Vitamin A, C and E levels in patients with Non-Alcoholic Fatty Liver Disease: An Indian Cohort Study

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This study aimed to investigate the levels of vitamins A, C, and E in patients with Non-Alcoholic Fatty Liver Disease (NAFLD) compared to healthy controls and to explore the correlation between these vitamin levels and various other parameters, including bone mineral density (BMD), adiposity (fat storage), insulin resistance and subclinical inflammation. The study involved 50 participants diagnosed with NAFLD and 50 healthy controls. Blood samples were collected to measure vitamin A, C and E levels, along with other parameters like insulin, inflammatory markers, and liver function tests. Additionally, participants underwent DEXA scans to assess BMD and body composition. Vitamin levels: The study found no significant deficiencies in vitamin A or C levels in either group. However, vitamin E levels were significantly higher in the NAFLD group compared to controls, although only one case of vitamin E deficiency was observed in the NAFLD group. No significant correlations were found between vitamin levels and BMD, adiposity parameters, insulin resistance, or subclinical inflammation markers in either group. The study acknowledges the limited data available on the association between NAFLD, vitamin levels and BMD in the Asian Indian population. The findings regarding vitamin A and C levels are consistent with some previous studies, whereas the higher vitamin E levels in the NAFLD group contradict other research. This discrepancy might be due to factors like sample size, dietary habits, or vitamin fortification programs. The lack of significant correlations between vitamin levels and other parameters suggests that further research is needed to understand the complex interplay between these factors in NAFLD development and progression.

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Key words: Vitamin A, C and E levels, Non-Alcoholic Fatty Liver Disease (NAFLD), Vitamin A, C and E correlations

Introduction

Low bone mineral density (BMD) has emerged as a significant health concern among individuals afflicted with Metabolic Syndrome (MS), affecting both men and women. Preliminary findings indicate a potential link between Non-Alcoholic Fatty Liver Disease (NAFLD) and an elevated susceptibility to osteoporotic fractures. This association may be attributed to chronic low-grade inflammation, concurrent vitamin D deficiency, and restricted physical activity. Disturbances in circulating markers of bone metabolism, including osteopontin, osteoprotegerin, osteocalcin and fetuin-A, have been observed in patients with NAFLD[1]. The role of antioxidants, such as Vitamin A, C, and E, assumes paramount importance in mitigating free radical-induced hepatic injury associated with fatty infiltration. Investigations have revealed that individuals with metabolic syndrome and NAFLD exhibit deficiencies in vitamin C and E compared to those without NAFLD[2]. Therefore, this study seeks to comprehensively evaluate bone mineral density, insulin levels, subclinical inflammation markers and levels of vitamins A, C and E.

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A comparative analysis of these parameters will be undertaken between subjects with and without NAFLD.

Vitamins A, C, E and NAFLD

Vitamin C, a water-soluble vitamin, serves as a crucial donor of reducing equivalents in various enzymatic reactions, along with Vitamin A and E[3]. Humans are reliant on dietary intake for Vitamin C, with deficiency defined as plasma levels below 23 µM[4]. Studies in Western populations have reported a prevalence of vitamin C deficiency ranging from 10.0% to 20.0%, correlating with increased all-cause mortality[5]. Notably, Vitamin C deficiency is more pronounced in obese individuals compared to their lean counterparts, establishing a potential link between obesity, NAFLD and oxidative stress[6].

A study based on NHANES III survey data linked metabolic syndrome to decreased levels of Vit C and other antioxidants like Vitamin E[7]. Furthermore, Vitamin C has shown an inverse association with inflammatory markers such as C-reactive protein (CRP) and myeloperoxidase[8]. Daily supplementation of 1 gm of Vitamin C has demonstrated positive effects on metabolic parameters, including HbA1c, fasting blood sugar, serum insulin, triglycerides and low-density lipoprotein[9].

In India, the literature on NAFLD and its natural history remains limited. Population-based studies indicate a prevalence of NAFLD ranging from 8.7% to 32.0%[10]. Despite being a significant hepatic component of the metabolic syndrome, there is a dearth of data regarding the relationship between NAFLD and vitamin levels, as well as BMD measured by DEXA scan, in Asian Indian patients. This study aims to address this gap by comparing blood levels of vitamins A, C and E in the two groups and evaluating their correlation with BMD, adiposity, insulin resistance and subclinical inflammation.

Methods

A cross-sectional study was conducted, involving a total of 100 participants, with 50 cases diagnosed with Non-Alcoholic Fatty Liver Disease (NAFLD) and 50 control subjects.

Patients diagnosed with NAFLD based on trans-abdominal ultrasonography were recruited from the Medicine Outpatient Department (OPD) at AIIMS. Inclusion criteria included subjects aged between 18-50 years, diagnosed with fatty liver on ultrasound, and a willingness to participate in the study. Exclusion criteria encompassed daily alcohol consumption exceeding 20 gm/day or 140 gm/week, intake of drugs known to induce fatty liver, severe end-organ dysfunction, HIV/HBV/HCV infection, pregnancy, and severe acute or chronic illnesses.

After obtaining written informed consent and applying the exclusion criteria, patients with NAFLD were recruited from the Medicine OPD at AIIMS. The recruited subjects underwent anthropometric and biochemical parameter measurements, abdominal ultrasound for grading fatty liver, dual-energy X-ray absorptiometry (DEXA) scan, and bioimpedance analysis.

Clinical History and Examination:

Detailed clinical history, including past illness, drug intake, and family history, was obtained using a predefined Performa. A thorough examination, including assessment for signs of metabolic syndrome such as acanthosis nigricans, double chin, buffalo hump, and xanthelasma, was conducted.

Serum retinol and tocopherol analysis

a) *Equipment used*: Shimadzu LC-6AD Binary Gradient System

b) *Reagents/chemicals used*: Retinyl Acetate Standard, Retinol Standard, n-Hexane, Methanol, Ethanol, Water

c. Chromatographic conditions: i) Column: Supelco C-18, 10 microns; ii) Flow rate: 1.2 ml per minute; iii) Pressure: 80-130 kgf; iv) Detector wavelength: 326 nm for 0-10 minutes, 290 nm for 10-20 minutes; v) Mobile phase: Methanol: water (95:5, v/v); vi) Internal standard: Retinyl acetate (RAC); vii) Injection volume: 20 µl and viii) Retention times: Retinol (4.4 minutes), Retinyl Acetate (6.1 minutes), Tocopherol (13.0 minutes).

d. Analysis: The method involved the extraction of retinol from serum, followed by HPLC analysis using C-18 column and UV-detector. Concentrations were calculated, and values were adjusted for the recovery factor.

Plasma ascorbic acid analysis: i) Equipments Used: Spectrophotometer, Analytical Weighing Balance; ii) Reagents/Chemicals Used: Distilled Water, Metaphosphoric Acid, Sulfuric Acid, 2,4 Dinitrophenyl Hydrazine Reagent, Thiourea, Anhydrous Copper Sulfate, Ascorbic Acid and iii) Analysis: Ascorbic acid in plasma was oxidized by Cu2+ to form dehydroascorbic acid, which reacted with acidic 2,4-dinitrophenyl hydrazine to

form a red bis-hydrazone, measured at 520 nm. Calibration curves were prepared using ascorbic acid calibrators. Concentrations of samples were obtained from the calibration curve and multiplied by 5 to correct for plasma dilution by metaphosphoric acid.

Parameters and reference ranges: i) Retinol (µg/dl): <20 (Pee and Dary, 2002); ii) Tocopherol (mg/dl): <0.5 (Centre for Disease Control, 2008) and iii) Ascorbic Acid (mg/dl): At risk of deficiency: 0.2-0.49; Deficiency: <0.2 (Donald et al. 1994).

Results

Vitamin levels were found to be adequate in all patients except for one case of vitamin E deficiency in the NAFLD arm. However, patients with NAFLD had significantly higher mean values compared with non NAFLD arms as shown in Table I.

Table I: Antioxidants in cases and controls

Parameters	Cases	Controls	p value
	Mean±SD	Mean±SD	
Vitamin-A	49.45±10.8	43.06±10.98	0.005
Vitamin- C	0.84±0.39	0.79±0.66	0.016
Vitamin-E	1.55±0.51	1.29±0.25	0.002

Table II: The correlation of the adiposity parameters with vitamin-A was not significant in cases and controls. Vitamin-A levels showed no correlation with T.BMD, T.BMC and T-score. The correlation of insulin resistance, HOMA-IR, hs-CRP with vitamin-A was not statistically significant.

Table II: Correlation vitamin A with of adiposity, DEXA parameters, insulin and hs-CRP in cases and controls

Variables	Cases (n=50)		Controls (n=50)	
	Pearson's coefficient	p value	Pearson's coefficient	p value
BMI	0.185	0.199	0.120	0.406
WC	0.016	0.195	0.186	0.195
HC	0.084	0.560	0.116	0.422
W/H ratio	-0.141	0.330	0.136	0.346
Biceps-skin fold thickness	-0.080	0.581	-0.083	0.566
Triceps-skin fold thickness	-0.092	0.525	0.265	0.063
Sub-scapular-skin fold thickness	-0.085	0.556	0.296	0.037
Suprailliac-skin fold thickness	-0.171	0.236	0.097	0.504
T-BMD	-0.151	0.296	0.063	0.664
T-Score (Bone)	0.151	0.294	-0.014	0.921
Fasting insulin	0.118	0.192	0.100	0.490
HOMA-IR	0.189	0.188	0.111	0.444
Hs-CRP	0.036	0.805	0.221	0.123

Table III shows the correlation of vitamin C levels with adiposity parameters, BMD, fasting insulin and hs-CRP. In both cases and controls, no significant correlation of vitamin C levels was observed with adiposity parameters (BMI, WC, HC, WHR and skin fold thickness). Similarly, no significant correlation was observed between vitamin C levels and bone mineral density. Correlation of the vitamin C with insulin and sub clinical inflammatory markers was not significant in the cases and controls.

Table III: Correlation of adiposity, DEXA parameters, insulin and hs-CRP with vitamin C in cases and controls

Variables	Cases (n=50)		Controls (n=50)	
	Pearson's coefficient	p-value	Pearson's coefficient	p-value
BMI	-0.096	0.506	0.092	0.525
WC	0.195	0.175	0.018	0.903
HC	0.183	0.203	-0.036	0.804
WHR	-0.091	0.532	0.158	0.272
Biceps-skin fold thickness	-0.082	0.574	0.244	0.087
Triceps-skin fold thickness	-0.100	0.491	0.188	0.192
Subscapular-skin fold thickness	-0.142	0.327	0.020	0.891
Suprailliac-skin fold thickness	0.006	0.966	0.015	0.920
T-BMD	0.082	0.572	-0.110	0.447
T-Score (Bone)	-0.150	-0.300	0.082	0.571
Fasting insulin	-0.100	0.491	-0.008	0.954
Hs-CRP	-0.069	0.636	0.260	0.068

Table IV shows the correlation of vitamin E levels with adiposity parameters, BMD, fasting insulin and hs-CRP. In both cases and controls, no significant correlation of vitamin E levels was observed with adiposity parameters (BMI, WC, HC, WHR and skin fold thickness). Similarly, no significant correlation was observed between vitamin E levels and bone mineral density. Correlation of the vitamin E with insulin and sub clinical inflammatory markers was not significant in the cases and controls.

Table IV: Correlation of adiposity, DEXA parameters, insulin and hs-CRP with vitamin E in cases and controls

Variables	Cases (n=50)		Controls (n=50)	
	Pearson's coefficient	p-value	Pearson's coefficient	p-value
BMI	-0.036	0.801	0.139	0.334
WC	0.195	0.175	0.168	0.245
HC	0.205	0.153	0.121	0.402
WHR	-0.053	0.716	0.083	0.569
Biceps-skinfold thickness	-0.011	0.942	0.047	0.745
Triceps-skinfold thickness	0.083	0.565	0.101	0.483
Subscapular-skinfold thickness	0.129	0.372	0.110	0.447
Suprailliac-skinfold thickness	0.004	0.975	-0.010	0.945
T.BMC	-0.120	0.406	0.103	0.478
T.BMD	-0.021	0.883	0.001	0.993
T-Score (Bone)	0.023	0.875	0.066	0.650
Z-Score (Bone)	0.008	0.955	-0.117	0.007
Fasting insulin	0.090	0.533	0.272	0.056
HOMA-IR	0.097	0.501	0.297	0.037
Hs-CRP	-0.169	0.240	0.178	0.217

Discussion

This study investigated the levels of Vitamins A, C and E in patients with Non-Alcoholic Fatty Liver Disease (NAFLD) compared to healthy controls. It was found that the absolute levels of these vitamins were significantly higher in the NAFLD group, although only one case of vitamin E deficiency was observed.

Vitamin A Levels

Our findings regarding vitamin A differ from some previous studies. Chaves et al. reported a vitamin A deficiency prevalence of 11.3% in morbidly obese patients, with a correlation observed between deficiency and total bilirubin and albumin levels[11]. Conversely, Souza et al. found an association between inadequate serum retinol levels and an increased trend of steatosis, although this association was not statistically significant[12]. Interestingly, Ford et al. based on NHANES III data discovered significantly elevated vitamin A levels in both metabolic syndrome patients and non-syndrome controls[13]. However, after adjusting for elevated lipids, the association between vitamin A and metabolic syndrome became non-significant in their study[13]. The observed differences in vitamin A levels across studies may be due to several factors. Our study population included fewer obese individuals compared to Chaves et al.[11]. Additionally, vitamin A fortification programs have become more prevalent in recent years, which could contribute to the absence of vitamin A deficiency in this cohort.

Vitamin C Levels

This study findings regarding vitamin C align with previous research. Da Silva et al. reported similar vitamin C levels in both NAFLD and non-NAFLD groups, regardless of obesity status, and observed a correlation with dietary vitamin C intake[14]. Similarly, a study conducted at our hospital by Madan et al. in 2006 found no significant difference in vitamin C levels between NAFLD cases and controls ($p=0.65$) and no correlation with the grade of NAFLD ($p=0.53$)[15].

Vitamin E Levels

Similar to vitamin C, this study's vitamin E findings were consistent with some previous studies. While Ford et al. reported higher absolute vitamin E levels in the metabolic syndrome group, similar to our findings, their analysis after correcting for elevated lipids revealed an inverse correlation between vitamin E and metabolic syndrome[13]. Conversely, Mager et al. observed decreased vitamin E levels in their NAFLD group[16]. These discrepancies in vitamin E findings may be attributed to differences in study populations, dietary habits, and the presence of other confounding factors.

Conclusion

This study contributes valuable insights into the complex relationship between NAFLD and vitamin levels. While our findings suggest higher absolute vitamin levels in the NAFLD group compared to controls, further research is necessary to elucidate the underlying mechanisms and potential causal relationships. Future investigations with larger sample sizes and detailed dietary assessments are warranted to gain a more comprehensive understanding of this intricate association.

Unique features of our study

There is very limited data on the association of NAFLD with BMD and vitamins A, C and E in Asian Indian population. We performed DEXA scan (which is considered to be most appropriate method) to determine percentage body fat and BMD in our study. There is only one other study in Asian Indians which assessed BMD with NAFLD by DEXA scan at hip and lumbar spine. We also performed direct estimations of blood levels of vitamins A, C and E in patients with and without NAFLD.

Limitations of the study

The sample size of the study was relatively small; it was because of limited time period. Ultrasonography was used to diagnose NAFLD instead of liver biopsy. Therefore it was not possible to determine the exact histological stage of the disease. We used fasting insulin levels and HOMA-IR as surrogate markers of insulin resistance instead of the gold standard (hyper-insulinemic euglycemic clamp study). Vitamin intake by dietary methods was not accounted for in our study and data on physical activity was not taken into account.

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