

STUDY OF SERUM VITAMIN B12 AND SERUM VITAMIN D LEVEL IN PATIENTS OF CHRONIC LIVER DISEASE AT A TERTIARY CARE HOSPITAL OF BIHAR, INDIA

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ABSTRACT

Background: The study aimed to study this relationship and was planned to evaluate serum vitamin B12 levels and Vitamin D in patients with NAFLD and their association with disease severity. **Materials and Methods:** This case control observational study was conducted in the department of medicine And Biochemistry during the period of August 2023 to July 2024 in NMCH, Patna, Bihar India. The study was conducted on randomly selected 150 subjects. 100 NAFLD patients of either gender, in the age group of 19 and above years and 50 aged and gender-matched healthy controls selected from the attendants of the patients visiting the OPD. To rule out other possible liver diseases, both cases and controls were evaluated by abdominal ultrasonography, clinical and laboratory findings as well. Data was analysed and statistical calculations were carried out using Microsoft excel 2020 software. Mean standard deviation, median was found out to appropriately represent the data. P value of <0.05 was considered significant. **Result:** Mean age of patients was 46.45±9.34 years. Out of the 100 subjects studied as cases, 46 were females, while there were 20 females (out of 50 subjects studied) under controls group. There was not much significant difference between NAFLD patients and controls regarding age (46.45±9.34 years; 42.41±8.37 years), Vitamin B12 levels in cases showed up as a significant factor of differentiation among the two groups (p<0.001), serum cholesterol, triglycerides also differing significantly (p<0.05). There was a significant variation among patients and controls with 81 (81%) of patients having lower vitamin D levels as compared to only 30 (59%) among healthy controls. **Conclusion:** Vitamin B12 levels were found lower in NAFLD patients, makes it important to assess vitamin B12 levels in all NAFLD patients for better management of the disease.

INTRODUCTION

NAFLD is currently the most common chronic liver disease in developed countries and obesity contributes maximum to this disease load.^[1] This disease increases the risk for co-morbidities like type 2 diabetes (T2DM) and cardiovascular disease and is greatly responsible for liver-related morbidity and mortality. Metabolic syndrome including insulin resistance, together with adverse genetic, hormonal and lifestyle factors is a kind of trigger that often precipitates to the development of NAFLD.^[2] NAFLD is defined as the presence of ≥5% steatosis in the absence of secondary causes of fat accumulation in the liver. The disease starts with fatty liver or hepatic steatosis and may progress to steatohepatitis with hepatic inflammation.^[3] Five to

twenty percent of patients with fatty liver develop nonalcoholic steatohepatitis (NASH) in their clinical course, of which 10-20% develop into higher-grade fibrosis and <5% progress to full blown cirrhosis.^[4] In the western world, NAFLD continues to be the most common liver disease, with rising incidence, mainly due to the sedentary life style, high fatty food and the accompanying obesity. Current global prevalence of NAFLD is 10.9%, higher in patients with a background of T2DM or with metabolic syndrome.^[5] A reduction in the energy content of diet, by itself alone, may not prevent NAFLD, nor could alleviate it. Dietary compositions, both macronutrient and micronutrient, may also be playing a crucial role in the manifestation and development of NAFLD. Recent studies are bringing to light the association, importance and role of dietary vitamins

and liver fat accumulation. Vitamins are essential micronutrients which help in the buildup and maintenance of health status of a person. Vitamin B12 or cobalamine is water soluble cobalt containing vitamin. Vitamin B12 is a cofactor for two enzymes. Firstly, in the cytoplasm, methionine synthase requires vitamin B12 in the form of methylcobalamin and catalyzes the conversion of homocysteine to methionine by transfer of a methyl group from methyltetrahydrofolate. Secondly, in the form of 5'-deoxyadenosylcobalamin, vitamin B12 is also required for the mitochondrial enzyme methylmalonyl, important for the oxidation of odd-chain fatty acids and catabolism of ketogenic amino acids.^[6]

Vitamin B12 is involved in the metabolism of every cell of the human body. It is one of eight B vitamins. It is a cofactor in DNA synthesis and in both fatty acid and amino acid metabolism.^[7] Unlike most other vitamins, B12 is stored in substantial amounts, mainly in the liver, until it is needed by the body. It is largely understood that the body's stores of this vitamin usually take about 3 to 5 years to exhaust.^[8,9] Besides, several liver diseases such as acute hepatitis, alcohol hepatitis, liver cirrhosis and hepatocellular carcinoma have been associated with changes in plasma vitamin B12 by different pathways.^[10,11] But, the influence of vitamin B12 deficiency on NAFLD has not been studied much. Therefore, the study aimed to study this relationship and was planned to evaluate serum vitamin B12 levels and Vitamin D in patients with NAFLD and their association with disease severity.

MATERIALS AND METHODS

This case control observational study was conducted in the department of medicine And Biochemistry during the period of August 2023 to July 2024 in NMCH, Patna, Bihar India. The study was conducted on randomly selected 150 subjects. 100 NAFLD patients of either gender, in the age group of 19 and above years and 50 aged and gender-matched healthy controls selected from the attendants of the patients visiting the OPD. Written informed consent was taken from all participants before their enrollment in the study. Ethical clearance was obtained from IEC and the study protocol conformed to the ethical guidelines. Medical history, physical examination and standard tests and procedures were performed during the screen visits of patients for determining their eligibility for the study. To rule out other

possible liver diseases, both cases and controls were evaluated by abdominal ultrasonography, clinical and laboratory findings as well.

Inclusion criteria for NAFLD patients (cases)

Patients with age >18 years, bright liver on ultrasound imaging, increased liver function tests for at least 6 months before enrolment and patients consenting for liver biopsy were included in the study.

Inclusion Criteria for Healthy Controls

Age, gender and body mass index (BMI) matched individuals with normal liver ultrasound imaging and normal liver function test were included in the study.

Exclusion Criteria

Patients having ethanol consumption for more than 20 g/day, patients with liver cirrhosis, any other liver disease (viral hepatitis, autoimmune hepatitis and sclerosis), patients having T2DM, pancreatitis, uncontrolled hypothyroidism or hyperthyroidism, renal failure, cancer, patients who are pregnant and patients with addiction to any drug or use of folate or on regula vitamin B supplementation were excluded from the study. Serum AST, ALT, GGT, and HDL-C, triglycerides, total cholesterol, glucose and vitamin B12 were measured by immunochemiluminescence by ADVIA Centaur immunoassay system.

Data was analysed and statistical calculations were carried out using Microsoft excel 2020 software. Mean standard deviation, median was found out to appropriately represent the data. P value of <0.05 was considered significant.

RESULTS

Mean age of patients was 46.45±9.34 years. Out of the 100 subjects studied as cases, 46 were females, while there were 20 females (out of 50 subjects studied) under controls group. There was not much significant difference between NAFLD patients and controls regarding age (46.45±9.34 years; 42.41±8.37 years), however slightly significant difference of data values were found for hemoglobin and platelet count among them. BMI also seemed to be an important variant among them here. Patients and controls almost matched in all clinical examinations like fever, jaundice, nausea, constipation or diarrhea. Vitamin B12 levels in cases showed up as a significant factor of differentiation among the two groups (p<0.001), serum cholesterol, triglycerides also differing significantly (p<0.05) [Table 1].

Table 1: B12 levels, lipid profile and liver functions in patients and controls (n=150).

S. no.	Parameters	Mean	SD	Mean	SD	P Value
1	Vitamin B12 (pg/ml)	376.7	184.13	542.88	287.5	0.0001
2	Cholesterol (mmol/l)	8.5	1.09	6.47	1.35	0.0012
3	Triglycerides (mmol/l)	4.1	1.12	2.16	1.03	0.0065
4	ALT (IU/l)	82.05	39.32	32.19	10.64	<0.001
5	AST (IU/l)	106.01	43.43	34.38	13.25	<0.001

There was a significant variation among patients and controls with 81 (81%) of patients having lower vitamin D levels as compared to only 30 (59%) among healthy controls, however, number of patients with diabetes mellitus and proton pump inhibitors (PPI) use to relieve symptoms of acid reflux or

gastro-esophageal reflux disease (GERD) was almost similar among the two. Inflammatory bowel disease (IBD) was showing in 59 patients (59%), which was also quite higher to 44% prevalence among controls [Table 2].

Table 2: Vitamin D level, diabetes, IBD and PPI use-variation among patients and controls.

S.no	Associated Risk	Cases	Control	P value
1.	Vitamin D Level			
	Low	81	30	0.0009
	Normal	19	20	
2	Diabetes mellitus			0.7976
	Yes	65	21	
	No	35	29	
3	IBD			0.0810
	Yes	88	20	
	No	12	30	
4	PPI Use			0.9439
	Yes	75		
	No	25	25	
			25	

DISCUSSION

B12 deficiency is extremely common in India as is vitamin D, folic acid or iron deficiency. This is because malnutrition is extremely common and related to diet, lifestyle and social and cultural issues. In spite of being a common disorder, its recognition is missed because the manifestations are diverse in nature, affecting all the organs and systems and is often subclinical. Laboratory estimations are often unreliable and doctors tend to rely on laboratory estimate of B12 levels.^[12] As an estimate, prevalence of vitamin B12 deficiency is at least 47% in Indian population and only 26% population may be vitamin B12 sufficient if people with levels between 200-300 pg/ml are considered borderline deficient.^[13] Lindsay et al in 2008 in the US, showed that the prevalence of vitamin B12 deficiency (serum vitamin B12<148 pmol/l) varied with age. It affected less than 3% of those aged 20-39 years and up to 6% of persons aged more than 70 years.^[14] B12 levels between 160 and 200 ng/l but up to 1000 ng/l can be normal but is false normal in majority of cases, especially if it is an autoimmune etiology, serum methyl malonic acid (MMA) is elevated (950 nmol/l) most often and is said to be more specific. Serum MMA could be used for early diagnosis, even in the absence of hematological abnormalities.^[15-17]

Vitamin B12 deficiency may be related to various causes such as insufficient nutritional intake due to vegetarian diets or increased metabolic demands among pregnant and lactating women. It can also be related to the malabsorption syndrome. Chronic pancreatitis could also reduce pancreatic enzyme secretion, leading to impaired degradation of HC-bound vitamin B12 and vitamin B12 trapping. Kavimandan et al in 2013 had related the low vitamin B12 levels, detected among Indian patients, to the prevalence of tropical sprue.^[18]

In our study, we found that patients with NAFLD have low vitamin B12 level. In a similar study with a comprehensive and comparative analysis, vitamin B12 levels were found significantly low among 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.^[19] In Western countries, NAFLD is the most common liver disease with a prevalence of 20-30%.^[20] Foster et al in 2013 showed a prevalence of 24% in the United States of America with more prevalence in African Americans.^[21] A study by Kopley et al in 2011 and Abdullah et al in 2015 found that more males (54.7%) have NAFLD than females (45.2%) and that serum vitamin B12 levels were significantly lower in the patients with NAFLD than in those of the control group, but remained within the reference range.^[10,19] Kopley et al in 2011 also concluded that low vitamin B12 levels could be associated with NAFLD especially in grade 2 to grade 3 hepatosteatosis.^[10]

Polyzos et al in 2012 measured serum vitamin B12 and folate levels in patients with biopsy proven NAFLD and their association with the disease severity.^[22] Furthermore, vitamin B12 and folate levels were not associated with either insulin resistance or the severity of liver disease. Our study obtained similar results with lower vitamin B12 and folate levels were observed in non-alcoholic steatohepatitis and non-alcoholic fatty liver patients. The course and content of this present study also revealed a significant relation of low serum vitamin D with non-alcoholic fatty liver disease and similar studies are there to suggest low serum vitamin D causing NAFLD. Hypovitaminosis D is seemed to be associated with the severity and incidence of NAFLD.^[23]

The limited geographical outreach of the community health center, in which it was conducted, was a major limitation of the current study. Besides, there was

very little prior research and investigations on the topic worldwide, to be able to directly compare the results and outcomes with.

CONCLUSION

Vitamin B12 levels were found lower in NAFLD patients, makes it important to assess vitamin B12 levels in all NAFLD patients for better management of the disease. More such studies are required to be conducted in a larger geographical and demographical expanse and more investigations are required to understand whether supplementation of vitamin B12 to patients with NAFLD could improve their health status.

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