VITAMIN B12 DEFICIENCY IN NONALCOHOLIC FATTY LIVER DISEASE
Malladi Subramanya Sharma¹, G. Jahnavi²

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ABSTRACT: INTRODUCTION: The prevalence of Non-alcoholic fatty liver disease (NAFLD) is increasing worldwide. This study aims to elucidate serum levels of Vitamin B12 in south Indian NAFLD patients. AIM: To investigate the correlation between Vitamin B 12 and NAFLD. MATERIALS AND METHODS: The study was designed as a case control study. Patients 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 was assayed using chemiluminiscence commercial kits. RESULTS: A total of 158 subjects were enrolled in the study. Cases constituted 79 and an equal number of controls were chosen by matching. Vitamin B 12 deficiency (<223 picograms/ml), was observed in 43% of NAFLD patients and none in the control group. CONCLUSION: Our results indicate that Vitamin B 12 deficiency may be one of the factors responsible for hepatic steatosis in NAFLD patients.

KEYWORDS: Vitamin B 12, Non-alcoholic fatty liver disease, Body mass index.

INTRODUCTION: Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in India[1] The disease begins with the accumulation of fat within the cells of the liver, but can progress to inflammation, the development of scar tissue, and in some cases death from liver failure or cancer[2-4] Simple accumulation of fat within the liver generally proceeds without producing any overt symptoms, but it may not remain harmless.

The liver regulates blood glucose and blood cholesterol levels, plays a critical role in burning fat for fuel, helps eliminate excess nitrogen, contributes to the metabolism of endocrine hormones, stores vitamin A, protects against infections, and detoxifies drugs and environmental toxins. Any type of damage to the liver is thus likely to impact whole-body health. Indeed, fatty liver disease increases the risk of cardiovascular disease three-fold in men, fourteen-fold in women, and seven to ten-fold in type-one diabetics.[5-6]

Fatty liver is a dangerous silent epidemic, and it is likely caused by the overabundance of calorie-rich, nutrient-poor refined foods and the banishment of traditional sources of choline like liver and egg yolks from the diet maybe due to adoption of western dietary pattern in our country. Epidemiological studies suggest prevalence of NAFLD is around 9% to 32% of general population in India with higher prevalence in those with overweight or obesity and those with diabetes or prediabetes[1] dyslipidemia and hypertension.[7,8]

NAFLD represents a wide spectrum of liver damage ranging from simple non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH), featuring steatosis, inflammation, cirrhosis, and hepatocellular carcinoma.[9] In addition, metabolic abnormalities mostly associated with NAFLD are insulin resistance (IR) and an increased supply of fatty acids to the liver.[10,11] However, the etiology and pathogenesis remains uncertain.[12]
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.

MATERIALS AND METHODS: The case control study was conducted at the outpatient clinic of star diagnostics Visakhapatnam between June and December 2013. Patients of South Indian origin who met the study criteria were included in the study.

Matched healthy adults were taken in the control group. Exclusion criteria included 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 for both the groups. Determination of eligibility was based on medical history, physical examination. Standard tests and procedures performed during the screening visit. All the participants provided an informed consent.

This study involved seventy nine NAFLD identified cases. The controls consist of twenty one genders matched healthy adults. For measurement of weight, subject 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 (m$^2$).

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 avacutainer 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 2000x g for 10minutes, 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 $^{[19]}$.

Statistical Analysis: All results are expressed as mean ± SD. The mean fasting glucose, 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: Clinical and Biochemical Parameters are summarized in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NAFLD (n=79)</th>
<th>Controls (n=21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year (range)</td>
<td>40.73±8.99</td>
<td>28.62±6.73</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Sex (M)</td>
<td>79</td>
<td>21</td>
<td></td>
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<tr>
<td>Height</td>
<td>165.13±6.28</td>
<td>160.86±7.85</td>
<td>*&lt;0.01</td>
</tr>
<tr>
<td>Weight</td>
<td>73.68±13.03</td>
<td>58.95±9.64</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>BMI, Kg/m</td>
<td>27.27±4.73</td>
<td>23.00±3.09</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>93.53±15.72</td>
<td>85.33±5.11</td>
<td>*&lt;0.02</td>
</tr>
<tr>
<td>Hip</td>
<td>97.42±11.19</td>
<td>87.52±5.13</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Glucose (ng/dL)</td>
<td>96.87±10.95</td>
<td>78.57±8.08</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Insulin ()</td>
<td>15.16±11.46</td>
<td>15.52±7.63</td>
<td>0.89</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>223.85±65.39</td>
<td>264.38±68.71</td>
<td>*&lt;0.014</td>
</tr>
<tr>
<td>ApoB</td>
<td>84.71±24.11</td>
<td>74.13±11.16</td>
<td>0.054</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>182.75±37.08</td>
<td>180.71±37.83</td>
<td>0.825</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>152.94±64.73</td>
<td>139.71±41.70</td>
<td>0.377</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>39.26±6.44</td>
<td>45.00±1.92</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1.27±0.69</td>
<td>0.88±1.64</td>
<td>0.099</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>63.65±47.67</td>
<td>19.71±2.41</td>
<td>*&lt;0.05</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>44.90±25.76</td>
<td>18.76±1.94</td>
<td>*&lt;0.05</td>
</tr>
</tbody>
</table>

RESULTS: In NAFLD groups, there were 27 subjects who had impaired glucose tolerance; no subject had diabetes, 27 subjects with lower Vitamin B12 deficiency. 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 18 subjects had a BMI over 25 Kg/m when compared to control group. Serum vitamin 12 were significantly lower in patients with NAFLD (223.85±65.39 pg/mL) as compared to control group 264.38±68.71, p=0.014). There is no significance in insulin, ApoB, Total cholesterol, Bilirubin and Triglycerides. Significantly higher values of FPG, AST and ALT were recorded in NAFLD subjects when compared to controls (p<0.05)(Table1). Vitamin B12 deficiency was observed in 43% of NAFLD patients and elevated ALT was found to be correlated with 67% of Vitamin B12 deficiency patients.

DISCUSSION: In our study we found that the in NALFD patients the serum B12 was significantly lower than in the control group. Vitamin B12 deficiency results from an inadequate intake of nutrition, abnormal nutrient absorption, and rare inborn errors of vitamin B12 metabolism. Vitamin B12 is mainly present in animal protein, particularly organ meats, bivalves and to a lesser extent, in seafood, milk and milk products. However, prevalence of vitamin B12 deficiency is not rare, and it is especially common the elderly. Vitamin B12 deficiency does not only cause anemia and megaloblastic anemia, but also causes neurological symptoms.
A correlation between NALFD and B12 deficiency has been investigated in a few studies. 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.\cite{13,14}

In our study it was observed that the cases were found to be overweight when compared to the controls which was statistically significant. 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.\cite{15}

Clinicopathological studies show that NAFLD is an important cause of unexplained rise in hepatic transaminases, cryptogenic cirrhosis and cryptogenic hepatocellular carcinoma in Indian patients.\cite{1} Studies by Ludwig et al demonstrate elevated plasma levels of alanine transaminase (ALT) in patients with negligible alcohol intake.\cite{16}

Further, studies demonstrate hyperhomocysteinemia in Indians living in India is more attributable to low concentrations of vitamin B12.\cite{17} 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.\cite{18} Studies reported that Hyperhomocysteinemia (HHcy) alters intracellular lipid metabolism.\cite{19}

In our study, significantly high levels of serum glucose, serum homocysteine ALT and AST were found in NAFLD patients compared to controls. Lower levels of Vitamin B12 were found in NAFLD patients when compared to control.

**CONCLUSION:** In our study we observed that there were low levels of Vitamin B12 in NALFD cases rather than controls and also obesity was more in cases than in controls. However, this study is not enough to explain a possible relationship between vitamin B12 and hepatic fat accumulation. Further studies are warranted to determine more clearly the role of Vitamin B12 on hepatic steatosis and steatohepatitis.

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15. Duseja A. Non-alcoholic fatty liver disease in India--is it different? Trop Gastroenterol. 2006 Oct-Dec; 27 (4): 142-6