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RESEARCH ARTICLE

Relation between vitamin B12 and Non-alcoholic fatty Liver Disease: A Hospital Based Study

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Abstract

Background/Aims: Nonalcoholic fatty liver disease is the most common liver disease worldwide. NAFLD is characterized by excessive fat accumulation in the form of triglycerides in the liver. A subgroup of NAFLD patients has liver cell injury and inflammation in addition to excessive steatohepatitis. The liver plays an important role in the storage and bioavailability of vitamin B12. The relation between NAFLD and vitamin B12 has not been adequately studied. Therefore the current study investigated the vitamin B12 levels in NAFLD.

Patients and Methods: Vitamin B12 levels were measured in patients with NAFLD at Salman Bin Abdul Aziz University Hospital, Al Kzharj, Kingdom of Saudi Arabia. All patients were assessed by liver function test, lipid profile, hepatitis viral markers and abdominal ultrasound. The levels of vitamin B12 were correlated with the stage of NAFLD, lipid profile and liver parameters.

Results: Vitamin B12 levels were significantly lower in patients with NAFLD. Vitamin B12 levels inversely correlated with cholesterol and triglyceride levels. Patients with vitamin B12 deficiency had more advanced NAFLD as well as gastric manifestations treated by PPI. Blood sugar levels were also higher in NAFLD patients.

Conclusion: Vitamin B12 levels are lower in NAFLD patients. This has important implications since it is important to assess vitamin B12 levels in NAFLD patients and provide vitamin B12 supplementation.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a pathological condition characterized by macrovesicular accumulation of triglycerides within hepatocytes (hepatic steatosis) (Angelico et al, 2004). NAFLD is rapidly becoming the most common liver disease worldwide (Bellentani et al, 2010). In developed countries, NAFLD is observed in 20-50% of the general population depending on the population studied and the diagnostic method utilized (Farrell et al, 2006). The diagnosis of NAFLD is based on the presence of evidence of hepatic steatosis, either by imaging or by histology and absence of secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders (Kleiner et al, 2006). NAFLD is frequently associated with metabolic risk factors such as obesity, diabetes mellitus, and dyslipidemia (Caldwell et al, 2004 and). NAFLD includes a wide spectrum of hepatic pathology extending from simple steatosis, steatohepatitis that may evolve in cirrhosis and liver failure in 20-25% of affected subjects (Gambino et al, 2011; Williams et al, 2011).

Vitamin B12 or cobalamin is a water-soluble cobalt- containing vitamin. Vitamin B12 is a cofactor for at least 3 enzymes that carry out these types of reactions, acting as a transitional carrier of the single carbon group (Fragasso et al, 2010). The liver plays an important role in the storage and bioavailability of vitamin B12 (Pflipsen et al, 2009).

Several liver diseases such as acute hepatitis, alcoholic hepatitis, liver cirrhosis and hepatocellular carcinoma have been associated with changes in plasma levels vitamin B12 by different pathways (Koplay et al, 2009; Cylwik et al, 2011). To date the influence of vitamin B12 deficiency on NAFLD has not been established. Some studies reported that there might be a relationship between the levels of vitamin B12 and NAFLD ((Koplay et al, 2009).
No studies have been conducted to assess the relation of vitamin B12 deficiency on NAFLD in the Kingdom of Saudi Arabia. Therefore, we designed the current study to evaluate serum vitamin B12 levels in patients with NAFLD and their association with the disease severity.

Patients and Methods

Vitamin B12 levels were assessed in 232 consecutive patients with proven NAFLD (105 women and 127 men) with age range 29 to 55 years (mean ± SD, 41.88±7.64 in addition 26 healthy controls ranging in age from 29 to 55 years (mean ± SD, 40.56±9.13; 9 women, 17 men). The study was conducted between December, 2012 and September 2013 in Salman Bin Abdul Aziz University Hospital, Al Kharj, KSA. . All participants provided informed consent before enrollment in the study.. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of Salman Bin Abdul Aziz University Hospital.
Determination of eligibility was based on medical history, physical examination, and standard tests and procedures performed during the screening visits. For the diagnoses of NAFLD, and to rule out other possible liver diseases, both the NAFLD group and the HC group were evaluated by abdominal ultrasonography (US), clinical and laboratory findings. The time interval between US and the laboratory study was less than 1 week. Apart from vitamin B12, the liver enzymes including aspartate aminotransferase, alanine aminotransferase (ALT), and alkaline phosphatase, as well as the total cholesterol, triglycerides, serum lipoproteins, glucose, folic acid and hepatitis markers, were evaluated. In addition, body mass index (calculated as weight in kilograms divided by the square of the height in meters) and the waist circumference (measured with a tape measure just above the umbilicus) were calculated.
Inclusion criteria included Inclusion criteria for the NAFLD patients were (1) age ≥ 18 years, (2) bright liver on ultrasound imaging and increased liver function tests for at least 6 months before enrollment and (3) patient's consent for liver biopsy. Age-, gender- and body mass index (BMI)-matched individuals were recruited for control group. Inclusion criteria for the controls were (1) age ≥ 18 years and (2) normal liver ultrasound imaging and normal liver function tests. Exclusion criteria for both NAFLD patients and controls were (1) ethanol consumption more than 20 g/day, (2) liver cirrhosis, (3) other liver diseases (viral hepatitis, autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis and overlap syndromes, drug-induced liver disease, haemochromatosis, Wilson's disease, α1-antitrypsin deficiency), (4) type I diabetes mellitus, (5) pancreatitis, (6) uncontrolled hypothyroidism or hyperthyroidism, (7) adrenal insufficiency, (8) renal failure, (9) thrombotic disorders, (10) cancer, (11) pregnancy, (12) addiction to any drug, (13) use of the following medications within a 12-month period before screening: folate or any vitamin B supplements, oestrogens, progestins, glucocorticosteroids, thiazolidinediones, insulin, sibutramine, orlistat, rimonabant, vitamin E, vitamin C, ursodeoxycholic acid, ferrum, interferon, tamoxifene, amiodarone, metronidazole, biologic agents, any medication against tuberculosis, epilepsy or viruses, or any medication affecting haemostasis, such as antiplatelet agents, aspirin or oral anticoagulants and (14) use of antibiotics, intravenous glucose administration or parenteral nutrition within a 1-month period before screening.Serum aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and glucose were measured within 1 h after blood was drawn, with standard methods using an automated analyser (Olympus AU2700; Olympus, Hamburg, Germany). Sera were also immediately frozen at − 30°C for the measurement of vitamin B12. Vitamin B12 was measured with immuno-chemiluminescence by ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics; vitamin B12: intra-assay CV 2.4–5.0%, inter-assay CV 2.7–9.2%; folate: intra-assay CV 4.5–7.9%, inter-assay CV 5.3–7.2%).BMI was calculated by the formula body weight (kg)/height^2 (m^2). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula.

Results

Table 1: Demographics of patients and controls

Parameter	NAFLD Patients (n=232)	Control (n=26)	P value
Age	41.88±7.64	40.56±9.13	0.4048
Sex Male : female	 127:105	 17:9	 0.0387*
Hemoglobin (gm/dl)	12.84±1.42	13.08±1.47	0.4147
Red blood cells	3.98±0.83	4.00±0.89	0.9014
White blood cells	8556.28±1970.03	8954.00±372.84	0.3283
Platelet	181.73±21.91	182.50±21.06	0.8652
Body mass index	31.45±2.23	28.46±2.21	0.05*

No significant difference was detected between patients and control subjects regarding age and blood picture. Patients and control subjects differed in BMI and gender

Table 2: Clinical data of patients and controls

Parameter	NAFLD Patients	Control	P value
Fatigue Yes No	 75 157	 7 19	 0.6616
Fever Yes No	 43 189	 4 22	 1.0000
Jaundice Yes No	 21 211	 3 23	 0.7191
Bleeding tendency Yes No	 1 0	 231 26	 1.0000
Nausea Yes No	 49 183	 4 22	 0.6145
Constipation/diarrhea Yes No	 19 213	 3 22	 0.2658

Table 3: Lipid profile, liver functions and B12 levels in patients and controls:

Parameter	NAFLD Patients (n=232)	Control (n=26)	P value
B12 (pg/ml)	469.87±267.88	770.35±380.27	0.0001*
Cholesterol (mmol/L)	7.60±1.13	6.15±2.08	0.032*
Triglycerides (mmol/L)	3.16±1.16	2.00±0.88	0.02*
ALT (IU/L)	115.02±48.74	31.12±10.13	0.0001*
AST (IU/L)	115.47±49.97	29.04±13.16	0.0001*

This table shows significant difference between vitamin B12 levels, lipid profile and AST and ALT levels.

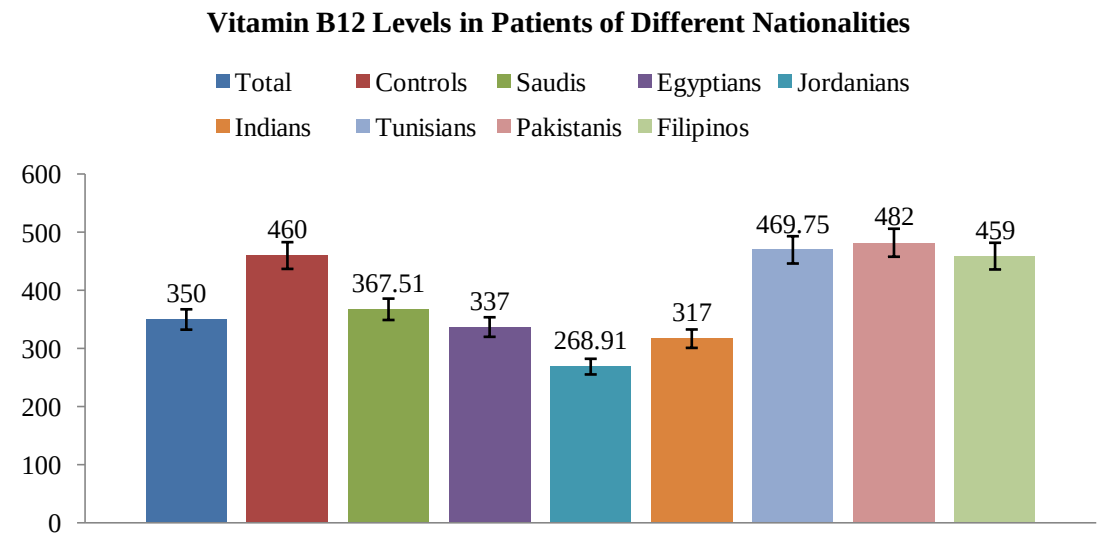
Table 4: Diabetes, it D level, PPI use , and IBD in patients and controls:

Risk Factor	NAFLD Patients	Control	P value

	(n=232)	**(n=26)**	
Diabetes mellitus Yes No	 91 140	 12 15	 0.6796
Vitamin D level Normal low	 43 189	 15 11	 0.0001*
PPI use Yes No	 108 124	 12 14	 1.000
IBD Yes No	 124 108	 12 14	 0.5377

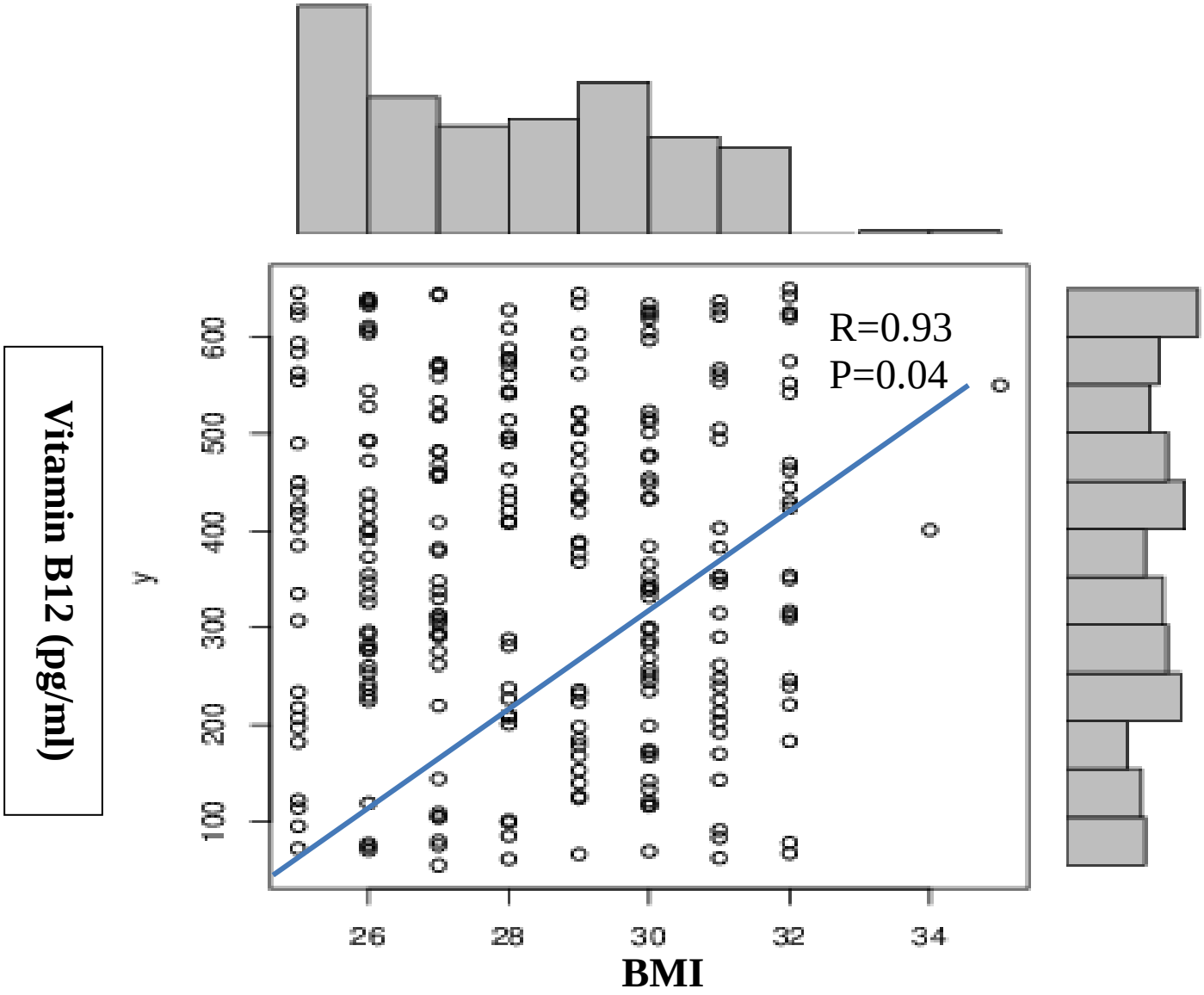
*There is a significant difference in vitamin D levels in NAFLD patients and controls

Figure 1: Vitamin B12 levels in Saudi and non-Saudi patients



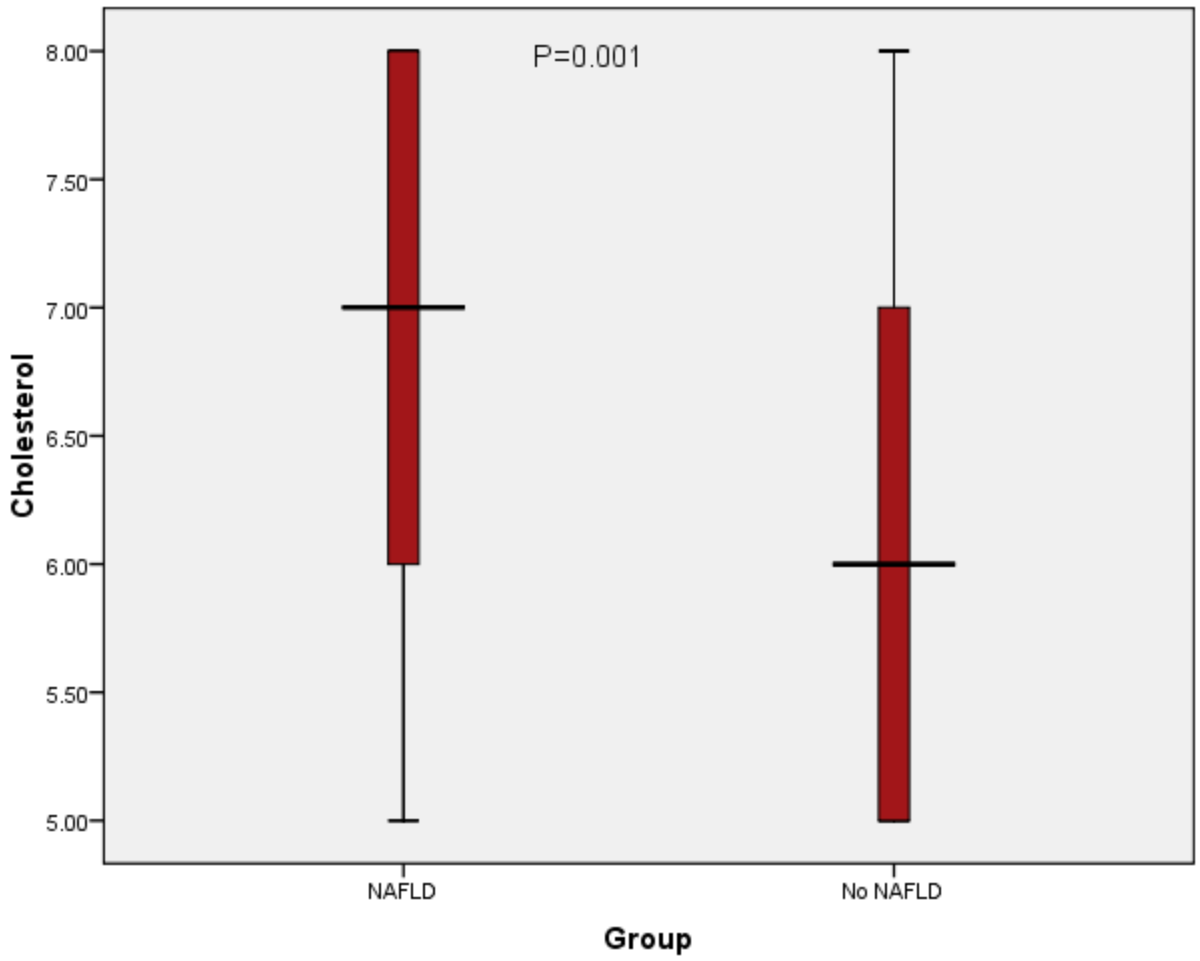
This bar diagram shows the mean vitamin B12 levels according to nationalities.

Figure 2: Relation between Vitamin B12 Levels and BMI



Correlation between BMI and vitamin B12 levels showed a strong positive linear correlation

Figure 3: Cholesterol Level in NAFLD Patients and Control



*The cholesterol levels significantly differ between the two groups.

Discussion

This study is the first to explore the relationship between NAFLD and Vitamin B12 deficiency in Saudi Arabia. We found that patients with NAFLD have low vitamin B12 level. In our study, vitamin B12 levels were significantly low in Saudis and Jordanian compared to other nationalities. The mean vitamin B12 levels in Saudis were 367.5 pg/L ranking lowest after Jordanians and Indians. Only one study by Ardawi et al, 2002 assessed vitamin B12 levels in the Kingdom of Saudi Arabia. This study showed that plasma vitamin B12 concentrations were significantly lower in Saudi patients with cardiovascular diseases. Stepwise multiple linear regression analysis showed that age, sex, serum folate, and waist-to-hip ratio and plasma vitamin B12 were significant determinants of cardiovascular disease. B12 related megaloblastic anemia in 18 of 25 cases reported from Saudi Arabia and the frequency of anti-intrinsic factor antibodies was very high, 89% (Sally et al, 2004).

In Jordan, a study investigated the levels of vitamin B12 deficiency in a Jordanian university hospital and they found that low serum levels of vitamin B12 (< 180 pg/mL) were detected in 374 patients (44.6%). (Barghouti et al, 2009). This study revealed that there was a high frequency of suboptimal serum vitamin B12 level, which is an early sign of negative B12 balance (Fora et al, 2005). A study from Kuwait described several cases of vitamin B12 pernicious anemia, and the authors suggested that it was underdiagnosed in Arabs (Sally et al, 2004).

In the US, the prevalence of vitaminB12 deficiency (serum vitamin B-12 < 148 pmol/L) varied by age group and affected ≤3% of those aged 20–39 y, ≈4% of those aged 40–59 y, and ≈6% of persons aged ≥70 y. Marginal depletion (serum vitamin B-12: 148–221 pmol/L) was more common and occurred in ≈14–16% of those aged 20–59 y and >20% of those >60 y (Lindsay et al, 2008).

In Western countries, NAFLD is the most common liver disease with a prevalence of 20-30% (Bellentani etal, 2010). In Asia-Pacific regions, the prevalence of NAFLD ranges from 5 to 30%. In the United States multiple studies were performed to assess the prevalence of NAFLD. , In one study, NAFLD represented 21.4% and 19.0% (Lazo et al, 2013). Another study showed a prevalence of 24 % in the United States of America with more prevalence in African Americans (Foster et al, 2013). In KSA only one study assists the prevalence of NAFLD. AL-Hamoudi et al, 2012 found that 7-10% of Saudis have NAFLD.

In our study, we found that 26% of our NAFLD patients have B12 deficiency. We found that more males (54.7%) have NAFLD than females (45.2%). Our findings are in agreement with the study Kopley et al, 2011 who found that serum vitamin B12 levels were significantly lower in the patients with NAFLD than in those of the control group; however, these still remain in the reference range. They concluded that low vitamin B12 levels may be associated with NAFLD especially in grade 2 to grade 3 hepatosteatosis. (Koplay et al, 2011).

Vitamin B12 deficiency may be related to various causes such as insufficient nutritional intake, due to vegetarian diets, increased metabolic demands pregnant and lactating women or defects in cobalamin absorption as in malabsorption syndrome. Chronic pancreatitis reduced pancreatic enzyme secretion leads to impaired degradation of HC-bound vitamin B12 and vitamin B12 trapping. Gluten-sensitive enteropathy, a permanent intolerance to wheat gliadins in genetically susceptible individuals, and chronic treatments with drugs such as para-aminosalicylic acid, used in inflammatory bowel disease or metformin.6-total gastrectomy, ileal resection or pernicious anemia and leads to a progressive depletion of the vitamin, cellular dysfunction, metabolic and clinical abnormalities.

The cause of low vitamin B12 levels among Saudi population has not been previously investigated. However, one may speculate that low consumption of oily fish in KSA, the relative frequency of malabsorption, inflammatory bowel disease and peptic ulcers may contribute to decrease intake and absorption of vitamin B12. The significant low vitamin B12 levels detected among our Indian and Filipino patients may be due to the prevalence of Tropical sprue. Vitamin B12 deficiency may also be related to parasitic Infection (Kavimandan et al, 2013).

In a study from Greece, they measure serum vitamin B12 and folate levels in patients with biopsy-proven non-alcoholic fatty liver disease (NAFLD) and their association with the disease severity. Similar vitamin B12 and folate levels were observed in non-alcoholic steatohepatitis and non-alcoholic fatty liver patients, and controls. Furthermore, vitamin B12 and folate levels were not associated with either insulin resistance or the severity of liver disease (Polyzos et al, 2012)

In conclusion, vitamin B12 levels are lower in NAFLD patients. This has important implications since it is important to assess vitamin B12 levels in NAFLD patients. Further studies may be conducted to investigate if supplementation of vitamin B12 to patients with NAFLD and vitamin B12 deficiency would improve the parameters of NAFLD in those patients.

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