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The Splenectomy Effects on Lipid Profile and Glucose Metabolism in the Major Thalassemia Patients

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ABSTRACT

Background: Splenectomy is a common treatment for beta thalassemia. It not only eliminates many complications by reducing the need for blood transfusion, but also causes new complications that threaten the patients' health. The aim of this study was to determine if splenectomy could alter the lipid profile and glucose metabolism in beta thalassemia major patients. **Methods:** In this case-control study, 41 splenectomized and 42 non-splenectomized eligible beta thalassemia patients were selected from Zafar Thalassemia Clinic, Tehran, Iran. Anthropometric, demographic, and biochemical data were collected using standard methods. Physical activity and food intake were measured using International Physical Activity Questionnaire (IPAQ) and food frequency questionnaires (FFQ), respectively. **Results:** Demographic characteristics and dietary intake were not significantly different between the two groups. However, triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), TC/HDL-C, LDL-C/TG, and LDL-C/HDL-C ratios were significantly higher, but HDL-C was significantly lower in splenectomized patients ($P < 0.05$). Furthermore, fasting blood glucose ($P < 0.39$) and oral glucose tolerance test ($P < 0.53$) did not significantly differ between the two groups. **Conclusions:** Reduced activity of the reticuloendothelial system and reduced removal of cholesterol might be the reason for higher plasma lipid profile and greater risk of cardiovascular diseases in splenectomized patients. On the other hand, glucose metabolism was not affected by splenectomy in adult patients. To clarify this relationship, prospective studies are suggested.

Keywords: *Thalassemia; Splenectomy; Lipid profile; Glucose*

Introduction

Thalassemia, as the most common hemoglobinopathy worldwide, has higher prevalence in the Mediterranean area, the Middle East, and Southern Asia (Wehr *et al.*, 2010). Beta thalassemia major is a sever hemolytic anemia, which causes a defective hematopoiesis and necessitates frequent blood transfusion (Muncie Jr and Campbell, 2009). Given that spleen performs vital hematologic activity and filters the defected blood cells, its activity is increased in this disease.

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This hyperactivity causes splenomegaly, so that the patient has to be splenectomized due to the discomfort, abdominal pain, and false satiety (Crary and Buchanan, 2009, Nishi *et al.*, 2010). Partial or complete removal of the spleen reduces the need for the blood transfusion, which reduces the iron overload consequently. However, it induces new complications such as increased pulmonary arterial pressure, sepsis, thrombosis, reduced response to encapsulated bacteria, and increased susceptibility to infection (Bhatia and Cairo, 2009, Cappellini *et al.*, 2014, Piga *et al.*, 2011).

Altered lipid profile seems to be another complication emerging after the splenectomy. Animal studies reported increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-C) in asplenic patients. This effect was stronger in animals fed with high-fat diets (Fatouros *et al.*, 1995, Kanichi *et al.*, 1988). Caligiuri et al. demonstrated that splenectomy accelerated atherosclerosis in mice (Caligiuri *et al.*, 2002). As the most frequent cause of death in thalassemia is cardiopathy (69%) (Ceci *et al.*, 2006), investigating the changes in lipid profile after the splenectomy is of high importance.

Splenectomy can indirectly affect the glucose metabolism as well (Rosa *et al.*, 2015). Observations suggest that serum lipopolysaccharide levels are increased in splenectomized patients (Feleder *et al.*, 2003, Yoshida *et al.*, 1995).The spleen is an important bacteria debugger and the absence of this lymphoid organ causes the liver's Kupffer cells to assume the role of clearing Lipopolysaccharides (LPS) (Ge *et al.*, 1997, Sivitz *et al.*, 1997). In turn, Kupffer cells increase the production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, interfering with insulin signaling and glucose transport (Feleder *et al.*, 2003, Sivitz *et al.*, 1997), which increases fasting blood glucose (FBG). Children with severe thalassemia who required splenectomy were noted to develop insulin resistance due to the interaction of spleen and pancreas (Lee *et al.*, 1985). We suspect that splenectomy might play a role in the high prevalence of diabetes mellitus in thalassemia patients.

Since splenectomy is prevalent among beta thalassemia major patients, we decided to find out the effect of splenectomy on these patients' health through glucose and lipid metabolism alteration.

Materials and Methods

Study population: This case-control study was conducted in Zafar Thalassemia Clinic, Tehran, Iran from January to February 2019. This clinic is a referral center for thalassemia patients to receive governmental and free health services. Forty one splenectomized and forty two non-splenectomized patients were recruited to participate in this study through convenient sampling method. Participants aged 18-30 years who had a history of using standard iron-chelating drugs, were non-alcoholic, non-smoker, and did not have any metabolic or infectious diseases were selected to participate in this research. Splenectomized patients had undergone the surgery at least a year before the study. All participants received multivitamin-mineral supplement during the past six months. *Measurements*: The participants' height was measured to the nearest 0.01 cm while they were wearing no shoes. Their weight was also measured in light clothes without shoes and with a digital scale to the nearest 100 g. Body mass index (BMI) was calculated using weight (kg) divided by squared height (m). All measurements were performed using standardized procedures. Other information including age, gender, iron chelator drug, and blood transfusion intervals of the patients were obtained from their medical records.

To control the effect of confounders, dietary intake and physical activity were measured using reliable and validated questionnaires. Dietary data was collected using food frequency questionnaire (FFQ) (168-item), which represented the food intake throughout the last year. The information was collected using a face-to-face interview by a trained interviewer.

Information on physical activity was obtained using the International Physical Activity Questionnaire (IPAQ) (Moghaddam *et al.*, 2012).

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Blood samples were collected at least 10 days after the last blood transfusion and were obtained after 12 hours of fasting overnight. Samples were analyzed for FBG, oral glucose tolerance test (OGTT), TG, TC, HDL-C, LDL-C, TC/HDL-C, LDL-C/TG, and LDL-C/HDL-C ratio using ELISA method (Ebioscience, USA). Hemoglobin (Hb), ferritin, white blood cells (WBC), and platelet were also measured as confounders.

Ethical considerations: All participants signed a written informed consent to enter the study. The protocol of this study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC 1397.97-04-207-31244).

Data analysis: The Kolmogorov-Smirnov test was run to test normality. The data for quantitative variables were reported in the form of mean ± Standard Deviation (SD). Independent *t-test* was applied to compare the mean of quantitative outcomes between the two groups. The ANCOVA analysis was used to examine the association between splenectomy, serum lipid, and glucose indices by adjusting the confounding variables. P- value < 0.05 was considered statistically significant. All analyses were performed using Statistical Package for Social Sciences (version 21.0, SPSS Inc, Chicago, IL).

Results

Comparison of the clinical and biochemical characteristics between splenectomized and non-splenectomized participants (**Table 1**) showed that 66.1% of them used desferrioxamine, 17.7% used deferiprone, and 16.1% used both drugs as iron chelator. Furthermore, 48.9% of the participants were male and 51.1% were female. No significant difference was observed between the two groups in clinical characteristics and physical activity. Splenectomy reduced the need for blood transfusion in splenectomized patients ($P = 0.001$) significantly. The WBC ($P = 0.001$) and platelet ($P = 0.02$) were significantly higher in splenectomized participants.

As observed in **Table 2**, no significant differences were found between the two groups regarding energy and macronutrients' intake.

The correlation of splenectomy with glucose and lipid profile of the participants is represented in **Table 3**. According to ANCOVA analysis, after adjusting for blood transfusion intervals, WBC, and Platelet, splenectomy had no significant association with FBG or OGTT, but HDL-C was significantly lower. Moreover, the TG, TC, LDL-C, VLDL-C, TC/HDL-C, LDL-C/TG, and LDL-C/HDL-C ratios were significantly higher in asplenic participants ($P < 0.05$).

Table 1. Comparison of mean ± SD of anthropometric, physical activity score and laboratyry variables between splenectomized and non-splenectomized patients.

Variables	Splenectomized (n=41)	Non-splenectomized (n=42)	P-value[a]
Age (y)	28.59 ± 6.33	29.79 ± 6.55	0.92
Weight (kg)	58.31 ± 6.08	57.15 ± 6.36	0.69
Height (cm)	157.44 ± 4.89	159.44 ± 4.81	0.63
Body mass index (kg/m^2)	23.51 ± 2.11	22.47 ± 2.21	0.44
Blood transfusion intervals (day)	93.21 ± 8.01	16.90 ± 3.49	< 0.001
Hemoglobin (g/dl)	9.80 ± 1.03	9.72 ± 0.92	0.79
Ferritin (ng/mL)	1249.87 ± 576.27	1385.56 ± 652.14	0.14
Wight blood cell (×10^3 cells/l)	27.95 ± 4.29	5.87 ± 0.89	< 0.001
Platelet (×10^3 cells/l)	528.70 ± 146.74	284.21 ± 21	0.003
Physical activity score	447.96 ± 393.74	376.6 ± 338.61	0.25

[a]: *Student t-test*

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Table 2. Comparison of mean ± SD of daily dietary nutrients intake between splenectomized and non-splenectomized patients.

Nutrients	Splenectomized (n=41)	Non-splenectomized (n=42)	P-value[a]
Energy (kcal)	1541.45 ± 518.86	1412 ± 401.52	0.21
Protein (g)	51.02 ± 13.48	54.30 ± 13.73	0.81
Carbohydrate (g)	236.35 ± 26.17	203.29 ± 24.02	0.34
Fat (g)	56.14 ± 13.19	51.25 ± 13.11	0.48
Cholesterol (g)	270.33 ± 118.45	224.38 ± 102.28	0.10
Fiber (g)	6.52 ± 3.10	5.63 ± 3.07	0.48

[a:] *Student t-test*

Table 3. Comparison of mean ± SD of biochemical characteristics between splenectomized and non-splenectomized patients.

Variables	Splenectomized (n=41)	Non-splenectomized (n=42)	P-value[a]
FBG (mg/dl)	79.54 ± 6.42	83.43 ± 6.96	0.39
OGTT (mg/dl)	138.70 ± 12.40	127.56 ± 14.37	0.53
TG (mg/dl)	2.59 ± 0.26	1.61 ± 0.26	0.01
TC (mg/dl)	3.73 ± 0.46	3.10 ± 0.44	0.04
HDL-C (mg/dl)	0.87 ± 0.25	1.47 ± 0.18	0.001
LDL-C (mg/dl)	2.72 ± 0.44	1.52 ± 0.34	0.001
VLDL-C (mg/dl)	0.54 ± 0.07	0.37 ± 0.05	0.02
TC/HDL-C ratio	4.64 ± 1.55	2.13 ± 0.42	0.001
LDL-C/TG ratio	1.10 ± 0.21	0.97 ± 0.30	0.04
LDL-C/HDL-C ratio	3.34 ± 1.10	1.03 ± 0.23	0.001

[a]*: ANCOVA test, FBG: fasting blood glucose, OGTT: oral glucose tolerance test, TG: triglycerides, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, VLDL-C: very low density lipoprotein cholesterol, LDL-C: low density lipoprotein cholestero,*

Discussion

According to our findings, splenectomy could not affect FBG and OGTT, but it significantly decreased HDL and increased TG, TC, LDL-C, VLDL-C, TC/HDL-C, LDL-C/TG, and LDL-C/HDL-C ratios in beta thalassemia major patients.

In the 1970s, Robinette and Fraumeni Jr. observed a high mortality rate due to the cardiovascular diseases along with reduction in the serum lipids in soldiers who were splenectomized during the World War II. (Dennis Robinette and Fraumeni, 1977). In addition, other researchers investigated the relationship between splenectomy and serum lipid profile in human and animal studies, most of which noted the same results. Asai K. et al. found that splenectomized rabits ended up with atherosclerosis due to higher TG, TC, LDL, and lower HDL in comparison with the non-splenectomized rabits (Asai, 1988). Petroianu A. et al. observed higher TC and LDL and lower HDL in splenectomized female rats but no differences were observed between the two groups in VLDL and TG. They also reported that partial splenectomy would not induce such metabolic alterations that represent the important role of spleen in the lipid metabolism (Petroianu *et al.*, 2006).

Our study also suggests a possible role for the spleen in lipid metabolism. A mechanism to explain the regulation of plasma lipids by the spleen can be the decreased activity of the reticuloendothelial system in the absence of the spleen. Under normal circumstances, macrophages absorb LDL and VLDL lipids via phagositisis, which transfers them to the spleen that is responsible for most of the LDL and VLDL catabolism. According to M. Aviram et al., this

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mechanism is responsible for the altered cholesterol metabolism in patients with myeloproliferative Disorder who have undergone splenectomy (Aviram *et al.*, 1986).

A. Petroianu et al. suggested that changes in serum lipidograms observed after splenectomy may be due to the catabolic effect of surgical trauma on the organism, not the absence of spleen itself (Petroianu *et al.*, 2008). However, this seems not to be the case in this study considering our inclusion criteria.

Higher TC/HDL-C, LDL-C/TG, and LDL-C/HDL-C ratios in asplenic patients suggest that splenectomized patients are in greater cardiovascular risk than non-splenectomized ones. Bordbar M. et al. proclaimed that LDL-C/TG ratio, which predicts LDL-C oxidation, is higher in young thalassemia patients than the healthy members of the control group (Bordbar *et al.*, 2012). Boudrahem-Addour N. et al. noted that splenectomy constitutes an additional high risk factor for cardiac complications regarding TC/HDL-C, LDL-C/TG, and LDL-C/HDL-C ratios in thalassemia patients (Boudrahem-Addour *et al.*, 2015). Splenectomy not only causes intravascular thrombosis and pulmonary hypertension, but also enhances the risk of coronary heart disease in thalassemia patients who are already at risk due to their heart's iron overload.

According to our findings, no significant differences were observed between splenectomized and non-splenectomized patients regarding FBG and OGTT. Findings suggest that spleen plays an important supportive role in the endocrine function of the pancreas and adjustment of blood glucose level in children (Ley *et al.*, 2012). This can be caused by the unique population of cells located in the splenic capsule capable of differentiation into pancreatic islets (Kodama *et al.*, 2003, Ley *et al.*, 2012). However, according to our participants' age, the glucose metabolism can be affected by factors other than splenectomy such as iron deposition in pancreas and inflammation caused by iron overload, which indirectly affect insuline signaling and blood glucose level (Mowla *et al.*, 2004, Zuppinger *et al.*, 1979). A review study suggested that serum ferritin levels and iron content of endocrine glands, especially liver, Thyroide, and kidney were important factors in predicting glucose intolerance in thalassemia patients (De Sanctis *et al.*, 2016). We could not assess the iron deposition in endocrine glands using the magnetic resonance imaging (MRI) method in our study due to the financial limitations. However, since the ferritin level of our participants was less than 2000 ng/ml, we could guess that the iron overload in the mentioned tissues has not been enough to cause dysfunction.

We faced some limitations in this study. First, the patients' diet was assessed using FFQ, which is based on self-reported dietary intake. Therefore, information bias might have occurred. Second, although we controlled many confounders, some other confounders might have been missed including the level of measured indices before the splenectomy in splenectomized patients. Retrospective design of this study was another defect because splenectomy could alter glucose metabolism in a longer period of time among older patients. Finally, lack of a direct and precise measurement of the amount of tissue's iron deposition confused us since we could not determine whether this finding was the effect of splenectomy or iron overload. The strengths of this study include adjusting for various confounders, using validated and reliable measurements, and sampling in a referral clinic.

Conclusion

According to our observation, splenectomy may affect the plasma lipid profile and the risk of cardiovascular disease, but not the glucose metabolism in adult beta thalassemia major patients. Further prospective studies are required with larger number of patients, longer follow up periods, and stronger analysis to better reveal the possible association between splenectomy and metabolic health of these patients.

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Conflict of interest

None

Author's contributions

All authors were involved in designing, developing, and drafting this manuscript. Sajedi-Hezaveh Z and Hadidi M cooperated in data collection and data analysis. Shidfar F was involved in interpretation of the results and critical revision. All authors approved the final draft of manuscript.

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