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TO STUDY 25 (OH) D LEVELS IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and its increasing incidence has been well documented from Asian countries. Diabetes mellitus (DM), obesity, hyperlipidemia are predisposing factors for NAFLD. Hypovitaminosis D has been recently recognized as a worldwide epidemic. Since vitamin D exerts significant metabolic activities, comprising free fatty acids (FFA) flux regulation from the periphery to the liver, its deficiency may promote fat deposition into the hepatocytes.Therefore this study was planned to estimate serum 25 (OH) D levels in patients with NAFLD.

Aims and objectives: To estimate the serum vitamin D levels in patients with NAFLD and to find out the association if any between the Vitamin D deficiency and NAFLD.

Material and method: This observational and analytical study was conducted in department of General Medicine, Himalayan Institute of Medical Sciences, Dehradun from November 2015 to November 2016. A total of 70 patients of NAFLD diagnosed by ultrasonography and 70 healthy volunteers were included in the study. Data was analyzed using SPSS version 22.0. Chi square and unpaired t test were used. A 'p' value of <0.05 was considered significant.

Results: NAFLD patients had low serum 25 (OH) levels as compared to healthy volunteers which was statistically significant. Higher BMI (>23 kg/m^2) was established as a risk factor for NAFLD. HDL and LDL levels were significantly lower in NAFLD patients. Higher plasma glucose level was risk factor for NAFLD but there was no significant difference in vitamin D levels.

Conclusion : The serum vitamin D levels were lower in NAFLD patients as compared to healthy volunteers and obesity was associated as a risk factor for NAFLD and vitamin D deficiency.

KEYWORDS : Non alcoholic fatty liver disease, serum 25 (OH) D, Diabetes mellitus, Obesity, Dyslipidemia

INTRODUCTION:

Vitamin D is metabolized to its active form through two consecutive hydroxylations exerted by liver and kidney, respectively (1). Non alcoholic fatty liver disease (NAFLD) is a pathological condition consisting a spectrum of liver diseases due to macrovesicular accumulation of triglycerides within hepatocytes (hepatic steatosis) (2). Nonalcoholic fatty liver disease (NAFLD) has become the most common form of chronic liver disease in Western countries with prevalence as high as 30% (3). NAFLD represents a continuum of hepatic injuries, which progress from simple fatty liver to steatohepatitis (NASH), cirrhosis or even hepatocellular carcinoma. The metabolic syndrome is universally considered as the key factor in the pathogenesis of NAFLD (4).

Since vitamin D exerts significant metabolic activities, comprising Free Fatty Acids (FFA) flux regulation from the periphery to the liver, its deficiency may promote fat deposition into the hepatocytes (5).NAFLD has been classified as primary and secondary; primary NAFLD is usually associated with insulin resistance or metabolic syndrome, whereas secondary NAFLD is caused by intake of some drugs, surgery, or total parenteral nutrition (6).

Epidemiological studies suggest prevalence of NAFLD in around 9% to 32% of general population in India (7). In developed countries NAFLD is observed in 20-30% of general population and 75% of type 2 diabetic patients (8).Given that vitamin D deficiency (VDD) and NAFLD have both direct and indirect associations with obesity and sedentary lifestyle, it is not unexpected that VDD would coexist with NAFLD. So in this study we have tried to assess the role of Vitamin D deficiency in the pathogenesis of NAFLD (9).

AIMS AND OBJECTIVES

1. To estimate the serum vitamin D levels in patients with Non Alcoholic Fatty Liver Disease (NAFLD).
2. To find out the association if any between the Vitamin D deficiency and NAFLD.

MATERIALS AND METHODS:

The study was conducted in department of General Medicine, Himalayan Institute of Medical Sciences, Swami Ram Nagar, Dehradun within a period of 12 months. Subjects were recruited amongst the patients attending General medicine / Gastroenterology OPD and IPD with primary diagnosis of NAFLD after obtaining written informed consent. A total of 140 subjects were recruited for statistical purpose by convenient sampling method out of which 70 were NAFLD patients above the age of 18 years diagnosed by ultrasonography. All the patients with history of current or past excessive alcohol drinking as defined by an average daily consumption of alcohol >40-80g/day in men and >20g/day in women, positive serology for the presence of hepatitis B surface antigen and antibody to hepatitis C virus, presence of history, clinical and imaging findings consistent with Cirrhosis, known cases of Auto Immune Hepatitis and other known causes of chronic liver disease, chronic kidney disease or any malignancy were excluded. 70 healthy volunteers were also included in the study who were non alcoholics with no fatty liver changes on the ultrasound.

RESULTS

Table 1 : Comparison of age, BMI and serum 25 (OH) D levels in NAFLD patients and Healthy volunteers

Parameter	NAFLD patients (n=70)	Healthy volunteers (n=70)	P value
Age (years)	48.50±11.53	46.02±13.18	0.240
BMI (Kg/m^2)	24.51±3.21	22.63±2.51	0.000
Serum 25 (OH) D (nmol/L)	39.48±12.38	54.91±18.83	0.000

Unpaired t test

Table 2 : Comparison of serum 25 (OH) D levels of males versus females in NAFLD patients and Healthy volunteers

Gender	Serum 25 (OH) D (nmol/L)				P value
	Male		Female		
	No.	mean±sd	No.	mean±sd	
NAFLD patients (n=70)	41 (58.57%)	**41.55±12.65**	29 (41.43%)	**36.31±11.50**	0.081
Healthy volunteers (n=70)	50 (71.43%)	**59.59±17.77**	20 (28.57%)	**43.22±16.48**	0.001

* Unpaired t test

Table 3 : Comparison of serum 25 (OH) D amongst smoker versus non-smokers in NAFLD patients and Healthy volunteers

Smoking	Serum 25 (OH)D (nmol/L)		P value
	Smokers	Non smokers	
NAFLD patients (n=70)	37.82±11.85 (n=40 ; 57.14%)	41.46±13.28 (n=30 ; 42.86%)	0.227
Healthy volunteers (n=70)	55.60±20.25 (n=32 ; 45.71%)	54.33±17.80 (n=38 ; 54.29%)	0.780

Unpaired t test

Table 4 : Association between severity of NAFLD on ultrasonography and serum 25 (OH) D levels

Grades of NAFLD on ultrasonography	Serum 25 (OH)D (nmol/L)	
	Deficient (<25)	Insufficient (25-74)
Mild	8	45
Moderate	1	10
Severe	1	5
Chi square =0.30, p= 0.861		

Table 5: Comparison of various biochemical parameters in NAFLD patients and Healthy volunteers

Parameter	NAFLD Patients (n=70)	Healthy volunteers (n=70)	P value
Total serum Bilirubin (mg/dL)	1.30±1.10	0.98±0.27	0.018
ALT (IU/L)	47.12±36.59	23.20±10.10	0.000
AST (IU/L)	49.98±34.46	25.77±8.10	0.000
Total serum Cholesterol (mg/dL)	146.32±40	137.11±33.98	0.144
Serum HDL (mg/dL)	43.05±16.41	55.54±11.74	0.000
Serum LDL (mg/dL)	98.45±33.87	120.31±18.60	0.000
Serum Triglycerides (mg/dL)	168.04±60.39	162.91±35.71	0.542

* Unpaired t test

Table 6 : Comparison of random, fasting and post prandial plasma glucose levels in non diabetic and diabetic NAFLD patients

Parameter	NAFLD patients		P value
	Non diabetic (n=50 ; 71.4%)	Diabetic (n=20 ; 28.5%)	
RBS (mg/dl)	123.78±20.85	169.85±27.90	0.0001
FBS (mg/dl)	88.26±9.63	145.50±17.73	0.0001
PPBS (mg/dl)	119.64±14.99	229.20±35.28	0.0001

Unpaired t test

Table 7 : Mean of serum 25 (OH) D levels in non-diabetic and diabetic NAFLD patients

Parameter	Non diabetic NAFLD patients (n=50; 71.4%)	Diabetic NAFLD patients (n=20 ; 28.5%)	P value
Serum 25(OH) D	40.07±13.22	36.97±10.42	0.352

* Unpaired t

DISCUSSION

In our study the mean age of NAFLD patients was 48.50± 11.53 years and of healthy volunteers was 46.02±13.18 years.The mean BMI in NAFLD patients was 24.51±3.21 kg/m^2 and in healthy volunteers was 22.63 kg/m^2. This difference was found to be statistically significant (p=0.000) indicating that the majority of patients in NAFLD group (62.86%) were overweight or obese and 37.14% patients had normal BMI.The result was similar to the study of Amarapurkar et. al who established BMI > 25 kg/m^2 as a risk factor associated with NAFLD (10).Uchil D et. al also in a study conducted on 1003 patients concluded the BMI was higher in NAFLD group with the mean of 28.58 ± 4.25 kg/m^2 and 25.67 ± 5.05 kg/m^2 in control group (p value <0.05) (11).

The mean serum 25 (OH) D levels in NAFLD patients were 39.48±12.38 nmol/L and in healthy volunteers were 54.91±18.83 nmol/L, and the level was lower in NAFLD than healthy subjects (p=0.000). Similarly Rhee et al. also found a minor but significant difference in 25(OH)D levels between patients with and without NAFLD (38.7±9.0 vs. 39.7±9.7 nmol/L) (12).

In another study conducted by Dasarathy J et.al, plasma vitamin D concentrations were quantified in 148 consecutive biopsyproven NAFLD patients and 39 controls. The researchers found that vitamin D levels were significantly lower in NAFLD patients compared with healthy controls. Higher NAFLD activity scores were associated with lower plasma concentrations of vitamin D (13).Targher et al. studied 60 consecutive patients with NAFLD and 60 clinically healthy volunteers during the winter months. The researchers performed a liver biopsy to confirm NAFLD. They found significantly lower levels of serum 25(OH)D (51.0 nmol/L) in NAFLD patients than in controls (14).

According to our study the maximum number of patients amongst NAFLD and healthy volunteers group were males (n= 41; 58.57% and n=50; 71.43% respectively) as compared to females in both the groups (n=29; 41.43% and n=20; 28.57% respectively) (Table 2).In our study the mean serum 25 (OH) D levels in male NAFLD patients and female NAFLD patients were 41.55±12.65 nmol/L and 36.31±11.50 nmol/L respectively, the difference was found to be statistically non significant (p= 0.081) whereas in healthy volunteers the mean serum 25 (OH) D levels were significantly high (p=0.001) in males than females (59.59±17.77 nmol/L and 43.22±16.48 nmol/L) respectively. Hence our study did not show any significant association between severity of serum 25 (OH) D deficiency among both the genders in NAFLD patients (p = 0.730).Similar observation was also made by Singh et.al who studied 159 healthy subjects, fatty liver was diagnosed in 39 patients with ultrasonography, of which 26.9% were males and 13.8% were females (15). Similar study was done by Amarapurkar et. al in 1168 subjects which showed higher male preponderance (47.8%) with prevalence of NAFLD in 16.6% patients (10).

Our study shows the number of smokers were more in the NAFLD group as compared to the healthy volunteers (n=40; 57.14% and n=32; 45.71% respectively). The mean serum 25 (OH) D levels of NAFLD patients who were smokers and non smokers were 37.82± 11.85 nmol/L and 41.46±13.28 nmol/L respectively whereas in healthy volunteers the mean serum 25 (OH) D levels were 55.60±20.25 nmol/L in smokers and 54.33±17.80 nmol/L in non smokers. This difference was found to be statistically non significant when compared between smokers and non smokers in NAFLD patients and healthy volunteers.Kassai EN et.al, found a strong correlation between 25(OH)D and smoking on 181 male patients. The study concluded that a young smoker (20-29 years) had 58% increased likelihood of having vitamin D deficiency compared to a non-smoker of the same age group (p=0.041) (16).

According to Saverymuttu et. al Liver steatosis was scored semi quantitatively on ultrasonography on a scale of 0-3 ; 0,absent ; 1,mild ; 2,moderate ; 3, severe. They evaluated 85 patients amongst which 48 patients (56.4%) showed steatosis ranging from mild to severe and fibrosis in 35 patients (41.1%) (17).However out study did not find any significant association between severity of NAFLD and serum 25 (OH) D deficiency (p=0.861).

Similar results were also observed by Metin Kucukazman et. al who evaluated 211 patients dividing them into two groups, NAFLD group had 154 subjects and the control group included 57 subjects. In this study significantly lower vitamin D levels (12.3±8.9 ng/dl, p<0.001) were achieved in NAFLD group with gradual decline but statistically insignificant mean of 25 (OH) D levels as the grade of NAFLD increased (18).

According to the study done by Dhiman RK et. al NASH and chronic viral hepatitis were the most common causes of asymptomatic rise in hepatic transaminases (19). Serum transaminase levels have a poor correlation with histological severity in patients with NAFLD. Patients with raised ALT levels may not have histological NASH, and normal ALT levels do not preclude severe disease. This was proved by Amarapurkar et al. who found histological evidence of cirrhosis in 23% of their 25 patients with NASH and normal ALT levels (20).

But our study showed the mean levels of ALT were significantly higher in NAFLD patients (47.12±36.59 IU/L) as compared to the healthy volunteers which were 23.20±10.10 IU/L (p=0.0001). Similarly

Deepak A et. al who evaluated 193 patients of NAFLD and found 16 percent had ALT more than the upper limit of normal and 9 percent had AST the upper limit of normal (21).In our study the AST levels in NAFLD patients were 49.98±34.46 IU/L compared to 25.77±8.10 IU/L in healthy volunteers which was highly significant (p=<0.0001). Total serum bilirubin levels were significantly higher statistically in NAFLD patients compared to healthy volunteers (p=0.018).

Also mean of random plasma glucose, fasting plasma glucose and post prandial plasma glucose were significantly high in both diabetic and non diabetic NAFLD patients (p=0.0001). No statistical significance between the mean serum 25 (OH) D levels amongst diabetic and non diabetic NAFLD patients was found. Hence our study established that raised plasma glucose levels could also be an independent risk factor for the development of NAFLD. However serum 25 (OH) D levels amongst the diabetic and non diabetic NAFLD patients were not significantly altered.

Similar observation was seen by Uchil D et. al who on 1003 patients of NAFLD found levels of fasting blood glucose and post prandial blood glucose were more in NAFLD group as compared to control group (p=<0.05 and <0.05 respectively) (11).BarchettaI et. al also concluded that patients affected by type II diabetes mellitus had serum 25 (OH) D levels 17±10.2 ng/ml similar to the non diabetic patients 17.5±8.8 ng/ml which was found to be statistically non significant (1).

According to our study the mean serum HDL was significantly low in the NAFLD patients compared to healthy volunteers (p=0.0001) but no significant association was established between low HDL and serum 25(OH)D levels in patients of NAFLD. According to Barchetta I et. al patients with NAFLD (n=162; 61.8%) had reduced serum 25 (OH) vitamin D levels compared to subjects without NAFLD (14.8 ± 9.2 vs 20.5 ± 9.7 ng/ml, p < 0.001, OR 0.95, IC 95% 0.92 – 0.98). The relationship between NAFLD and reduced 25 (OH) D levels was independent from age, sex, triglycerides and high density lipoproteins (HDL).Triglycerides, HDL, LDL were found to be an independent factor for lower levels of 25(OH) D proving the further need to study these parameters for future reference (1).

CONCLUSION

- Our study showed male preponderance in NAFLD patients and healthy volunteers.
- This study did not show any significant difference in serum 25 (OH) D levels between male and female NAFLD patients.
- Our study established obesity (higher BMI >23 Kg/m^2) as a risk factor for NAFLD.
- NAFLD patients had significantly low serum 25 (OH) levels as compared to controls.
- No statistical significance on serum 25 (OH) D deficiency amongst NAFLD patients was observed between smokers and non-smokers in NAFLD patients and healthy volunteers.
- Also no significant association was found between severity of NAFLD grading on ultrasonography and serum 25 (OH) D deficiency.
- The serum HDL and LDL was significantly low in NAFLD patients as compared to healthy volunteers.
- RBS, FBS and PPBS in NAFLD patients was higher when compared to healthy controls and was found to be statistically significant.
- The mean of serum 25 (OH) D levels was not statistically altered when compared between diabetic and non diabetic NAFLD patients.

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