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RESEARCH ARTICLE

SERUM LIPID PROFILE AND FERRITIN IN β- THALASSEMIA TRAIT PATIENTS

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ABSTRACT

Introduction: Thalassemia is one of the heterogenic groups of inherited anemia which occurs mainly dueto mutation in the gene related to the synthesis of hemoglobin beta- chains.
Objectives: The aim of our study was to evaluateserumlipid profile andferritin levels in β-thalassemia trait (BTT) and compare them with that ofage and sex matched controls.
Methodology: Totally 120 young adults were selected for the study, in which 60 wereBTT and other 60 were healthy controls. Serum lipid profile was analyzed using ERBA kit and serum ferritin was estimated withMeril Diagnostic kit.
Results: Total cholesterol and LDL values were lower in BTT when compared to controls and TG and HDL values were higherin BTT with that of controls.Serum ferritin values were found to be higher in BTT than that ofcontrols.
Conclusion: The presentstudy revealed that there were significant changes in serum lipid profile and ferritin levels in BTT when compared to controlswhich should be a cause for anxiety of better assessment of cardiovascular risk factors in the patients.

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INTRODUCTION

Thalassemia is one of the heterogenic groups of inherited anemia which mainly occurs due to mutation in the gene related to the synthesis of hemoglobin beta- chains. The prevalence of thalassemia varies in different places which are between 4-15% among different populations.Patients with minor β-thalassemia have usually no problem clinically, but show a type of microcytic hypo chromic anemia (Hashemi and Shirzadi, 2007).From past few years many scientific evidences have raised the adverse effect of abnormal blood lipid levels, like total-cholesterol and other lipids and lipoproteins on atherosclerotic disease (Gotto, 1994. Ginsberg, 1994 and Wilson *et al.,* 1988).The serum total cholesterol, LDL and HDL are found to be low in patients with β-thalassemia major (Maioli *et al.,* 1984) and β-thalassemia intermedia (Amendola *et al.,* 2007 and Hartman *et al.,* 2002). At this point it should be mentioned that the relationships between blood lipids and atherosclerosis might be influenced by several other lifestyle-related factors, like glucose intolerance; blood pressure levels, dietary and smoking habits (JAMA, 2001). Arecent study performed on β- thalassemia trait (BTT) in Sardinia, LDL (low density of lipoprotein) level of thalassemia patients was lower compared to the control group, Apo A and Apo B also significantly decreased (Deiana *et al.,* 2000). In β-thalassemia major, liver damage accounts for the low total-cholesterol (TC), high- density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) serum levels (Goldfarb *et al.,* 1991). The average values of serum totalcholesterol, TG, LDL, HDL and VLDL in thalassemia minor subjects were 163.63±34.28,159.74±157.54, 90.97±23.94, 34.97±8.07 and 73.44±72.43 mg/dL respectively (Hashemieh *et al.,* 2011). Non-fasting blood samples can be normally used for estimation of plasma lipid profiles. Laboratoryreports muststandardize abnormal values based on desirable concentration cut-points. Measurements ofnon-fasting and fastingvalues must be complementary but not commonly exclusive (Borge *et al.,* 2016). Serum ferritin levels are related to the quantity of iron stored in the body. However, countless other genetic and acquired conditions, with and without iron overload, can influence these results. An increase in ferritin concentration with no excess iron in the body can be observed in acute and chronic inflammatory processes, autoimmune diseases, neoplasia, chronic renal insufficiency, hepatopathies and metabolic syndrome. In these conditions, transferrin saturation generally is normal or decreased. On the other hand, when there is an iron overload, except in ferroportin disease, the increase in ferritin concentration is associated with increased saturation of transferring (Camaschella and Poggiali, 2009). Serum ferritin evaluations

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have a huge clinical significance for measurement of body iron storage (Porter, 2001). In β– thalassemia major patients, mean value of serum ferritin was found to be 4098.67 ± 1598.63 ng/ml, least level was 1212ng/ml and highest was 7560ng/ml (Sultana *et al.,* 2011). Inβ-homozygousthalassemia patients, the mean value of serum ferritinwas 281.8 ± 219.9 µg/L (Anil *et al.,* 1985). In Bhopal, India it was found that mean serumferritin level was 2767.52(SD 1849.1) ng/ml. Only 12.5% patients had serum ferritin value lower than 1000 ng/ml, 44.4% patients' serum ferritin level was between 1000-2500 ng/ml,whereas 43.05% patients had serum ferritin values more than 2500 ng/ml(Amit and Archana, 2013).In case of β– thalassemia trait females, the mean level of serum ferritin was 97.9µg/L and in normal adult females, it was 55.9 µg/L(Sonay *et al.,* 1976). In our study, we estimated the serum lipid profile as well asferritin concentrationin β– thalassemia traitpatients of DakshinaKannada and Kodagu population, Karnataka, and compared those values with healthy controlsto check for the cardiovascular risk factors in these patients.

MATERIALS AND METHODS

Sample collection

Total of120 young adults were selected for the study, in which 60 wereBTT and the other 60 were healthy controls. Forty-one BTT from Dakshina Kannada district and nineteen from Kodagu district population were collected and among them, 11 were males and 49 werefemales. Human ethical clearance was obtained from Mangalore University Institutional Human Ethics Committee (No.MU-IHEC-2016-3 dt.18/04/2017). Informed consent was obtained from all the subjects who had participated in this study.

Methodology

Two ml of blood was drawn and collected in plain containers. Serum was separated and stored in deep freezer at -20°C until further use. Samples were allowed to attain room temperature before use. Serum lipid profile was analyzed usingERBA kit in STAR-20 for HDL and ERBA (Manheim) Chem 5X for cholesterol/triglycerides. Serum ferritin was estimated using a kit from Meril Diagnostics in ERBA (Manheim) LisaScan EM ELISA plate reader.

Statistical analysis

The obtained values were analyzed bynon-parametric test (Mann-Whitney U test) using statistical software SPSS version 22. IBM Corp. released 2013. Armonk, NY: IBM Corp.P>0.05 was considered as statistically significant.

RESULTS

Evaluation of serum lipid profile and ferritin values of β-thalassemia trait and control groups inoverall population of Dakshina Kannada and Kodagu districts:

The lipid profile and ferritin levels were analyzed in the serum samples of the patients and the control subjects. It was found that in BTT, HDL, VLDL, Total Cholesterol/HDL and LDL/HDL(4.42, 2.95) median values were slightly higherwhen compared to healthy controls(3.61, 2.14). Cholesterol and LDL have slightly lower median value in BTT (169.10, 110.38mg/dL) with that of controls (177.40, 110.46mg/dL). TG in BTT was higher (116.30mg/dL) than controls (76.04mg/dL).In BTT, ferritin level was very high (45.50 ng/mL) compared to controls (6.14 ng/mL) (Table 1).

Table 1. Serum lipid profile and ferritin values of BTT and controls in overall population

Parameters	Groups	Q1	Median	Q3
Cholesterol(mg/dL)	BTT	151.20	169.10	192.10
	Control	155.25	177.40	257.65
Triglycerides(TG)	BTT	66.90	76.04	100.10
(mg/dL)	Control	66.38	116.30	165.30
HDL (mg/dL)	BTT	46.53	55.10	62.95
	Control	32.80	41.15	45.48
VLDL (mg/dL)	BTT	11.40	17.15	23.82
	Control	14.03	15.82	20.25
LDL (mg/dL)	BTT	83.71	110.38	138.36
	Control	91.87	110.46	136.58
T.Chol/HDL	BTT	3.71	4.42	5.60
	Control	3.11	3.61	4.24
LDL/HDL	BTT	2.34	2.95	4.05
	Control	1.59	2.14	2.51
Ferritin	BTT	23.84	45.50	95.25
(ng/mL)	Control	4.61	6.14	20.84

where Q1 and Q3 are inter quartile

Table 2. Mann-Whitney U test for lipid profile and serum ferritin values of BTT and controls in the overall population

Statistical tests	Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	T.Chol/HDL	LDL/HDL	Ferritin (ng/mL)
Mann-Whitney U	1396.500	1192.000	793.000	1757.000	1797.500	1051.000	947.500	393.000
Wilcoxon W	3226.500	3022.000	2623.000	3587.000	3627.500	2881.000	2777.500	2223.00
Z	-2.118	-3.191	-5.286	-.226	-.013	-3.932	-4.475	-7.385
P	.034	.001	.001	.821	.990	.001	.001	.001

Cholesterol, TG, HDL, T.Chol./HDL, LDL/HDL and ferritin levels showed statistically significant change (Table 2).

Evaluation of serum lipid profile and ferritin concentration of β-thalassemia trait and control groups in DakshinaKannada district:

In Dakshina Kannada population, it was observed that, in BTT TG, VLDL, HDL, T.Chol./HDL and LDL/HDL (86.34, 18.51, 53.30 mg/dL; 4.73, 3.13) median values were slightly elevated when compared to controls (76.04, 15.82, 41.10 mg/dL; 3.65,

2.14). Cholesterol and LDL had slightly lower median value in BTT (173.00, 109.58 mg/dL) than that of control (177.80, 113.23 mg/dL). Ferritin concentration in control was very low (5.47 ng/mL) compared to BTT (63.49 ng/mL; Table 3). Only HDL, T.Chol./HDL, LDL/HDL and ferritin had significant changes (Table 4).

T.Chol./HDL, LDL/HDL levels were slightly elevated in BTT (60.20, 3.96, 2.59 mg/dL) compared to controls (41.20, 3.53, 2.14 mg/dL). Ferritin concentration in BTT patients was significantly elevated (39.90 ng/ml) than the controls (7.26 ng/ml; Table 5). Cholesterol, TG, HDL and ferritin levels showed highly significant changes (Table 6).

Table 3. Lipid profile and serum ferritin values of BTT and controls in Dakshina Kannada

Parameters	Groups	Q1	Median	Q3
Cholesterol(mg/dL)	BTT	152.30	173.00	240.75
	Control	157.20	177.80	202.01
Triglycerides(TG)	BTT	57.18	86.34	142.45
(mg/dL)	Control	66.61	76.04	98.27
HDL (mg/dL)	BTT	44.25	53.30	58.35
	Control	32.45	41.10	46.20
VLDL (mg/dL)	BTT	11.70	18.51	31.08
	Control	14.15	15.82	20.48
LDL (mg/dL)	BTT	84.44	109.58	135.73
	Control	97.96	113.23	159.85
T.Chol/HDL	BTT	3.77	4.73	6.48
	Control	3.11	3.65	4.30
LDL/HDL	BTT	2.46	3.13	4.59
	Control	1.61	2.14	2.51
Ferritin	BTT	28.10	63.49	114.90
(ng/mL)	Control	4.60	5.47	19.95

Table 4. Mann-Whitney U test for Lipid profile and serum ferritin values of BTT and controls in Dakshina Kannada population

Statistical tests	Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	T.Chol/HDL	LDL/HDL	Ferritin (ng/mL)
Mann-Whitney U	825.500	693.000	388.000	706.000	717.500	413.000	380.000	149.000
Wilcoxon W	1686.500	1554.000	1249.000	1567.000	1578.500	1274.000	1241.000	1010.00
Z	-.139	-1.368	-4.197	-1.247	-1.141	-3.965	-4.271	-6.413
P	.889	.171	.001	.212	.254	.001	.001	.001

Table 5. Lipid profile and serum ferritin values of BTT and controls in Kodagu population

Parameters	Groups	Q1	Median	Q3
Cholesterol(mg/dL)	BTT	140.40	154.80	169.40
	Control	168.50	212.50	264.20
Triglycerides(TG)	BTT	116.40	130.90	192.55
(mg/dL)	Control	66.80	76.04	104.70
HDL (mg/dL)	BTT	48.30	60.20	65.60
	Control	33.40	41.20	44.20
VLDL (mg/dL)	BTT	10.02	13.75	19.27
	Control	13.94	15.82	20.32
LDL (mg/dL)	BTT	79.58	105.80	118.06
	Control	82.90	120.15	147.50
T.Chol/HDL	BTT	3.00	3.96	4.69
	Control	2.80	3.53	3.99
LDL/HDL	BTT	2.03	2.59	3.82
	Control	1.58	2.14	2.77
Ferritin	BTT	18.90	39.90	63.16
(ng/mL)	Control	5.26	7.26	25.10

Table 6. Mann-Whitney U test for Lipid profile and serum Ferritin values of BTT and controls in Kodagu population

Statistical tests	Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	T.Chol/HDL	LDL/HDL	Ferritin (ng/mL)
Mann-Whitney U	68.500	59.000	73.000	135.000	122.000	139.000	134.500	58.000
Wilcoxon W	258.500	249.000	263.000	325.000	312.000	329.000	324.500	248.000
Z	-3.270	-3.547	-3.139	-1.328	-1.708	-1.212	-1.343	-3.576
P	.001	.001	.002	.184	.088	.226	.179	.001

Evaluation of serum lipid profile and ferritin values of β-thalassemia trait and control groups in Kodagu district:

In Kodagu population, when lipid profile was estimated it was observed that TG was very high in BTT (130.90 mg/dL) when compared to controls (76.04 mg/dL). Cholesterol, VLDL and LDL (154.80,13.75, 105.80 mg/dL) levels were lower in BTT patients than controls (212.50, 15.82, 120.15 mg/dL). HDL,

Evaluation of serum lipid profile and ferritin values of females withβ -thalassemia trait and control groups in overall female population of Dakshina Kannada and Kodagu districts.

It was observed that in females cholesterol, VLDL, LDL, T.Chol./HDL, LDL/HDL (169.10, 17.03,107.59, 4.30, 2.88 mg/dL) in BTT was almost same as in controls (169.40, 15.50, 3.63, 2.14 mg/dL) it did not show any significant difference,

whereas TG and HDL in BTT had shown the significant difference (112.30, 53.30 mg/dL) compared to controls (76.03, 41.10 mg/dL). BTT patients have higher concentration of serum ferritin (40.07 ng/mL) than controls (6.02 ng/mL; Table 7). TG, HDL, T.Chol. /HDL, LDL/HDL and serum ferritin had shown statistically significant difference (Table 8)

Cholesterol and LDL were significantly lowered in BTT patients (170.60, 93.48 mg/dL) compared to controls (340.50, 235.10 mg/dL). VLDL in BTT patients was slightly higher (25.08 mg/dL) compared to controls (20.02 mg/dL). Serum ferritin concentrationwas significantly lowered (8.70 ng/mL) in control subjects compared to BTT (102.50 ng/mL; Table 9).

Table 7. Lipid profile and serum ferritin values of BTT and controls in overallfemale population

Parameters	Groups	Q1	Median	Q3
Cholesterol(mg/dL)	BTT	147.75	169.10	190.60
	Control	152.30	169.40	218.00
Triglycerides(TG)	BTT	58.48	112.30	155.40
(mg/dL)	Control	66.41	76.04	98.27
HDL (mg/dL)	BTT	45.00	53.30	62.30
	Control	29.20	41.10	46.75
VLDL (mg/dL)	BTT	11.31	17.03	23.27
	Control	13.97	15.50	19.18
LDL (mg/dL)	BTT	81.91	107.59	120.67
	Control	87.07	112.01	137.79
T.Chol/HDL	BTT	3.19	4.30	5.45
	Control	3.21	3.63	4.30
LDL/HDL	BTT	2.17	2.88	3.87
	Control	1.64	2.14	2.51
Ferritin	BTT	23.14	40.70	83.28
(ng/mL)	Control	4.61	6.02	18.67

Table 8. Mann-Whitney U test for lipid profile and serum ferritin values of BTT and controls in overallfemale population

Statistical tests	Chol. (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	T.Chol/HDL	LDL/HDL	Ferritin (ng/mL)
Mann-Whitney U	1075.500	838.000	602.000	1190.000	1022.000	793.000	673.500	254.000
Wilcoxon W	2300.500	2063.000	1827.000	2415.000	2247.000	2018.000	1898.500	1479.00
Z	-.888	-2.576	-4.253	-.075	-1.268	-2.896	-3.745	-6.725
P	.374	.010	.001	.941	.205	.004	.001	.001

Table 9. Lipid profile and serum ferritin values of BTT and controls in overall male population

Parameters	Groups	Q1	Median	Q3
Cholesterol(mg/dL)	BTT	150.90	170.60	247.00
	Control	212.50	340.50	441.90
Triglycerides(TG)	BTT	85.86	128.30	233.30
(mg/dL)	Control	73.66	90.14	113.50
HDL (mg/dL)	BTT	49.10	57.10	65.30
	Control	38.90	42.20	44.20
VLDL (mg/dL)	BTT	14.95	25.08	34.82
	Control	15.13	20.02	23.38
LDL (mg/dL)	BTT	80.64	93.48	157.60
	Control	113.23	235.10	302.54
T.Chol./HDL	BTT	4.03	6.57	9.60
	Control	2.80	3.13	4.05
LDL/HDL	BTT	2.41	5.24	7.45
	Control	1.48	1.80	2.63
Ferritin	BTT	23.14	40.70	83.28
(ng/mL)	Control	4.61	6.02	18.67

Table 10. Mann-Whitney U test for lipid profile and serum ferritin values of BTT and controls in overall male population

Statistical tests	Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	T.Chol/HDL	LDL/HDL	Ferritin (ng/mL)
Mann-Whitney U	12.000	30.000	5.000	56.000	24.000	19.000	27.000	10.000
Wilcoxon W	78.000	96.000	71.000	122.000	90.000	85.000	93.000	76.000
Z	-3.187	-2.003	-3.646	-.295	-2.397	-2.725	-2.200	-3.316
P	.001	.045	.001	.768	.017	.006	.028	.001

Evaluation of serum lipid profile and ferritin values in males of β-thalassemia trait and control groups in overall male population of Dakshina Kannada and Kodagu districts:

In male population of BTT, it was observed that TG, HDL, T.Chol./HDL, LDL/HDL levels were significantly higher (128.30, 57.10mg/dL;6.57, 5.24) than that of controls (90.14, 42.20 mg/dL; 3.13, 1.80).

Cholesterol, TG, HDL, T.Chol./HDL, LDL/HDL and serum ferritin showed significant altered values (Table 10).

DISCUSSION

In the present study, the samples positive for BTT, were analyzed for serum lipid profile and ferritin. Since it does not follow the normal distribution as variation is high,

nonparametric test (Mann-Whitney U test) was applied and so the descriptive measures were in median and inter quartile range. Hashemieh*et al*., (2011) in their historical cohort study of minor thalassemic patients in Iran, observed significantly lower values of total cholesterol and LDL compared to normal subjects, but other factors such as TG, HDL and VLDL showed no difference. In β-thalassemia minor patients of Turkey, Sinam *et al*., (2014) found hypocholesterolemia and low LDL levels which could be due to acceleratederythropoiesis and an increased LDL uptakeby macrophages of the reticuloendothelial system. Mohammad *et al.,* (2014) discovered in a cross sectional study in Iran that β-thalassemia minor patients had significantly lower total cholesterol and LDL values. The results in our study indicated the similar trend with hypocholesterolemia and low LDL concentration but TG and HDL levels were significantly high. There was no difference in VLDL values when compared to healthy controls.The pathogenesis of these abnormalities can be caused by many mechanisms including plasma dilution because of anemia, accelerated erythropoiesis resulting in increased cholesterol uptake by macrophages and histiocytesof the reticuloendothelial system, defective liver functioning because of iron overload, macrophage system activation with cytokine release, and hormonal disturbances (Deiana *et al.,*2000; Al-Quobaili and AbouAsali, 2004 and Shalev *et al.,* 2007).

Anemiadrives the thalassemic patients at risk for decreased extrahepatic lipolytic activity, resulting in high serum triglycerides (Quobaili and AbouAsali, 2004). BTT patients are generally asymptomatic but sometimes they have mild anemia, associated with microcytic anemia with an elevated RBC number and a small decrease in the hemoglobin concentration (Sinam *et al*., 2014). Earlier studies proved that lipids and lipoproteins play a main role in pathogenesis and development of atherosclerosis and cardiovascular diseases (Amendola *et al*., 2007). In our study, some factors of lipid profile in BTT were lower when compared to a control which reduces the risk of cardiovascular diseases. No significant difference in lipid profile was established between males and females with beta thalassemia trait. Further, there was no difference in lipid profile between different BTT populations of two districts of Karnataka. It was observed in the study of Amit and Archana, (2013) that serum ferritin levels were high in β- thalassemia intermedia when compared to controls. Sonay*et al*., (1976) found that serum ferritin level in BTT and controls was almost same. High levels of serum ferritin have been observed in beta-thalassemia trait comparative studies, and even those who had never been transfused developed clinical and laboratory signs of iron overload (Pipern*oet al.,* 2000). Based on the results, Estevao*et al.,* (2011) concluded that ferritin concentrations have a heterogeneous pattern in heterozygous beta –thalassemia, and are higher in men than in women. In our study, there was a significant increase in serum ferritin concentration in BTT patients when compared to controls and further, higher in men than in women. This sex difference in body iron stores is well known.

Ferritin is considered the most important indicator of iron status as even in the first stage of iron deficiency, its concentration decreases (Knovich *et al.,* 2011). Ferritin is a storage form of iron and serum ferritin levels normally correlate well with total iron stores. The accumulation of iron results in progressive dysfunction of the heart, liver and endocrine glands. The iron burden on the body can be assessed by means of elevated levels of serum transferrin saturation, serum ferritin, iron and Total Iron Binding Capacity (TIBC) levels (Sagare and Trivedi, 2014). Iron overload is responsible for the most damaging effects of thalassemia, making iron chelating a focal point of the management of these diseases.

Conclusion

Based on the present study, low levels of total cholesterol and LDL and high levels of triglycerides, HDL and ferritin in patients with β- thalassemia trait should be a cause for anxiety of better assessment of cardiovascular risk factors in the patients. These patients should also be monitored for diseases associated with iron overload and may be considered for iron chelating therapy to minimize further complications caused by excess iron. However, a larger number of BTT cases could have helped in extrapolating the results to other populations.

CONFLICT OF INTEREST: None declared

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