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Original Article

Disturbances of Lipid Profile, Hemoglobin and Serum Ferritin Levels in Thalassemia Patients in Misan City, Amara, Iraq

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ABSTRACT

Background: One of the most common hereditary hematological disorders is beta thalassemia major manifested by severe hemolysis and profound anemia for which the regular blood transfusions are required that leading to the iron accumulation in blood and body organs as well as increased serum ferritin. Thalassemic patient's lipid profile levels are significantly variable in many studies.

Aims: The study aimed to detect any alteration of serum lipid profiles in thalassemic major patients and to find out if they are correlated with serum ferritin and Hb concentration.

Method sand patients: Sixty-two Beta thalassemia major patients were enrolled in the study, aged (6months-15 years) randomly selected (65) matched control children. Obtaining serum samples for lipid profile, and ferritin estimation, as well as blood test of hemoglobin concentration was done. Statistical data analysis was performed using standard methods.

Results: The most presented age was (>5-10 years.) followed by (6mo-5years) then (>11-15 years.) (27, 21, 14) patients, respectively. Female were slightly predominant than male (54.8%), (45.2%), respectively mainly in group 2 (55.9%), while male slightly predominates in the younger and older groups (26.5%), (17.7%), respectively. Serum ferritin levels were very high in (98.4%) of cases, mainly in group 2 (43%), then in group 1 (34%), followed by group 3 (23%). The majority of patients had normal cholesterol level (95.16%). Lipoprotein were Low in (35.5%) of patients mainly in group 2 (54.5%) and normal in (64.5%) mainly equally distributed in groups, although not statistically significant, (p.value=0.7). Low Density Lipoprotein was normal in most patients (95.16%), and not significant statistically (P value=0.8). Triglyceride was high in most patients (83.9%), while normal in (16.1%), it was with no statistically significant (p. value=0.4).

Conclusion: The present study concluded a positive correlation between (high Triglyceride, low, and high density Lipoprotein) and (low Hb and high ferritin levels) which may attribute to the future fatal cardiac complication.

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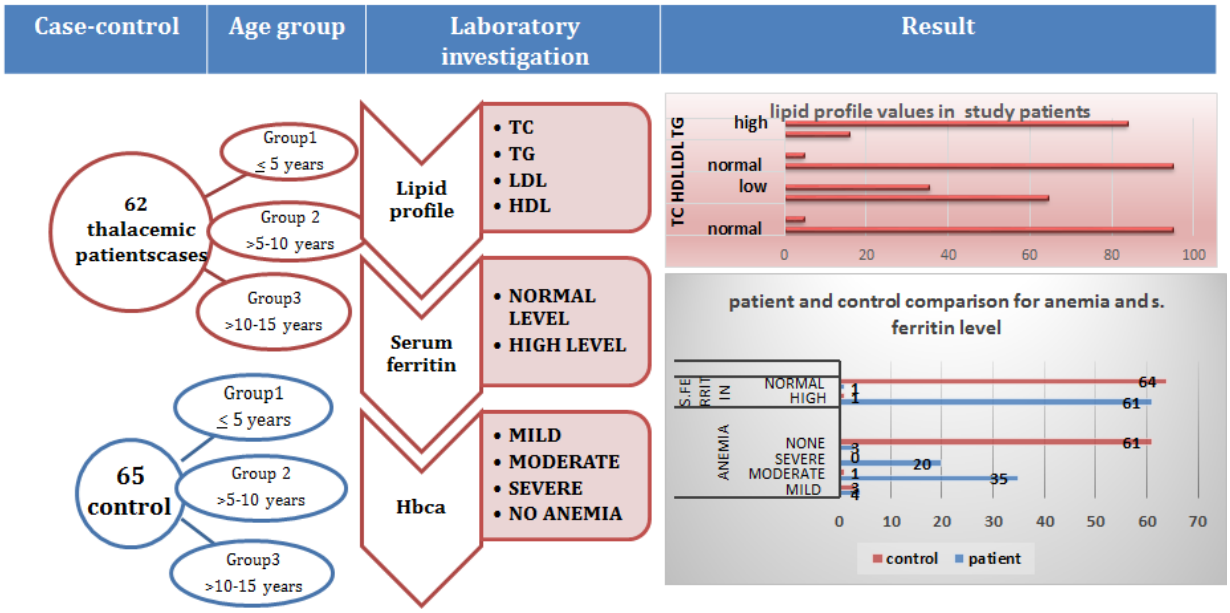
GRAPHICAL ABSTRACT



Introduction

Beta thalassemia syndromes are a group of hereditary blood disorders characterized by a genetic deficiency in the synthesis of beta-globin chains. The homozygous state results in beta thalassemia (thalassemia major) a common, severe, life-threatening anemia with ineffective erythropoiesis, and rapid erythrocyte breakdown [1]. It is a transfusion-dependent anemia which eventually led to iron overload manifested by increased serum ferritin level, this continuous iron deposition causes progressive damage and dysfunction of vital organs (heart, liver, and endocrine glands). Lipid abnormalities have been detected in different types of beta thalassemia, and also in various hematological disorders including sickle cell disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hereditary spherocytosis, aplastic anemia, and myelodysplastic syndrome [2]. The underlying pathogenesis may be attributed to the following mechanisms including anemia and related plasma dilution, cholesterol uptake by macrophages as well as histiocytes of the reticuloendothelial system is further increased in response to severe fast hemolysis with superadded compensatory accelerated erythropoiesis, defective liver functioning due to iron overload, macrophage system activation with cytokine release, and hormonal disturbances [3]. A high incidence of thrombo-embolic event commonly observed in *β*-thalassemia patients who are not receiving regular transfusions or splenectomies patients, strongly supporting the pro-coagulant activity of circulating damaged red blood cells [4]. Vascular dysfunction with increased arterial stiffness and endothelial dysfunction has been found in patients with β thalassemia [5]. Peroxidative tissue injury in response to the continuous blood transfusions causes endothelial dysfunction. Children with β thalassemia are at risk of developing premature atherosclerosis because of dyslipidemia [6]. Abnormal lipid profiles, including low total cholesterol, low high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and high triglycerides have long been observed in β thalassemia [7]. There is a variation among studies regarding alteration in serum lipid profile level in B-thalassemia patients; they need careful monitoring to prevent future atherosclerotic complications. The present study had been undertaken to detect any alteration in serum lipid profile levels in our patients and if it is correlated to serum ferritin, Hb level, or not.

The aim of the study

To demonstrates serum lipid profile, hemoglobin, ferritin levels in B-thalassemia major patients attended Misan thalassemia center and compare them with results of matched control pediatric participants to find out if any correlation is existed.

Materials and Methods

A case-control study undertaken in the Thalassemia Center in Misan city, sixty-two children who chronically attended in the center with long standing β thalassemia major were included in the proposed study. Their ages were ranged from (6 months-15 years), including 34 female and 28 male genders selected among patients recorded children through a period from January to July 2021, they were subdivided into 3 groups according to their ages: group1= (6mo-5 years.), group 2= (>5-10 years.), group 3= (>10-15 years.). Their medical history and data information were obtained from the thalassemia center medical records or from patients themselves or their parents and\or any caregivers, this information including age, gender, Hb level, serum ferritin level**,** any family member with thalassemia**,** and any relative with thalassemia**.**

On the other hand, a randomly selected (sixty-five) group of healthy non-thalassemic children as control group from outpatient's clinic visitors attended (the child and maternity hospital) in Misan city during the same period. The control group were matched for age and sex and nearly the same socioeconomic state and were apparently healthy individuals neither thalassemia trait nor carrier, with no history of blood transfusion, anemia, infection and no chronic disease state including 39 females and 36 males.

We checked patients' hemoglobin level to assess the anemia severity as it is the most common manifestation, and then we subdivided them into (mild, moderate, and severe anemia). Blood samples were obtained from the study subjects after 12 hours of overnight fasting. Serum triglyceride and cholesterol were measured in semi-automated Clinical Chemistry Analyzer using standard commercial reagents by Glycerol-3-phosphate oxidase-peroxidase (GPO-PAP) and Cholesterol oxidase-peroxidase (CHOD-PAP) method respectively. Measurement of serum HDL-C was performed by phosphotungstic acid precipitation method. The levels of LDL-C and VLDL-C were estimated by calculation using the formula of Fried Ewald and Levy. Hemoglobin of whole blood was assayed in a blood analyzer (KX-21 Sysmex Auto Hematology Analyzer). For ferritin estimation, serum was separated and stored at –200 °C Ferritin levels were performed by ELISA.

Data analysis was carried out by using the available Statistical packages version 17.0 (SPSS-17.0). The data were arranged in number and percentage, and also were presented in the form of tables. Chi-square test (χ^2-test) was used for testing the significance of association between variable under study. The statistical significance was considered whenever the P-value was equal or less than 0.05.

Informed consents were obtained from the parents or caregivers of the children, the ethical committee in our medical school approved the study.

Results and Discussions

Age and gender predominance among age groups: The most presented age was (>5-10 years.) followed by (6mo-5years) then (>11-15 years.), patients respectively (27, 21, 14). Overall, Females were slightly predominant than males (54.8%)**,** (45.2**%**), respectively, mainly in group 2 (55.9%), while males slightly were predominate in the younger and older groups (26.5%), (17.7%), respectively.

Regarding anemia

Most patients had anemia (95.2%), anemia of moderate severity was found in (56.5%) mainly in younger groups (1, 2), while severe anemia was found in (32.2%) mainly in older groups (2, 3), those with mild anemia were (6.5%) and lastly (4.8%) had no anemia.

Serum ferritin levels were very high in (98.4%) of cases, mainly in group 2 (43%), then in group 1 (34%), followed by group 3 (23%).

Family history of thalassemia was positive in (45.2%), and negative in (54.8%) of cases.

Relative history of thalassemia was positive in (43.5%), and negative in (56.5%) of cases.

Table 1: Characteristics of patients correlated with their age groups

Variables		Age groups								P value
		Group 10-5 years		Group 2 >5-10 years		Group 3 >11-15 years		Total		
		no	%	no	%	no	%	no	%	
Gender	Male	12	42.8	8	28.6	8	28.6	28	45.2	0.09
	Female	9	26.5	19	55.9	6	17.6	34	54.8	
Anemia	Mild	1	25	2	50	1	25	4	6.5	0.2
	Moderate	16	45.73	15	42.85	4	11.42	35	56.5	
	Severe	3	15	9	45	8	40	20	32.2	
	No anemia	1	33.3	1	33.3	1	33.3	3	4.8	
S. Ferritin	High	21	34	26	43	14	23	61	98.4	0.5
	Normal	0	0	1	1	0	0	1	1.6	
Thalassemia in Family member	Positive	10	35.7	14	50	4	14.3	28	45.2	0.3
	Negative	11	32.4	13	38.2	10	29.4	34	54.8	
Thalassemia in relatives	Positive	9	33.4	10	37	8	29.6	27	43.5	0.4
	Negative	12	34.3	17	48.6	6	17.1	35	56.5	

Table 2: Serum lipid profiles in correlation with thalassemia patient age groups

Lipid profile		patients age groups								P value
		Group1 < 5 years		Group 2 >5-10 years		Group 3 >11-15years		Total		
		no	%	no	%	no	%	no	%	
Total cholesterol	Normal	20	33.9	26	44	13	22	59	95.16	0.8
	High	1	33.3	1	33.3	1	33.3	3	4.84	
HDL	Normal	15	37.5	15	37.5	10	25	40	64.5	0.7
	High	6	27.2	12	54.5	4	18.1	22	35.55	
LDL	Normal	20	34	26	44	13	22	59	95.16	0.8
	High	1	33.33	1	33.33	1	33.33	3	4.84	
Triglyceride	Normal	5	50	3	30	2	20	10	16.1	0.4
	Low	16	30.8	24	46.2	12	23	52	83.9	

Regarding patient's lipid profile correlation with age groups: Table 2

Regarding cholesterol levels

The majority of patients had **normal cholesterol** level (95.16%), which demonstrated as follows: (33.9%) in group 1, (44%) in the group 2 and (22%) in the group 3; while **high cholesterol** level was found only in 3 patients (4.48%), distributed as one patient in each group. However, the results were not significant (p.value = 0.8).

Low HDL was found in (35.5%) of patients mainly in group 2 (54.5%) followed by group 1(27.2%) and lastly group 3 (18.1%). **HDL was normal** in (64.5%) mainly equally distributed in groups 1& 2 (37.5%) for each, followed by (25%) in group 3. Although it was not statistically significant (p.value=0.7).

LDL was normal in most patients (95.16%) while it was **high** in (4.84%) equally distributed through all groups. However, it was not statistically significant (p value=0.8).

Triglyceride was high in most patients (83.9%) mainly in group 2 (46.2%) followed by group 1 (30.8%) and lastly group 3 (23%). While it was **normal** in (16.1%) mainly in group 1 (50%) followed by group 2 (30%), then group 3 (20%). It was not statistically significant (p. value=0.4).

Table 3: Serum lipid profiles in relation to hemoglobin in thalassemia patients

Lipid profile		HB level						P value
		Low	%	Normal	%	Total	%	
Total Cholesterol T-C	Normal	54	91.5	5	8.5	59	95.2	0.1
	High	2	66.6	1	33.3	3	6.5	
	Total	56	90.3	6	9.7%	62	100	
HDL-C	Normal	38	95.1	2	4.9	40	64.5	0.09
	Low	18	81.8	4	18.2	22	35.5	
	Total	56	90.3	6	9.7	62	100	
LDL-C	Normal	54	91.4	5	8.5	59	95.2	0.1
	High	2	66.6	1	33.3	3	4.8	
	Total	56	95.1	6	9.7	62	100	
Triglyceride TG	Normal	7	70	3	30	10	16.1	0.01
	High	49	94.2	3	5.8	52	83.9	
	Total	56	90.3	6	9.7	62	100	

Regarding correlation of lipid profile and patients HB level: as in (table 3)

Cholesterol CT: 56\62 of patient were anemic, while only 2\56 had high cholesterol (TC), the remaining 54\56 had normal TC levels, so cholesterol &anemia are not correlated (p-value=0.1) and thus, it was not significant.

While HDL was low in 22\62 (35.5%) of all cases, (81.8%) among them were anemic, while 4\22 (18.2%) were not anemic. Therefore, anemia is correlated to low HDL-C levels, but it was not of statistical significant (P-value=0.09).

Triglycerides (TG): was high in (83.9%) of cases, (high TG & anemia) were found in (94.2%) of cases, while (high TG & no anemia) were found in (5.8%), which meant anemic correlated significantly to TC values (P- value=0.01).

LDL-C were normal in 59\62 patients of which 54 (91.4%) were anemic and 5 (8.5%) were not, which indicates no correlation was found between anemia and LDL-C levels (p -value=0.1).

Table 4: Correlation of Thalassemia Patients' Ferritin and Lipid profiles

Lipid Profile	S. Ferritin Level		
	Level	High	%
Total Cholesterol TC	Normal	58	95
	High	3	5
HDL-C	Normal	39	64
	Low	22	36
LDL-C	Normal	58	95
	High	3	5
Triglyceride TG	Normal	10	16.4
	High	51	83.6

Relations of S. ferritin and lipid profile values in patients demonstrated in (Table 4)

Patients with high S. ferritin levels:

They had high TG in (83.6%) which was a positive correlation, as well as it had low HDL in (36%) of cases, which is inverse correlation, while no correlation was found between serum ferritin and (TC), (LDL-C) levels.

Table 5: Comparison of patient & control regarding anemia severity and ferritin level

		patient		control		P value
		No.	%	No.	%	
Anemia	Mild	4	6.5	3	4.64	0.0001
	Moderate	35	56.5	1	1.53	
	Severe	20	32.2	0	0	
	No Anemia	3	4.8	61	93.83	
S. Ferritin Level	High	61	98.4	1	1.5	0.0001
	Normal	1	1.6	64	98.5	

Anemia was found in (95.2%) of patient and (6.1%) of controls [P-Value=0.0001] as statistically significant. Hence, anemia is much higher in patients' group.

Ferritin was high in (98.4%) of patient, while it was normal (98.5%) of controls [P-Value=0.0001] which was highly significant. Thus, ferritin is much higher in thalassemia group.

Table 6: Comparison of patient and controls lipid profile values

Lipid profile levels patient& controls								
Lipid type	**Severity**	**Patient=62**		**Control=65**		**Total=127**		**P value**
		No.	**%**	**No.**	**%**	**No.**	**%**	
Cholesterol T-C	Normal	59	95.16	65	100	124	97.7	0.1
	Intermediate	2	3.22	0	0	2	1.6	
	High	1	1.6	0	0	1	0.7	
Triglycerides TG	Normal	10	16.2	50	79.9	60	70.88	0.003
	Intermediate	40	64.5	15	23	27	21.25	
	High	12	19. 3	0	0	10	7.87	
HDL-C	Normal	0	0	24	36.9	24	18.9	0.00003
	Intermediate	38	61.3	30	46.1	68	53.54	
	Low	24	38.7	11	17	35	27.56	
LDL-C	Normal	59	95.16	65	100	124	97.7	0.1
	Intermediate	2	3.22	0	0	2	1.6	
	High	1	1.6	0	0	1	0.7	
	Total	62	48.8	65	51.2	127	100	

S. lipid profile values

Cholesterol: All controls had normal levels of (Cholesterol) TC, while patients had 3\62 of cases had intermediate & high TC, which is not statistically significant (p-value = 0.1).

Triglyceride: was high and intermediately high in (83.9%), while normal level in (76.9%) of control group which is highly significant (p-value =0.003).

LDL-C: Was at optimal normal level in (95.2%) of cases and normal in (100%) of control group, while it was high and intermediately high in (4.8%) of patients group only, this was not statistically significant (P- Value = 0.1).

HDL: All patients (100%) had low or intermediately low levels, while controls had normal levels in (37%) that are strongly significant (P-Value= 0.00003).

We found **slight female predominance** over male in a ratio of (1.2:1) which was agreed a study [9] in which (56.4%) of children were female, while it was disagreed another study [10] in which male: female ratio was (2.5:1). Our female predominance was probably not real; it may be related to our higher female gender in our studied group and in our society as well.

Patient mean age was (mean ±SD= 10.975 ± 8.044142) agreed a study [11] in which mean age was 10 years, while it was disagreed [12] mean + SD = 7.74±4.03 [12], this probable age may be related partly to the delayed diagnosis because of poor family's awareness of thalassemia diseases especially in early life, and partly related to the fact that older children will develop fatal problems like cardiac complications or other major problems as neural and hormonal deficiencies (hypothyroidism) or psychosocial disturbances which affect their attendance to thalassemia center as well as therapy compliance.

95.2% of patients were **anemic** because of the continued hemolysis as thalassemia is a hemolytic process due to hemoglobinopathy, this high anemia rate especially in older age groups in spite of assumed regular blood transfusion needs further evaluation to identify the real cause whether doctors are not following strictly the treatment protocol of super transfusion (Hb> 9 g\dl) or due to the possibility of patients and caregivers' poor compliance and ignorance or due to other reasons.

The high Serum ferritin in (98.4%) of cases indicates failure of iron chelating protocol therapy assumed mostly due to deferoxamine shortage or to patients' poor compliance, agreed by a study [13].

Thalassemia was positive in (45.2%) of **family members,** while positive in (43.5%) of **relatives** was disagreed a study in south of Iraq [14]. They found it much higher in family members (59.9%)

while less in relatives (36.5%). This may be related to the higher consanguinity marriages in Misan society specifically which correlates to a higher percentage in relatives, as well.

We also found significantly **low Hb level in patients compared to controls** (p <.0.0001) was agreed with other studies [12], [15], because it is a chronic hemolytic disease with rapid decline in Hb concentration that is why they need frequent blood transfusion (hypertransfution).

Serum ferritin levels: were significantly higher in patients compared to controls (p<0.0001) that was agreed [12], this indicated an existing iron overload due to frequent blood transfusions and increased iron gut absorption in spite of iron chelating drugs intake. This required further studies to know whether it is related to poor compliance or poor drug efficacy and responses or dietary habits which might interfere with iron chelating drug activity. **LDL and TC** had no correlation with Hb level, disagree a study [12] in which there was a significant decrease in TC with a decrease in hemoglobin. We also found a positive and strong significant correlation between (TG & LDL-C) and low Hb levels which was agreed [12].

After comparing lipid profile between cases and control

The majority of patients had **lower (HDL-C)** values than controls. The low HDL-cholesterol levels in our patients were presumably due to excessive clearing of HDL by the activated macrophages.it considered as a predictive value of cardiovascular risk in patients with (B-thalassemia major). This was in agreement with another study [12]

The study was found relatively **mild increase in (LDL) and (TC)]** levels in patients compared to controls which was agreed with a study [10], while it was disagreed another study [12] in which TC were lower in patients than controls. The pathogenesis of these abnormalities is not exactly clear; the purposed mechanisms include increased erythropoietic activity because of chronic hemolysis resulting in increased cholesterol requirements and production.

The current study also revealed slight increase in **(TG)]** compared to controls probably due to the extra hepatic lipolytic activity, this result was agreed with 2 studies [16], [17]. While disagreed with other [18] as it revealed nearly the same triglyceride levels in both groups.

We found negative correlation between high **serum ferritin and (TC), (LDL-C)** which are the same result of a study [12], while **positive** correlation between high **ferritin and high (TG), low (HDL-C)** similar to result of another study [19]. Thalassemic children with high serum ferritin and pathological indices of lipid profile should be considered as risk factors for heart disease, and thus require careful and regular monitoring and follow-up.

Conclusion

The present study concluded a positive correlation between (high TG, low HDL) and (low Hb, high ferritin levels) which may attribute to the future fatal cardiac complication. Thus, lipid profile monitoring as well as Hb and s. ferritin level are necessary to prevent or at least enhance the early detection of cardiac complications.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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