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Research Article

ASSOCIATION BETWEEN DYSLIPEDEMIA, VITAMIN D DEFICIENCY AND CALCIUM METABOLISM IN β-THALASSEMIA PATIENTS IN PUBERTAL AND POSTPUBERTAL YOUNG ADULTS

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ABSTRACT

Introduction: Thalassemia is a hematopoetic disorder which leads to rapid haemolysis and anaemia. As per recommended treatment, regular blood transfusions lead to iron accumulation in different vital organs followed by oxidative damage and metabolic derangement. Although, there is evidence of oxidative stress induced dyslipidemia, vitamin D deficiency along with altered calcium homeostasis in thalassemic albeit without any significant correlation between them.

Aims and objectives: In the present study we focussed on these incongruent observations and hypothesized that apart from the oxidative stress due to iron overload, lipid dysfunction acts also as one of the major cause of significant vitamin D deficiency in pubertal and post-pubertal (above 14 yrs) age group of thalassemic patients.

Material and methods: 50 diagnosed cases of beta-thalassemia and 50 age and sex matched control subjects selected. Serum ferritin, vitamin D, total cholesterol, LDL was estimated by standard laboratory procedure.

Result and analysis: Vitamin D, total cholesterol, LDL were found significantly lower (P value <0.001) in cases than controls. Multiple linear regression analysis showed vitamin D level was significantly decreased only with serum LDL (regression coefficient β =0.505, *P* =0.049).

Conclusion: Vitamin D synthesis is directly depend on cholesterol level inside the tissues, decreased levels of cholesterol within tissues in thalassemic subjects further complicate the cholesterol mediated vitamin D synthesis. Synthesis of 7- dehydrocholesterol, precursor molecule of vitamin D is also reduced due to oxidative damage leading to insufficient vitamin D level in thalassemic subject.

INTRODUCTION

Thalassemia is a heterogenous hematopoetic disorder characterized by decreased synthesis of adult haemoglobin HbA ($\alpha 2\beta$)[1], that leads to rapid red blood cell destruction followed by anaemia. Rapid red blood cell destruction and regular blood transfusions lead to iron accumulation and hemosiderosis in organs mainly, endocrine glands, liver, heart and gonads. This, in turn, leads to oxidative stress induced damage in different tissues and causes metabolic derangement like dyslipidemia[2], vitamin D deficiency[3], insulin resistance[4], liver dysfunction[5] etc. It is well established that beta-thalassemia is associated with low cholesterol levels caused by a significant reduction in the synthesis and circulating levels of both low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels have been consistently reported[6-7]. Importantly, TC and LDL-C seemed similarly decreased in beta-thalassemia trait carriers compared with controls[8]. Vitamin D deficiency (VDD) is reported to be high in thalassemic patients in many countries despite the presence of good sunshine and routine prescription of 400-1,000 IU vitamin D per day[9-10]. Although, there is evidence of oxidative stress (due to iron overload) induced dyslipidemia and vitamin D deficiency along with altered calcium homeostasis in thalassemia patients albeit without any significant correlation between them.

In the present study we focussed on these incongruent observations and hypothesized that apart from the oxidative stress due to iron overload, lipid dysfunction acts also as one of the major cause of significant vitamin D deficiency in pubertal and post-pubertal (more than 14 yrs) age group of thalassemic patients with their adverse metabolic consequences.

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MATERIALS AND METHODS

Study design: The present study was performed as a hospital-based, observational, case-control study from July 2015 to June 2016 in an urban metropolitan area.

Selection of study population

Selection of cases: We selected only diagnosed cases of beta-thalassemia on convenience basis from the Thalassemia clinic of Pathology department of a Tertiary Care Medical College & Hospital according to specific inclusion and exclusion criteria. The inclusion criteria were (1) diagnosed cases of beta-thalassemia on the basis of HPLC report and clinical findings, (2) both male and female patients in the age group of 14-35 years with equal preference, (3) patients belonging to similar socioeconomic status and ethnicity. Exclusion criteria were (1) severe nutritional deficiency, (2) autoimmune haemolytic anaemia, (3) sickle cell anaemia, (4) kalaazar, (5) chronic lymphocytic leukaemia, (6) tropical splenomegaly, (7) first degree relative of the cases.

Selection of control subjects: Control subjects were selected from the age and sex-matched healthy subjects accompanying the patients to the Thalassemia clinic. First degree relatives were not considered to avoid any genetic predisposition. To ascertain the similar nutritional and economical status, both cases and control subjects were selected from the similar socioeconomic status with the similar ethnic background.

Ethical consideration

Informed consents were taken from all participants following the protocol and guidelines of the Helsinki declaration 1975, revised in 2000. Before starting, the study was approved by the institutional ethical committee.

Measurement of Biochemical Parameters

1. Serum ferritin was measured by sandwich Elisa method (CALBIOTECH, 10461 Austin Drive, Spring Valley CA 91978 USA)
2. Serum vitamin D was measured by Elisa Method (CALBIOTECH Inc, Austin Drive, Spring Valley, CA, 91978 USA)
3. Total cholesterol, HDL, LDL were measured by CHOD, PAP method &Triglyceride was measured by lipase GOD-PAP method by using standard spectrophoto-metric technique in autoanalyser XL600
4. Inorganic phosphate was measured by Molybdate U.V. method.
5. Ionised calcium was measured by ions specific electrolyte analyser (9180 from ROSCH)

Coefficient of variance (CV) for each of the test was measured to monitor the precision of each parameter and was found to be lower than 10% for each analyte.

RESULTS AND STATISTICAL ANALYSIS

The data obtained were first checked for normal distribution and then compared for the significance of difference between the mean values of case and control groups by independent Student's *t*-test (for parametric distributions). The predictive values of different study parameters on the serum vitamin D levels were analyzed by multiple linear regression tests. All statistical analyses were done with the SPSS 17 software for Windows from IBM, USA. *P* value was considered statistically significant at a level <0.05 for a 95% confidence interval.

Tests for normal distribution of the data obtained

From the tests for normal distriburtion (Smirnov-Kolmogrov's test and Shapero-Wilk's test, data not shown in the tables), data appeared to follow the normal distribution pattern except serum ferritin values. Their overall distribution has been shown through the Box-Whisker plot that also suggests that the data are approximately normally distributed apart from serum ferritin values.(in Figure)

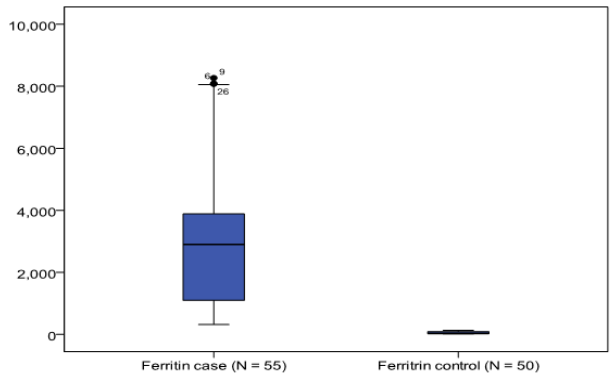

Figure 1 Boxplot showing the distribution of serum ferritin values in cases and controls

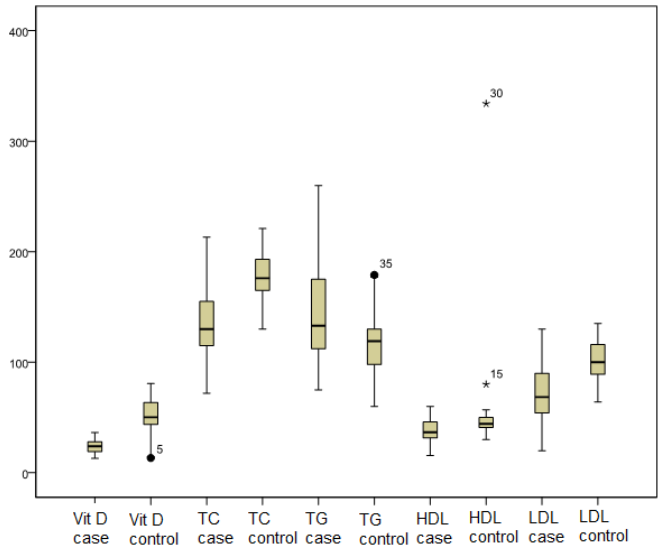

Figure 2 Boxplot showing the distribution of study parameters other than serum ferritin.

Tests for significance of mean value differences

In the independent *t*-test, these were reflected by the *t*-values and their corresponding *P* values in table 1. The result exhibited that in the case group, ionised Ca^{++}, vitamin D, total cholesterol, HDL, LDL are found significantly lower in case group compared to control group with p value <0.001 whereas serum phosphate level, triglyceride and VLDL level are found significantly higher in case group compared to control group with p value <0.001 (except p value for phosphate which is 0.002).

Table 1 comparison of the serum levels of study parameters between cases and controls by independent-t test

	Case (n=50) Mean ±SD	Control n=50 Mean ±SD	t value	P value
Ca^{++} (mmol/l)	1.044 ± 0.179	1.177 ± 0.079	-4.77	<0.001*
$Po4^{=}$ (mg/dl)	4.596 ± 1.293	3.872 ± 0.908	3.24	0.002*
Vitamin D (ng/dl)	23.879 ± 6.041	49.475 ± 16.679	-10.203	<0.001*
Total Cholesterol (mg/dl)	136.226 ± 33.897	179.558 ± 22.479	-7.533	<0.001*
Triglyceride (mg/dl)	150.82 ± 52.07	119.15 ± 30.37	3.714	<0.001*
HDL (mg/dl)	37.9 ± 9.73	45.4 ± 8.02	-4.194	<0.001*
LDL (mg/dl)	53.16 ± 26.16	100.8 ± 19.26	-10.368	<0.001*
VLDL (mg/dl)	47.41 ± 25.45	24.54±6.37	6.165	<0.001*

*p value is considered to be significant at a level of $p < 0.05$ for a 95% confidence interval.

Test for finding out the dependence and predictive effects of study parameters

To ascertain the relative importance of predictive values of the triglyceride, LDL, total cholesterol taken together on the vitamin D level *in vivo,* we performed the multiple linear regression analysis. Results showed that vitamin D level was significantly decreased only with serum LDL (regression coefficient β =0.505, *P* =0.049) and TG and TC show no significant predictive value on serum vitamin D level. This indicated strong positive and negative predictive effects of LDL, TG and TC on Vitamin D level, respectively.

Table 2 Multiple regression analysis analysing between vitamin D (as dependable) and TC, TG , LDL

Model	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	Std. Error	Beta		
Vit D (Constant)	17.089	3.501		4.881	0.000
TG case	.009	.027	.065	0.354	0.725
LDL case	.115	.057	.505	2.019	0.049*
TC case	-.022	.051	-.125	-.435	0.665

DISCUSSION

In our present study we found significantly decreased values for vitamin D (P value <0.001) and LDL cholesterol in our study population(P value<0.001).Decreasing LDL cholesterol level is probably due to an overall reduction in cholesterol biosynthesis reflected by decreased cholesterol level (Table 1). Regarding the relationship between the levels of vitamin D and lipid profile in thalassemia patient the observations are incongruent till now. Some studies have reflected lower level of LDL cholesterol[9-13] while some observed increased value of serum cholesterol[14-16]. Furthermore, in thalassemia patients of pubertal and post pubertal age group, relationship between vitamin D values and cholesterol level are not also consistent among different studies. Importantly our observations indicate clearly that both LDL cholesterol and vitamin D level were significantly decreased in the beta-thalassemia patients of pubertal and post pubertal age groups. To explore whether there was any significant linear relationship between these two parameters in our case group, we performed multiple linear regression analysis, result of which showed significant dependence of vitamin D level on LDL cholesterol (β-coefficient=0.505, P value=0.049). These values clearly indicate that decrease in vitamin D in β-thalassemia patient is directly dependant on decreased LDL cholesterol level. A recent study on patients heterozygous for beta-thalassemia measured levels of an artificially made labelled cholesterol-rich microemulsion (LDE) that mimicked LDL metabolism and binds to the LDL receptors. A 25% increase was noted in the removal of LDE cholesterol ester compared with healthy controls[17-18]. This may be the one of the important cause for decreased LDL level in thalassemic patient.

As vitamin D synthesis is directly depend on cholesterol level inside the tissues, decreased levels of cholesterol within tissues in thalassemic subjects further complicate the cholesterol mediated vitamin D synthesis. This finding can be further corroborated by the molecular basis of synthesis of vitamin D. The precursor molecule of vitamin D is 7- dehydrocholesterol, an intermediate of cholesterol synthesis pathway. As oxidative stress damage due to iron overload cause severe attenuation of cholesterol synthesis, so this is obvious that 7 dehydrocholesterol syntheses is also reduced[19-20], further which may lead to insufficient vitamin D level in thalassemic subject. In contrast to normal subject where lipid profile and vitamin D levels are inversely correlated, as per the finding of the present study, hypocholesterolemia in thalassemia due to iron induced hepatic damage and other causes may be one of the causative factors for vitamin D insufficiency.

Implications of the present study

The present study implicated that the iron induced tissue damage occurs on a wide scale basis affecting almost each part of the cell component including the membrane lipids, cellular proteins and nucleic acids and vitamin D metabolism. A derangement in lipid metabolism in beta thalassemia is found to be closely linked to alterations in the vitamin D levels and its consequences. Thus from the results, hypothesis of the present study may be accepted that apart from the oxidative stress due to iron overload, vitamin D deficiency and altered Ca^{++} metabolism may also be one of the major cause of significant lipid dysfunction in pubertal and post-pubertal(more than 14 yrs) age group of thalassemic patients with their adverse metabolic consequences. As a direct implication of this, it can be further suggested that a proper follow up for the dyslipidemia as well as vitamin D levels are needed to restrict the metabolic complications associated with beta thalassemia in these patients. The exact etiopathogenesis of the disease at the genetic level must be more extensively explored to understand and manage the iron overload and its related toxicity in beta thalassemia patient.

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