ORIGINAL RESEARCH PAPER

Gastroenterology

"PREVALENCE OF VITAMIN A DEFICIENCY (VAD) IN NON-ALCOHOLIC CHRONIC LIVER DISEASE (CLD) PATIENTS AND COMPARE IT WITH HEALTHY CONTROLS"

KEY WORDS: Chronic liver disease; Vitamin A deficiency; Night blindness; Serum Retinol

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ABSTRACT

Background- Liver plays an important role in vitamin A homeostasis and few previous studies have shown Vitamin A deficiency (VAD) in patients of CLD. The aim of the present study was to find the prevalence of VAD in non-alcoholic chronic liver disease (CLD) patients and compare it with healthy controls. We also studied the relationship between Vitamin A level with disease severity and duration. **Methodology-** This was a Case Control cross sectional study conducted on forty five patients of liver cirrhosis. Detailed clinical history, physical examination and blood investigations were done. The severity of CLD was assessed by Child Turcotte Pugh (CTP) score and Model for End stage Liver Diseases (MELD) score. Samples for estimation of serum Vitamin A levels were collected and Serum Vitamin A Levels were measured using commercially available ELISA kit. Vitamin A deficiency (VAD) was defined if serum vitamin A levels were < 30 µg/dl **Results-** Forty five patients with CLD were enrolled. Mean serum vitamin A level was lower in cases as compare to controls (28.96 ± 9.63 vs 62.36 ± 42.19µg/dl, p = 0.001). Mean serum bilirubin level and total duration of illness was higher in patients with VAD as compare to those with normal serum Vitamin A levels, ($p < 0.05$). Serum vitamin A levels in CHILD group A, B and C were 36.33±5.78, 30.00± 8.83 and 25.88±10.71 µg/dl respectively, there was a trend towards decrease in serum vitamin A levels with increase in Child Class (p value=0.16), statistically not significant. Patients with VAD had higher MELD score as compare to those without VAD, (p value=0.004). **Conclusion-** Patients with cirrhosis had a high prevalence of VAD (prevalence was 48.89% in present study). Cirrhotic patients with VAD had more severe disease with higher MELD score. Patients with longer duration of illness had severe VAD.

INTRODUCTION

Chronic liver disease (CLD) is a significant cause of mortality and morbidity throughout the world. Liver diseases are recognized as the second leading cause of mortality amongst all digestive diseases in the United States.[1] In India, prevalence of CLD varies from 8.7 to 32%.[2] Mortality data is most often used to assess the disease burden and there had been a 46% increase in CLD mortality in the world between 1980 to 2013. Vitamin A deficiency (VAD) is one of the world's great malnutrition problems. Vitamin A and its retinoid derivatives are essential for physiological functions, including vision, cellular proliferation and differentiation and immune system activity. Night blindness (NB) is a disorder frequently caused by VAD and is defined as diminished vision in individuals with normal daytime vision.[3] The liver plays a central role in the uptake, storage and also the oxidation site of vitamin A catabolism and responsible for the regulated release of this vitamin to other tissues.[4] Dietary intake is the exclusive source of vitamin A in humans.[5] Around 50-90% of the retinol ingested is absorbed via the lymphatics and carried by chylomicrons and chylomicron remnants, as retinyl esters, to the liver and taken up by the parenchymal cells. 6 Notably 60-95% of vitamin A is stored in the liver, while only minor fractions are stored in the extrahepatic tissues, and under normal conditions Stellate cells contain about 90% of hepatic retinol.[7]

The decrease in serum levels of retinol is frequently found in patients with liver cirrhosis and it can be explained by the reduced hepatic vitamin storage, synthesis and/or diminished release of binding proteins by the liver.[8] Another factor could be due to deficient enzymatic conversion of beta-carotene into retinol, which also occurs in liver.[9] Other factors such as chronic inflammation and infection, which are a part of clinical manifestations of liver cirrhosis, are also responsible for the reduction of the serum levels of Retinol due to reduction in the synthesis and release of retinol binding proteins.[10,11] The decrease in serum levels of Vitamin A can be aggravated by the degree of severity of liver disease. There was a progressive drop in serum retinol levels according to the severity of liver disease, and a greater prevalence of severe vitamin A deficiency was noted in Child class C cirrhosis.[12]

Considering the importance of VAD in CLD characteristics and progression, the aim of the present study was to find the prevalence of VAD amongst non-alcoholic CLD patients and compare it with healthy controls. The relationship of serum retinol levels with disease severity and duration were also studied.

MATERIALS AND METHODS

Experimental methods

This was a Case Control cross sectional study conducted on forty five patients of liver cirrhosis attending Gastroenterology OPD or emergency department of SAMC & PGI, Indore over a period of 18 months from Feb 2017 to Sep 2018. Institutional ethics committee approval was taken and written consent was obtained from all patients. Forty five age and sex matched healthy controls were also included in the study. All patients of chronic liver disease, ≥18yrs those who were willing to give informed written consent for study were included. Patients under 18 years of age, with any kind of addictions e.g. alcohol; smoking; tobacco chewing; those with

any active source of infection or sepsis in the body; those with any kind of malignancy, renal disease and those with history of any kind of drugs that potentially has any effect on vitamin A metabolism were excluded from the study. We excluded patients with alcoholic liver disease as these patients are associated with reduction in the concentration of vitamin A in liver even though the concentration of serum retinol, retinol binding protein and transthyretin are normal. [13, 14] As a result the serum retinol level may not reflect the exact vitamin A status in alcoholic subjects with or without liver disease since serum retinol level is kept nearly constant until vitamin A stores are nearly exhausted in liver. [15]

Assessment Of Liver Function

Detailed clinical history and physical examination was done, including complete blood count, serum bilirubin, aminotransferases, prothrombin time, serum Albumin, serum globulin, serum urea, serum creatinine, serum electrolytes, viral markers (HIV, HBsAg, Anti-HCV) and Ascitic fluid analysis. Cirrhosis of liver with portal hypertension was diagnosed on the basis of standard clinical features (history of any decompensation), biochemical alterations (raised bilirubin, aminotransferases, INR, reversal of AG ratio), Radiological evidences (shrunken liver, dilated portal vein with periportal or other collaterals), and endoscopic evidences (presence of esophageal/gastric/ectopic varices and or portal hypertensive gastropathy). [16] The severity of CLD was assessed by Child Turcotte Pugh (CTP) score and Model for End Stage Liver Disease (MELD) score.

- Evaluation of vitamin A nutritional status
- Evaluated by functional indicator i.e. Night Blindness and biochemical indicator i.e. serum retinol concentration.
- Assessment of Night blindness

The functional indicator, NB was assessed by a standardized questionnaire which contained set of 4 questions. [17]

1) Do you have any difficulty seeing objects at late dusk or early dawn?
2) Do you experience difficulty seeing objects in a room light by a weak candle, lantern or other light?
3) Do you have difficulty seeing objects when driving a car at dusk or night?
4) Do you have difficulty with vision adaptation in a dimly lit room?

Cases were defined as subjects who answered YES to 2 out of 4 questions.

Assessment of Serum Retinol

Samples for estimation of serum Vitamin A levels were collected after overnight fasting (of minimum 8 hours) in a plain tube (red colored vial). Serum from the samples was segregated and stored in a deep freezer with temperature of minus 4 degree centigrade. Serum Vitamin A Levels were measured using commercially available ELISA kit (Qayee-bio for life Science, Shanghai, China). Prepared sample, standard and HRP (Horseradish peroxidase)-Conjugate Reagent were incubated for 60 minutes. Plate was washed five times and Chromogen Solution A and B was added and incubated for 10 minutes at 37□C.After this stop solution was added. Measurement was made by ELISA reader within 15 minutes and calculation was done. Vitamin A deficiency was defined if serum vitamin A levels were < 30 µg/dl. [18]

Statistical Analyses

Data was presented as mean and standard deviation for Continuous variables and number and percentages for discrete variables. Normality of data was checked by Kolmogorov smirnov test. Student t test and Mann Whitney U test were applied to see the significant difference in mean and median of continuous data for parametric and non-parametric data respectively. Chi Square test was used to see the significant difference in frequency of discrete variables between two groups. SPEARMAN Correlation test was done to see the correlation of Vitamin A levels with MELD score. Multivariate logistic regressions were applied to see the independent associated factors for CLD. P value <0.05 was considered significant.

RESULTS

A total of 45 patients with CLD were enrolled and 22 (48.9 %) were males and 23 (51.1 %) were females with a mean age of 48.64±14.32 years, mean height was 1.44±0.07 meters, mean body weight was 55.69±7.04 Kilograms and mean BMI of 26.88±2.71 kg/m2. Mean serum vitamin A level in cases was 28.96 ± 9.63 µg/dl vs 62.36 ± 42.19 µg/dl in control group, (p = 0.001). [figure1]

Patients having night blindness had mean serum vitamin A levels of 23.73 ±8.70 µg/dl vs 33.96±7.74 µg/dl ,those without having night blindness (p <0.001). [figure2]. Mean serum bilirubin level and total duration of illness was higher in patients with VAD as compare to those with normal serum Vitamin A levels (p < 0.05).

In the present study, the frequency of VAD was 48.89% .Serum vitamin A levels in CHILD group A, B and C were 36.33±5.78, 30.00± 8.83and 25.88±10.71 µg/dl respectively. A Trend towards progressive decrease of Vitamin A levels with increase in Child Class was observed, however it was not statistically significant (p=0.16). [Figure3].MELD score was significantly (p = 0.004) elevated amongst those with vitamin A deficiency (14.86±4.25) compared to those with normal vitamin A levels (13.29±2.27). [Figure4]

On studying various laboratory and clinical parameters of chronic liver disease few variables found statistically significant between those with Vitamin A deficiency and those with normal vitamin A levels. [Table1].Vitamin A deficiency was not associated with etiology of CLD in our cohort of nonalcoholic CLD patients.

DISCUSSION

The present study aimed to investigate the prevalence of vitamin A deficiency (VAD) in patients with chronic liver disease (CLD), and its correlation with disease characteristics and measures of disease severity. The study found a high prevalence of VAD in CLD patients, with no significant association of serum vitamin A levels with the etiology of CLD. However, a decreasing trend in serum vitamin A levels was observed with increasing severity of CLD as suggested by CHILD scores, although this trend was not statistically significant. On the other hand, MELD score was found to be significantly higher in those with serum vitamin A deficiency compared to those with normal serum vitamin A levels. (p < 0.05). Moreover, patients with night blindness were found to have significantly lower serum vitamin A levels than those without night blindness. (p= < 0.05). In addition, the study found that serum bilirubin levels and total duration of illness were significantly higher (p < 0.05) in patients with serum vitamin A deficiency compared to those with normal serum vitamin A levels.

Our study endeavors to investigate the nutritional status of vitamin A according to biochemical and functional indicators in CLD patients and assessed its correlation with disease characteristics and measures of disease severity, CTP and MELD. The study revealed a prevalence of VAD among CLD patients of 48.89%, which is in agreement with earlier research. [19] Moreover, our findings corroborated prior investigations, demonstrating a declining trend in serum vitamin A levels with escalating severity of CLD, as indicated by CHILD score and MELD. The decrease in serum vitamin A levels in CLD patients may be due to factors such as decreased hepatic storage capacity (due to loss of vitamin A storing cells) and decreased hepatic vitamin A uptake, as well as protein-calorie malnutrition, which can impair the synthesis of retinol-binding protein essential for the release

of retinol from the liver to serum. [20]. It should be noted that our study did not measure retinol-binding protein levels. The observed vitamin A deficiency in cirrhosis patients may stem from insufficient intake of dietary vitamin A, which typically exists in the form of retinyl esters or carotene rather than preformed retinol. Furthermore, impaired intestinal absorption of vitamin A may occur in cirrhosis patients due to disruptions in lipolysis and emulsification processes. [21]Serum retinol is subject to homeostatic regulation, whereby the depletion of hepatic vitamin A stores may occur to sustain adequate serum retinol levels in instances of inadequate dietary vitamin A intake.

In our study, a questionnaire was utilized to evaluate the functional status of vitamin A, which revealed a prevalence of 48.9% for Night Blindness (NB) among the sample population. [22] This manifestation of vitamin A deficiency is significant as it can be detected early on, potentially even before a decrease in serum vitamin A levels occurs. The present study demonstrated a significant elevation in mean serum total bilirubin levels among individuals with retinol deficiency compared to those with normal vitamin A levels (2.24±1.34 vs 1.83±0.76 mg/dl; $p < 0.05$), which is in agreement with a previous study. [23] Additionally, our findings indicated that the mean total duration of illness (in months) was significantly longer in individuals with retinol deficiency compared to those with normal vitamin A levels (25.29±26.85 vs 12.46±23.14 months; $p < 0.05$). To the best of our knowledge, no previous studies have reported such a correlation. These results may suggest the progressive depletion of retinol-storing cells from the liver due to fibrosis as a component of the disease process.

Several limitations were identified in our study. Firstly, the sample size was relatively small, and the study was solely financed by the researchers. Secondly, we only measured serum retinol levels and did not evaluate hepatic retinol levels, which may have yielded divergent outcomes. Notably, previous research has demonstrated a substantial negative correlation between hepatic retinol levels and the histological classification of the disease, which was not observed for serum retinol levels. The histological classification included mild and severe steatosis, nonalcoholic steatohepatitis (NASH), and hepatocyte necrosis. [24]

In conclusion, our study corroborates previous research indicating that individuals with cirrhosis exhibit diminished serum retinol levels. Additionally, our study revealed a novel association between longer illness duration and severe vitamin A deficiency. Given these findings, it is imperative that serum retinol levels not be the sole criterion for determining the necessity of vitamin A supplementation. Further investigations, employing larger sample sizes or alternative sampling techniques, are warranted to elucidate the role of serum vitamin A in chronic liver disease.

Abbreviations: CLD: Chronic liver disease; VAD: Vitamin A deficiency; NB: Night blindness; CTP: Child Turcotte Pugh; MELD: Model for End stage Liver Diseases; NASH: Nonalcoholic Steatohepatitis;

Table 1: Baseline Characteristics Of Patient

Parameters	Vitmian A Deficiency (Vad)	Normal Vitamin A	P Value
HB (gm/dl)	9.67±3.09	9.04±2.56	0.42
TLC (/cumm)	5528.57±2901.23	5862.50±3007.93	0.37
PLATELETS (/cumm)	93428.57±77955.48	110666.67±69773.34	1.0
MCV	83.86±12.86	83.00±10.60	0.82
UREA (mg/dl)	27.57±11.70	28.13±2.29	0.23
CREATININE (mg/dl)	0.86±0.36	0.92±0.82	0.22
SODIUM (meq/l)	135.29±7.36	139.00±7.78	0.69
BILIRUBIN TOTAL (mg/dl)	2.24±1.34	1.83±0.76	0.01
SGOT (IU/ml)	54.52±22.65	72.67±46.07	0.03
SGPT (IU/ml)	50.19±28.75	57.17±24.07	0.42
SAP	132.14±57.74	135.88±45.58	0.83
S. PROTEIN-TOTAL (gm/dl)	6.19±0.81	6.42±0.97	0.29
S. ALBUMIN	2.76±0.54	2.75±0.73	0.06
S. GLOBULIN	3.71±0.78	3.75±0.60	0.23
A/G	1.00±0.32	0.92±0.28	0.47
INR	2.10±0.63	1.79±0.51	0.76
Total Duration Of Illness (TDI)- Months	25.29±26.85	12.46±23.14	0.02

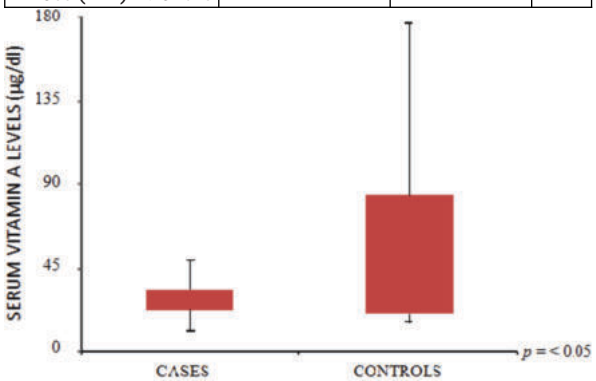

Figure 1: Serum vitamin A levels in cases and controls

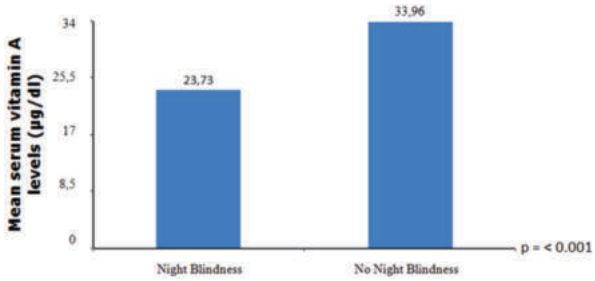

Figure 2: Mean serum vitamin A levels (µg/dl) according to night blindness

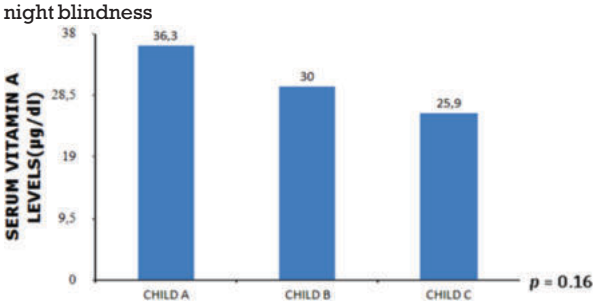

Figure 3: Serum vitamin A levels (µg/ dl) according to CTP class

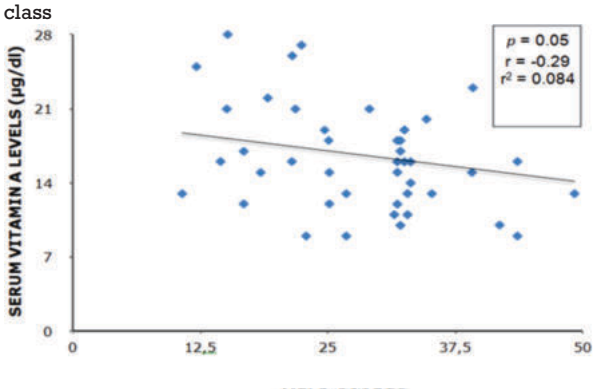

Figure 4: Mean serum Vitamin A levels according to MELD score

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