Research Article

Evaluation of serum trace elements, lipids profile, ferritin and 8-hydroxydeoxyguansine levels in Yemeni patients with beta thalassemia major

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Abstract

Objective: The aims of this study was to evaluate the serum level of some trace elements (Copper, zinc and magnesium), lipid profile and 8-hydroxydeoxyguansine(8-OHdG),ferritin levels in Yemeni patients with BTM. **Materials and Methods:** In this comparative cross sectional study, forty BTM blood transfusion dependent patients 4 to 22 years old who attended the Yemeni Society Centers for Thalassemia and Genetic Blood Disorders in Yemen were included. Forty healthy subjects served as control group .The serum level of trace elements (Copper, zinc and magnesium) were performed by humolyzer 3500 using spectrum diagnostic reagent kit. The serum levels of 8-OHdG was measured manually by enzyme linked immunosorbent assay (ELISA) kit. Chemiluminescent Microparticle Immunoassay (CMIA) was used for the quantitative determination of ferritin in human serum and plasma .Serum levels of lipid profile were enzymatically determined using reagent kit. **Results:** There was a significant elevation in serum level of zinc, 8-OHdG and ferritin in thalassemic patient as compared to controls. Copper and magnesium serum level were significantly decreased in BTM patient as compared to control. In addition total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein(HDL) were significantly lowered in BTM patient. **Conclusion:** The study showed Yemeni patients with BTM have high serum level of 8-OHdG and ferritin which reflect an increase oxidative stress status. Also the results showed low serum level of copper and magnesium, and a high serum level of zinc and dyslipidemia.

Keywords: Beta Thalassemia Major, Trace Elements**,** 8-hydroxydeoxyguansine, oxidative stress

Introduction

Beta-thalassemia is a group of frequent genetic disorders resulting in the synthesis of little or no β-globin chains. Beta Thalassemia Major (BTM) is the most common chronic hemolytic anemia among children and adolescents worldwide (Cassinerio et al., 2012) ,most prevalent in certain malaria-prone parts of the world including Africa, all Mediterranean countries, the Middle East, the Indian subcontinent, and Southeast Asia (Modell and Darlison, 2008).

Patients with BTM need repeated blood transfusions for survival due to severe anemia. Recurrent blood transfusions lead to accumulation of excess iron in the body tissues. Overload of iron is responsible for lipid peroxidation and oxidative stress by increased production of reactive oxygen species within the erythrocytes (Shazia et al., 2012). Many studies showed a fluctuation in the trace elements in BTM patients that led to many complications of disease. BTM is one of the important hemoglobinopathies in Yemen and with its clinical severity; it is considered a major health problem in Yemen. The prevalence of BTM among hereditary hemoglobinopathies patients in Yemen was reported to be 13% (8.6% for α-thalassemia and 4.4% for β-thalassemia traits) in Sana'a city (Al-Nood, 2009).

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The prolonged instability in Yemen because of conflict has led to an obvious deterioration in the health services in general with the absence of essential drugs such as chelating drugs specifically. These problems are the cause of major tragedies among Yemeni patients. There have been no studies evaluating the levels of trace elements, oxidative stress status and lipid profiles in BTM Yemeni patients. So the aims of this study to evaluate serum level of some trace elements, 8-OHdG and ferritin (indicator of oxidative stress and iron over load), and lipid profiles in BTM Yemeni patients.

Materials and Methods

This Comparative cross-sectional study was carried on forty with BTM blood transfusion dependent patients with the ages of 4-22 years old recruited in 2017 from the Yemeni Society Centers for Thalassemia and Genetic Blood Disorders in Sanaa city.

Exclusion criteria of this study include Subjects who have hepatitis B, C virus, cirrhosis, diabetes mellitus and malignancy.

The patients were included in the study if their parents agreed and signed the consent form. All parents were given clear explanations regarding the methodology of the research. The present study was approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana'a University.

A questionnaire for all participants was filled including the subject's personal and clinical information including the age, number of blood transfusion per year, iron chelator therapy, complications, surgery, drug supplements and family history along with anthropometric measurements.

Under sterile conditions, 5 ml of venous blood sample were collected from each subject (BMT patient and from control group) in the morning (after 10 to 12 hour fasting), into suitable blood collection test tubes. These samples were divided into two test tubes, one samples with EDTA anticoagulant materials for hematological profiles that were determined for all samples using a hematological analyzer Sysmex (KX-21N) for complete blood counts (CBC)and another without anticoagulants materials for biochemical assay.

The serum was separated within 30 minutes at 3000 rpm for 10 minutes and then dispensed them into two separated labeled cups one for 8-OHdG, Ferritin and the other for the measurement of zinc, copper, magnesium, and lipid profile .After separation, all sera immediately were frozen at -20$^{C^{\circ}}$ until usage.

Biochemical assays

Serum levels of 8-OHdG was measured by using Microplate ELISA reader (HumaReader HS) and enzyme linked immunosorbent assay (ELISA) commercial kit according to the manufacturer's instructions (Sunlong Biotech CO, LTD, Cat. No: SL2044Hu).

Serum ferritin was measured by immunoassay analyzer (Architect i 1000 SR) and Human ferritin commercial kit according to the manufacturer's instructions (CMIA) (Abbott-Germany Ref. 7K59).

Serum level of zinc was measured by Spectrum diagnostic Kit (Colorimetric Method with 5-Brom-PAPS) (Egyptian Company for Biotechnology) (Burtis et al., 2005; Johnsen and Eliasson, 1987).

Humolyzer 3500- and Spectrophotometer Spectrum diagnostic Kit colorimetric test with Dibrom-PAESA (Egyptian Company for Biotechnology) was used for measured the serum level of copper (Abe et al., 1989).

Serum level of magnesium, TC, triglyceride (TG) and HDL was measured by Humolyzer 3500- Spectrophotometer and commercial kit according to the manufacturer's instructions (Biochemistry Reagents QCA). LDL was calculated by the Friedwald formula, LDL (mg/dl) = TC -[HDL + TG/5] (Friedewald et al., 1972).

Statistical Analysis

The statistical analyses were performed on Social Package of Social Sciences (SPSS) version 11.5 (SPSS Inc, Chicago, IL, USA). The variables parameters were skewed and therefore normalized by logarithmic transformation. Means were subsequently back transformed for presentation as mean Stander division and geometric means 95% confidence interval. Independent t-test was used to compare differences in variables. The significant differences were indicated if p-value was < 0.05. The pearson was use for correlation relationship between different variables.

Results

The results showed a significant decrease in Weight, Height, BMI, Hemoglobin, Hematocrit and Red blood cells (RBC) ($P=1x10^{-3}$, $P=2x10^{-2}$, $P=2x10^{-3}$, $P=9.6x10^{-31}$, $P=1x10^{-25}$, $P=6.8x10^{-24}$) respectively in BMT patients compared to the control by (24.5%, 6.3%, 20.6%, 37%, 34.2%, 30.4%) respectively (Table 1).

Serum level of copper and magnesium were significantly decreased (P=7.4x10-21, P=1.9x10-9) in BMT patients compared to the control by (20.7%, 21%) respectively, which the results shows the serum level of zinc was significantly increased ($P=5.2x10^{-14}$) in BMT patients compared to the control by (46.7%) (Table 2).

The data in the table 3 show the serum level of 8-OHdG and ferritin that were significantly elevated ($P=3.8x10^{-12}$, $P=2.4x10^{-21}$) respectively, in BMT patients compared to the control by more than two fold.

As illustrated in table 4 TC, HDL and LDL were

Table 1. Comparison of Anthropometrical data and hematological parameters of BMT patients with their control subjects

Parameters	Control group (n=40)	BMT group (n=40)	P-value
Age(years)	9.5(8.3 -10.7)	9.3(8.1 -10.7)	0.903
Weight / kg	28.2 7.2	21.3 6.3	1x10 -3
Height /meter	128 15.8	120 17.0	2x10 -2
BMI(Kg/m2)	21.4 5.3	18.0 3.9	2x10 -3
Hb (g/dl)	12.7 1.0	8.0 1.1	9.6x10 -31
Hct %	38.0 3.6	25.0 3.8	1x10 -25
RBCs (10 -12/L)	4.6 0.38	3.2 0.45	6.8x10 -24

Data are expressed as mean ±SD and Geometric means (95% confidence interval of mean) P-value is significant at the <0.05.

Table 2. Comparison of trace elements of BMT patients with their control subjects.

Parameters	Control group (n=40)	BMT group (n=40)	P-value
Zn (g/dl)	105.1 9.1	154.2 32.6	5.2x10 -14
Cu (g/dl)	134.7 5.5	106.7 12.6	7.4x10 -21
Mg (mg/dl)	1.9 0.3	1.5 0.2	1.9x10

Data are expressed as meanSD. P-value is significant at the level < 0.05.

Table 3. Comparison of 8-OHdG and Ferritin of BMT patients with their control subjects.

Parameters	Control group (n=40)	BMT group (n=40)	P-value
8-OHdG (pg/dl)	239.8 (218 -263)	1023 (741 -1412)	3.8x10 -12
Ferritin (ng/dl)	38.9 (18.6 -83.1)	4897 (4073 -5754)	2.4x10 -21

Data are expressed as Geometric means (95% confidence interval of mean). P-value is significant at the level < 0.05

Table 4: Comparison of lipids profile of BMT patients with their control subjects.

Parameters	Control group (n=40)	BMT group (n=40)	P-value
TC (mg/dl)	147.5 32.6	104.6 19.2	1.8x10 -20
TG (mg/dl)	102.3 (97.7 -107.1)	114.8 (102.3 -128.8)	0.069
HDL (mg/dl)	39.8 (30.0 -41.6)	28.1 (25.1 -31.6)	1x10 -7
LDL (mg/dl)	85.1 (81.2 -89.1)	44.6 (38.1 -52.4)	3.8x10 -12

Data are expressed as meanSD and Geometric means (95% confidence interval of mean). P- Value is significant at the level < 0.05.

significantly decreased ($P=1.8x10^{-20}$,$P=1x10^{-7}$, $P=3.8x10^{-12}$) in BMT patients compared to the control respectively by (29.2%, 29.3%, 47.5%) respectively. However the serum level of TG showed an insignificant increase (P=0.069) in BMT patients compared to the control subjects.

A positive correlations was seen between copper and TG ($P=1x10^{-3}$), and also between 8- OHdG and Zinc ($P=2x10^{-2}$), however there were no such correlations between other parameters (Table 5).

Discussion

BMT is a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis (Galanello and Origa, 2010). It is characterized by metabolic disorders, iron overload; chronic hypoxia and cell damage. All physiological changes result in ineffective erythropoiesis, hemolysis, reduced Hb in red blood cells (RBC), decreased RBC production and anemia (Karim et al., 2016).

In the current study the patients showed low levels of Hb,

Table 5: Pearson,s correlation coefficients (r) between Zinc, Copper, Magnesium, 8-OHdG with the rest parameters in BMT patients

Parameters	Zn(r) (P. value)	Cu(r) (P. value)	Mg(r) (P. value)	8-OHdG(r) (P.value)
TC (mg/dl)	0.026 (0.874)	0.202 (0.202)	0.056 (0.736)	-0.108 - (0.508)
TG (mg/dl)	0.010 (0.951)	0.525 **(1x10-3)**	0.070 (0.669)	-0.033 -(0.841)
HDL(mg/dl)	-0.051 - (0.753)	-0.151 - (0.352)	-0.180 -(0.267)	-0.063 -(0.699)
LDL (mg/dl)	0.088 (0.588)	0.083 (0.611)	0.102 (0.502)	-0.023 -(0.889)
Zn (g/dl)	1	0.197(0.223)	0.249(0.121)	0.346 **(2x10-2)**
Cu (g/dl)	0.197(0.223)	1	0.178(0.271)	-0.021 -(0.895)
Mg (mg/dl)	0.249(0.121)	0.178(0.271)	1	0.083(0.609)
FBT/year	-0.065 -(0.690)	-0.182 -(0.261)	0.015(0.928)	-0.223 -(0.167)
Ferritin (ng/dl)	0.079(0.630)	0.181(0.263)	0.236(0.143)	-0.004 -(0.980)

Correlation is significant at the 0.05 level.

Hct and RBCs where compared to control subjects. These results are in alignment with previous results (Galanello and Origa, 2010; Karim et al., 2016; Shodikin et al., 2016). Anemia in the BMT children was caused by erythrocyte damage and ineffective erythrocyte synthesis due to globin chain defect (Galanello and Origa, 2010). Iron overload starts another pathological mechanism leading to oxidative damage of erythrocyte membranes (Petkov et al., 1990).

In contrast, Yemeni BMT patient suffers from poor or lack healthcare services and irregular administration of chelating agents. These situations result in over load of iron, and the results of this study showed a significant elevation of serum ferritin in BMT patients compared to control group. Serum ferritin levels are the most commonly employed test to evaluate iron overload in BMT (Mishra and Tiwari, 2013).

Meanwhile iron overload is responsible for peroxidative damage by increased production of reactive oxygen species, 8- OHdG is one of the predominant forms of free radical induced oxidative lesions and has therefore been widely used as a biomarker for oxidative stress (Valavanidis et al., 2009). The results of this study showed a significant serum elevation of (8- OHdG) in BMT in comparison to normal subjects. These results are in agreement with the results (El Gindi et al., 2015; Ferro et al., 2012), who found 8-OHdG was significantly increased in patients with BMT in comparison to normal control. However our results disagree with studies (El Gindi et al., 2015; Ferro et al., 2012) who found a positive correlation between 8-OHdG and ferritin in thalassemic patients.

The present study indicated a significant elevation of serum zinc and a significant decrease in serum level of magnesium and copper. Zinc is one of the essential micronutrients in human and considered as the most important mineral preceded by iron (Mahyar et al., 2010). It takes part in various important body functions including protein synthesis, DNA synthesis, and cellular growth (B WR, 2004). 84% of BMT patients had zinc deficiency most likely due to insufficient dietary zinc of intake, (Tabatabei et al., 2003) or due to hyperzincuria resulted from the release of zinc from hemolyzed red cells (Al-Samarrai et al., 2008).

The results of present study come in an agreement with other (Mansi et al., 2009). The study indicates that zinc level was significantly increased in BMT patients compared with controls. These finding may be explained by the decreasing rate of glomerular filtration of zinc seen in chronic hemolysis and the disturbances in its metabolism in BMT patients.

Copper is one of the essential micronutrients of human body. Its acts as the cofactor for at least 30 enzymes (JB, 2004). It possesses important antioxidant properties. It is a central component of the antioxidant superoxide dismutase molecule and also helps in the formation of a protein called ceruloplasmin thereby protecting the cells from free radical injury (Shazia et al., 2012). Our study reveals significantly reduce levels of serum copper in BMT patients as compared to control .This is an agreement with previous results (Bekheirnia et al., 2004; Eshghi et al., 2007), but disagree with reported studies (Al-Samarrai et al., 2008; Khaleel, 2014; Naji, 2012). Copper deficiency may be due to inadequate dietary copper intake due to gastrointestinal tract surgery, prolonged total parenteral nutrition and malabsorption enteropathies (Dembinski et al., 2012).

Our study indicated serum magnesium levels to be significantly lower in BMT patients as compared to control, which was in agreement with previous study by De Franceschi et al. (1998), but disagree with reported study by Khaleel (2014), who observed increase serum magnesium levels in patients with BMT compare to control.

Hypomagnesemia may be due to renal tubular dysfunction (Cetin et al., 2003). Decreased dietary intake, renal or gastrointestinal loss, and antacid therapy may also cause hypomagnesaemia (Al-Ghamdi et al., 1994; Jin-no Y et al., 1999).

Since the patients with beta-thalassemia major has multiple risk factors, like iron overload and oxidative stress that are the main implicated risk factors, as a result there are many alterations in carbohydrates and lipids. The present study showed significant decrease in serum concentration of TC, HDL and LDL receptively in BMT patients as compared to control subjects, this finding is in agreement with previous studies (Ragab et al., 2014; Sherief et al., 2017; Suman et al., 2017).

Dyslipidemia in BMT patients has been previously explained by several researchers who speculated different pathophysiologic pathways; plasma dilution because of anemia, accelerated erythropoiesis, increased uptake of LDL by macrophages and histiocytes of the reticuloendothelial system, hepatic damage due to iron overload and oxidative stress (Al-Quobaili and Asali, 2004; Amendola et al., 2007; Suman et al., 2017).

Our data showed increased serum concentration of TG in BMT compare to control, this results was in an agreement with reported studies (Ragab et al., 2014; Sherief et al., 2017; Suman et al., 2017). However our results disagreement to the reported studies (Asha Khubchandani et al., 2014; Paolo Ricchi et al., 2009) and studies done by (AB Patne et al., 2012) explained that the elevation of serum TG in BMT was due to reduce extrahepatic lipolytic activity.

Conclusion

The study revealed that the Yemeni patients with BMT have high serum level of 8-OHdG and ferritin which reflects over load of iron and increase oxidative stress status. Also the results showed that the Yemeni patient with BMT have a low serum level of copper and magnesium, and a high serum level of zinc and dyslipidemia. Deterioration in the health services in general with the absence of essential drugs such as chelating drugs play a big role in the increase of the complications in Yemeni beta thalassemia patients.

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Conflict of Interest

No conflict of interest associated with this work.

References

Abe A, Yamashita S, Noma A. 1989. Sensitive, direct colorimetric assay for copper in serum. Clinical Chemistry 35:552-554.

Al-Ghamdi SMG, Cameron EC, Sutton RAL. 1994. Magnesium deficiency: pathophysiologic and clinical overview. American Journal of Kidney Diseases 24:737-752.

Al-Nood H. 2009. Thalassemia trait in outpatient clinics of Sana'a City, Yemen. Hemoglobin 33:242-246.

Al-Quobaili FA, Asali IEA. 2004. Serum levels of lipids and lipoproteins in Syrian patients with beta-thalassemia major. Saudi Medical Journal 25:871-875.

Al-Samarrai AH, Adaay MH, Al-Tikriti KA, Al-Anzy MM. 2008. Evaluation of some essential element levels in thalassemia major patients in Mosul district, Iraq. Saudi Medical Journal 29:94-97.

Amendola G, Danise P, Todisco N, D'Urzo G, Di Palma A, Di Concilio R. 2007. Lipid profile in beta-thalassemia intermedia patients: correlation with erythroid bone marrow activity. International Journal of Laboratory Hematology 29:172-176.

Bekheirnia R, Shamshirsaz AA, Kamgar M, Bouzari N, Erfanzadeh G, Pourzahedgilani N, Tabatabaie SM, Shamshirsaz AA, Kimiagar M, Ezzati F. 2004. Serum zinc and its relation to bone mineral density in beta-thalassemic adolescents. Biological Trace Element Research 97:215-224.

Burtis CA, Ashwood ER, Bruns DE. 2005. Tietz Textbook of Clinical Chemistry and MolecularDiagnostics 4th ed. Philadelphia: W.B. Saunders.

Cassinerio E, Roghi A, Pedrotti P, Brevi F, Zanaboni L, Graziadei G, Pattoneri P, Milazzo A, Cappellini MD. 2012. Cardiac iron removal and functional cardiac improvement by different iron chelation regimens in thalassemia major patients. Annals of Hematology 91:1443-1449.

Cetin T, Oktenli C, Ozgurtas T, Yenicesu M, Sanisoglu SY, Oguz Y, Yildiz O, Kurt I, Musabak U, Bulucu F. 2003. Renal tubular dysfunction in beta-thalassemia minor. American Journal of Kidney Diseases 42:1164-1168.

De Franceschi L, Cappellini MD, Graziadei G, Manzato F, Olivieri O, Corrocher R, Fiorelli G, Beuzard Y, Brugnara C. 1998. The effect of dietary magnesium supplementation on the cellular abnormalities of erythrocytes in patients with beta thalassemia intermedia. Haematologica 83:118-125.

Dembinski K, Gargasz AE, Dabrow S, Rodriguez L. 2012. Three distinct cases of copper deficiency in hospitalized pediatric patients. Clinical pediatrics 51:759-762.

El Gindi HD, Hassanin AI, Mostafa NO, El Kassas GM, El Wakeel MA, El Batal WH, Galal EM, Elmalt HA, Amer MF. 2015. Oxidative DNA damage in beta-thalassemic children. Medical Research Journal 14:41-46.

Eshghi P, Alavi S, Ghavami S, Rashidi A. 2007. Growth impairment in beta-thalassemia major: the role of trace element deficiency and other potential factors. Journal of

pediatric hematology/oncology 29:5-8.

Ferro E, Visalli G, Civa R, La Rosa MA, Papa GR, Baluce B, D'Ascola DG, Piraino B, Salpietro C, Di Pietro A. 2012. Oxidative damage and genotoxicity biomarkers in transfused and untransfused thalassemic subjects. Free Radical Biology and Medicine 53:1829-1837.

Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18:499-502.

Galanello R, Origa R. 2010. Beta-thalassemia. Orphanet journal of rare diseases 5:11.

Jin-no Y, Kamiya Y, Okada M, Hirako M, Takada NMK. 1999. Primary hypomagnesemia caused by isolated magnesium malabsorption: atypical case in adult. Internal Medicine 38:261-265.

Johnsen Ã, Eliasson R. 1987. Evaluation of a commercially available kit for the colorimetric determination of zinc in human seminal plasma. International Journal of Andrology 10:435-440.

Karim MF, Ismail M, Hasan AKMM, Shekhar HU. 2016. Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. International Journal of Hematology-Oncology and Stem Cell Research 10:7-12.

Khaleel KJ. 2014. Biomarkers and trace elements in beta thalassemia major. Iraqi Journal of Cancer and Medical Genetics 6:81-86.

Khubchandani A, Solanki V, Parmar V, Solanki M, Patel J, Gangwani S. 2014. Estimation of Serum lipid profiles in patients with Beta-thalassemia major. International Journal of Research in Medical Sciences 3:65-67.

Mahyar A, Ayazi P, Pahlevan A-A, Mojabi H, Sehhat M-R, Javadi A. 2010. Zinc and copper status in children with Beta-thalassemia major. Iranian Journal of Pediatrics 20:297.

Mansi K, Aburjai T, Barqawi M, Naser H. 2009. Copper and zinc status in Jordanian patients with Beta-thalassemia major treated with Deferoxamine. Research Journal of Biological Sciences 4:566-572.

Mishra AK, Tiwari A. 2013. Iron overload in Beta thalassaemia major and intermedia patients. Maedica 8(1):328-332.

Modell B, Darlison M. 2008. Global epidemiology of haemoglobin disorders and derived service indicators. Bulletin of the World Health Organization 86:480-487.

Naji ZMA. 2012. Serum trace elements (zinc, copper and magnesium) in Iraqi patients with thalassemia major receiving desferrioxamine and its relation with growth state. Iraqi Journal of Medical Sciences 10:375-382.

Patne AB, Hisalkar PJ, Gaikwad S. 2012. lipid abnormalities in patients of beta thalassaemia major. International Journal of Pharmacy and Biological Sciences 2:106-112.

Petkov GH, Efremov GD, Efremov DG, Dimovski A, Tchaicarova P, Tchaicarov R, Rogina B, Agarwal S, Kutlar A, Kutlar F. 1990. beta-Thalassemia in Bulgaria. Hemoglobin 14:25-33.

Ragab SM, Safan MA, Sherif AS. 2014. Lipid profiles in beta- thalassemic children. Menoufia Medical Journal 27:66-72.

Ricchi P, Ammirabile M, Spasiano A, Costantini S, Di Matola T, Cinque P, Pagano L, Prossomariti L. 2009. Hypocholesterolemia in adult patients with thalassemia: a link with the severity of genotype in thalassemia intermedia patients. European Journal of Haematology 82:219-222.

Shazia Q, Mohammad ZH, Rahman T, Shekhar HU. 2012. Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in Beta thalassemia major patients: a review of the literature. Anemia 2012:1-7.

Sherief LM, Dawood O, Ali A, Sherbiny HS, Kamal NM, Elshanshory M, Alazez OA, Alhady MA, Nour M, Mokhtar WA. 2017. Premature atherosclerosis in children with beta-thalassemia major: New diagnostic marker. BMC Pediatrics 17:69.

Shodikin MA, Suwarniaty R, Nugroho S. 2016. Correlation between serum ferritin and cardiac troponin I in major beta thalassemia children. Journal of Tropical Life Science 6:10-14.

Suman R, Sanadhya A, Meena P, Singh J, Jain R, Meena S. 2017. Lipid profile in children of beta-thalassemia mayor and their correlation with serum ferritin. International Journal of Contemporary Pediatrics 4:543-547.

Tabatabei M, Kamkar M, Habibzadeh MR. 2003. Metabolic and endocrine complications in beta thalassemia major in a multicenter study. Tehran University Medical Journal 5:72-73.

Valavanidis A, Vlachogianni T, Fiotakis C. 2009. 8-hydroxy-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. Journal of Environmental Science and Health Part C 27:120-139.