrifugation at 2000 x g for 10 minutes, frozen, and stored at –20°C until analysis. Biochemical analysis of fasting blood glucose (FBG), total cholesterol (TC), serum triglycerides (TG), high-density lipoprotein (HDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were done as previously described [22].

Statistical Analysis:

All results are expressed as mean ± SD. The mean fasting glucose, homocysteine levels, vitamin B12, insulin and other serum parameters in both groups were tested using student t-test. All analyses were evaluated with the help of statistical packages SPSS 11 for windows (SPSS, Inc., Chicago, IL, USA). A p value of less than 0.05 was accepted as statistically significant.

RESULTS

In NAFLD groups, there were 27 subjects who had impaired glucose tolerance, no subject had diabetes, 27 subjects with lower Vitamin B12 deficiency and 31 subjects were with hyper homocysteinemia. Control group had neither impaired glucose tolerance nor diabetes. Further, obesity is taken into consideration for NAFLD, in the study group all subjects except for 18subjects had a BMI over 25 Kg/m when compare to control group. Serum homocysteine levels were significantly higher in patients with NAFLD (21.83±8.43umol/Las compared with the control group (17.24±5.06, p= 0.019. Similarly, serum vitamin 12 were significantly lower in patients with NAFLD (223.85±65.39pg/mL) as compared to control group 264.38±68.71, p=0.014). There is no significance in an insulin, ApoB, Total cholesterol, Bilurbin, and Triglycerides. Significantly higher values of FPG, AST and ALT were recorded in NAFLD subjects when compare to controls ($p<0.05$) (Table.1). Vitamin B12 deficiency was observed in 43% of NAFLD patient and 72% of these Vitamin B12 deficiency patients had hyper homocysteinemia and elevated ALT was found to be correlated with 67% of Vitamin B12 and 70% of hyper homocystenemic NAFLD patients.

Table.1: Clinical and Biochemical Parameters are summarized in

Variable	Controls (n=21)	NAFLD (n=79)	P
Age, year (range)	28.62±6.73	40.73±8.99	
Sex(M)	21	79	
Height	160.86±7.85	165.13±6.28	*<0.01
Weight	58.95±9.64	73.68±13.03	*<0.05
BMI	23.00±3.09	27.27±4.73	*<0.05
Waist Circumference (cm)	93.53±15.72	85.33±5.11	*<0.02
Hip	87.52±5.13	97.42±11.19	*<0.05
Glucose (mg/dL)	78.57±8.08	96.87±10.95	*<0.05
Insulin (µU/ml)	15.52±7.63	15.16±11.46	NS
HOMA IR	1.88±0.83	1.90±0.90	NS
Homocysteine (umol/L)	17.24±5.06	21.83±8.43	*<0.019
Vitamin B12 (pg/mL)	264.38±68.71	223.85±65.39	*< 0.014
ApoB (mg/dL)	74.13±11.16	84.71±24.11	NS
Total Cholesterol (mg/dL)	180.71±37.83	182.75±37.08	NS
Triglyceride (mg/dL)	139.71±41.70	152.94±64.73	NS
HDL (mg/dL)	45.00±1.92	39.26±6.44	*<0.05
Total Bilurubin (mg/dL)	0.88±1.64	1.27±0.69	NS
ALT(U/L)	19.71±2.41	63.65±47.67	*<0.05
AST(U/L)	18.76±1.94	44.90±25.76	*<0.05

DISCUSSION

Nonalcoholic fatty liver disease has been reported in coastal eastern India, studies demonstrate that males appeared to have a greater prediction for fatty liver than females [23,24]. Studies reported that Indian patients with NAFLD have overweight or obesity as per Asian Pacific criteria even though they do not have the kind of morbid obesity as seen in patients from the West [25]. Clinico-pathological studies show that NAFLD is an important cause of unexplained rise in hepatic transaminases, cryptogenic cirrhosis and cryptogenic hepatocellular carcinoma in Indian patients (5). Studies by Ludwig et al demonstrate elevated plasma levels of alanine transaminase (ALT) in patients with negligible alcohol intake [26]. Further, studies demonstrate hyperhomocysteinemia in Indians living in India is more attributable to low concentrations of vitamin B12 [27]. In India, hyperhomocysteinemia has been commonly observed in markedly decreased intakes of folic acid and vitamin B12 in the vegetarians and urban middle class residents [17, 18]. Studies

reported that Hyperhomo cysteinemia (HHcy) alters intracellular lipid metabolism [13]. In our study, significantly high levels of serum glucose, serum homocysteine ALT and AST were found in NAFLD patients compare to controls. Lower levels of Vitamin B12 were found in NAFLD patients when compare to control.

In conclusion; our data suggest that serum homocysteine level is increased in NAFLD patients. The low levels of Vitamin B12 may be associated with metabolic abnormalities. Further studies are needed to determine more clearly the role of homocysteine on hepatic steatosis and steatohepatitis.

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