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Ferric reducing capacity and oxidative stress index in sera and saliva of Iraqi patients with homozygous β-thalassemia major

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Abstract

Background and aim**:** β-thalassemia major is an autosomal recessive disease that leads to a severe hemolytic anemia in early infancy. Study of the oxidative stress in sera and saliva of homozygous β-thalassemia major patients is the aim of the present study.

Materials and methods**:** Serum and saliva specimens were collected from 91 males, 51 cases were β-thalassemia major patients, aged 11 to 26 years (mean= 15.275 ± 4.4501 years) and 40 were age- and gender-matched healthy controls.

Results**:** The results for the serum specimen showed a highly significant decrease ($P<0.001$) in total antioxidant status (TAS), and a highly significant increase ($P<0.001$) in ferric reducing capacity (FRC), total oxidant status (TOS), and oxidative index (OSI) in all studied patients compared with that of control group. While in saliva specimen, a non-significant decrease ($P>0.05$) in TAS with a non-significant increase ($P>0.05$) in FRC while a significant increase ($P<0.05$) in both TOS and OSI in the patients compared with that of control group.

Conclusion**:** The following can be concluded based on the results of the current study: the measured oxidative stress in Iraqi thalassemia patients due to the increase in FRC and the decrease in TAS. Furthermore, saliva can be used as an alternative fluid instead of serum for measurement of FRC in β-thalassemia major patients.

Key words

Thalassemia, oxidative stress, ferric reducing capacity, total oxidant status, total antioxidant status

الخلاصة

الخلفية والهدف: بيتا-الثلاسيميا الكبرى هو مرض وراثي متنحي يؤدي إلى فقر الدم الانحلالي الحاد في مرحلة الطفولة المبكرة. إن دراسة الإجهاد التأكسدي في الأمصال واللعاب لدى مرضى بيتا -الثلاسيميا الكبرى هو الهدف من هذه الدراسة.

المواد والطرق: تم جمع عينات المصل واللعاب من ٩١ من الذكور، وكانت ٥١ حالة من مرضى الثلاسيميا الكبرى، الذين تتراوح أعمارهم بين ١١ إلى ٢٦ سنة (متوسط = ١٥.٢٧٥ ± ٤.٤٥٠١ سنة) و٤٠ كانت الضوابط الصحية العمرية والجنسية المتطابقة.

النتائج: أظهرت نتائج عينة المصل انخفاضاً معنوياً ($P <0.001$) في الحالة الكلية لمضادات الأكسدة (TAS)، وزيادة معنوية ($P <0.001$) في القدرة الاختزالية للحديديك (FRC)، وحالة الأكسدة الكلية (TOS)، ومؤشر الجهد التأكسدي (OSI) في جميع المرضى الذين تمت دراستهم مقارنة مع مجموعة التحكم. بينما في عينة اللعاب، انخفاض غير معنوي ($P> 0.05$) في TAS مع زيادة غير معنوية ($P> 0.05$) في FRC بينما زيادة معنوية ($P <0.05$) في كل من TOS وOSI في المرضى مقارنة مع ذلك مجموعة السيطرة.

الخلاصة: يمكن استخلاص ما يلي بناءً على نتائج الدراسة الحالية: الجهد التأكسدي المقاس في مرضى الثلاسيميا العراقيين بسبب الزيادة في FRC والانخفاض في TAS. علاوة على ذلك، يمكن استخدام اللعاب كسائل بديل بدلا من المصل لقياس FRC في مرضى الثلاسيميا الكبرى.

الكلمات الدالة:

ثلاسيميا، الجهد التأكسدي، القدرة الاختزالية للحديديك، حالة الاكسدة الكلية، الحالة الكلية لمضادات الأكسدة

Introduction

Thalassemia is a group of inherited hemoglobin disorders characterized by reduced synthesis of one or more of the globin chains leading to imbalanced globin synthesis which is the major factor in determining the severity of the disease in the thalassemia syndromes. Beta-thalassemia results from a defect in beta globulin chain production and ranges from clinically silent heterogeneous thalassemia minor to severe transfusion-dependent thalassemia major **(Karim *et al*., 2016)**.

β-thalassemia major is an autosomal recessive disease that leads to a severe hemolytic anemia in early infancy **(Ghone *et al*., 2008)**. Depletion or impaired synthesis of β-globin chain can result in an imbalanced production of globin chains towards higher production of α-chain, which converts hemoglobin from a normal oxygen transporting function into toxic inclusion bodies, causing peripheral erythrocyte hemolysis **(Bazvand *et al*., 2008)**.

Oxidative stress has been reported to play an important role in many diseases such as renal diseases, cancer, cardiovascular failure, infections, neurological diseases, and non-alcoholic fatty liver disease etc. **(Phumala *et al*., 2003; Hasan and Mahmoud., 2017)**. This condition results from overproduction of different free radicals and deficiency of antioxidant defense system and causes damage of different biomolecules, such as DNA, lipids, and proteins. And this activates different damaging processes in the cells including oxidation of cell surface component of the erythrocytes in patients with β-thalassemia major **(Pavlova *et al*., 2007)**. Pavlova *et al*, and Ghone *et al*, measured a significant increase in the levels of lipid peroxide and a significant decrease in levels of total antioxidant capacity and vitamin E in Bulgaria patients with β-thalassemia major (**Pavlova *et al*., 2007**; **Ghone *et al*., 2008**).

The main cause of death in patients with thalassemia major is reported to due to the iron overload **(Taher *et al*., 2009).** In order to reduce the effect of iron overload, iron chelating therapy such as deferasirox has been used to treat patients with a various of haemoglobinopathies including thalassemia. This type of the therapy has been reported to reduce cardiac and hepatic iron levels **(Fragomeno *et al*., 2015).**

The aim of the present work: Is study of the oxidative stress status in sera and saliva of Iraqi patients with homozygous β-thalassemia major

Materials and Methods

Patients and healthy controls

This study included, 51 cases β-thalassemia major (males), aged 11 to 26 years (mean 15.275 ± 4.4501 years) attending Hereditary Hematology Center in Maysan/Iraq with 40 age- and gender-matched healthy controls. After diagnosis, all patients received approximately 350 to 500 ml of packed red blood cells (at 3 to 4-week intervals), and all were on iron chelation therapy using deferasirox (25-45 mg Kg^{-1} day^{-1}) and folic acid tablet (1mg/day).

Exclusion criteria

Patients and controls individuals who were thalassemia minors and had history of blood transfusion, anemia, liver disease, or active inflammatory conditions. Cushing's disease, acromegaly, chronic pancreatitis, pancreatactomy, chronic renal failure, malignancies and chronic, or acute inflammatory disease, as well as those patients who were taking drugs, lipid lowering therapy, history of smoking, or alcohol drinking were excluded. The study protocol conforms to the ethical guidelines, endorsed by the College of Science, University of Baghdad ethics committee**.**

Methods

Collection of samples

Sera and saliva samples were collected in plain tubes from thalassemia patients' samples before the next blood transfusion session. Blood and unstimulated saliva samples were taken from the individuals of all groups after overnight fasting and the participants were asked to rinse their mouth with saline before collecting the saliva samples. The saliva samples were centrifuged at (2400×g) for 10 minutes, then the supernatant was kept frozen to be used for the desired measurements. At the same time, 10 mL of blood were withdrawn from the same individual in serum tube then centrifuged at (3000 x g) for 5 minutes. The sera samples were collected and kept frozen to be used for the determinations of the different studied parameters.

Determination of total oxidant status (TOS)

Total oxidant status was measured as described by Erel (**Erel., 2005**).

Determination of total antioxidant status (TAS)

Total antioxidant status was determined according to the method described by Erel **(Erel., 2004)**.

Calculation of oxidative stress index (OSI)

Oxidative stress index (OSI) in serum and saliva samples was calculated by using the following equation**:**

$$OSI = TOS\ (mmol\ H_2O_2\ Eq./L) / TAS\ (mmol\ glutathione\ Eq./L)$$

Determination of ferric reducing capacity (FRC)

This method was accomplished as described by Benzie and Strain **(Benzie and Strain, 1996)**.

Statistical analyses

The program SPSS by Licensed Materials version 22 computer software was used to analyze the present study data. Data in this study was presented as Mean± Standard deviation (Mean± SD) using Independent-samples T-Test to compare the mean. A value of ($p<0.05$), was considered as statistically significant, while a value of ($p<0.001$), was considered as a highly significant.

Results and discussion

In order to estimate the oxidative stress status in the present studied groups, several parameters related to this status were measured and as follows:

The total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI), were determined in serum and saliva samples of the studied patients according to Erel method **(Erel., 2004; Erel., 2005)**. Moreover, the ferric reducing capacity (FRC), based on ferric to ferrous iron reduction, was recently used to determine the antioxidant capacity was also measured **(Kang and Saltveit, 2002)**.

The results in the Table (١) show the comparison of the antioxidants status between the patients and control groups. These results for the serum specimen show a highly significant decrease ($P<0.001$) in TAS in all patients compared with that of control group, and a highly significant increase ($P<0.001$) in FRC. While in saliva specimen, a non-significant decrease ($P>0.05$) in TAS with a non-significant increase ($P>0.05$) in FRC are observed in the patients compared with that of the control group.

Table (١): Mean values± SD of ferric reducing capacity (FRC) and total antioxidant status in the serum and saliva of β-thalassemia major patients

Parameters	Controls group (N=40) Means ± standard deviation	Patients group (N=51) Means ± standard deviation	P-Value
Total antioxidant status (mmol Glutathione Eq./L) in serum	0.4935±0.0519	0.4114±0.1077	0.000**
Total antioxidant status (mmol Glutathione Eq./L) in saliva	0.6537±0.0361	0.6402±0.0655	0.216
Ferric Reducing Capacity(mM) in serum	0.3840±0.0631	0.4543±0.1027	0.000**
Ferric Reducing Capacity(mM) in saliva	0.1568±0.0411	0.1642±0.0385	0.380

*The difference is significant at the 0.05 level. **The difference is highly significant at the 0.001 level

The result of serum total antioxidant status agrees with some reported studies, such as the study of Manafikhi *et al* who depend on systematic review of the available literature and meta-analysis of conducted case-control studies included their work **(Manafikhi *et al*., 2017)**, and other results study such as by Jouda when he determined the TAS in male and female Iraqi patients with β-thalassemia major (4-30 years) using colorimetric detection kit **(Jouda., 2017)**, as well as a study by Ghone, *et*

al which showed a decrease in total antioxidant status in their Indian children patients **(Ghone *et al*., 2008)**. The results of serum specimen TAS also agree with the results of Selek *et al* who studied Turkish individuals who suffered from β-thalassemia minor **(Selek *et al*., 2007)**.

While the result of serum total antioxidant status (TAS) disagrees with some studies, such as study by Karakas *et al*, in which total antioxidant status (TAS) was measured and reported to be of a significant increase in Iranian thalassemic patient **(Karakas *et al*., 2015)** and of Darvishi-Khezri *et al*, study who determined TAS in Iranian β-thalassemia major patients (**Darvishi-Khezri *et al*., 2017)**. And disagree with a study in Turkey, by Cakmak *et al*, on their Turkish thalassemia major patients where no significant variation in TAS was reported **(Cakmak *et al*., 2010)**.

The results in the saliva specimen agree with the only study that has been found in the literature which dealt with measurement of TAS in the saliva of β-thalassemia major patients. This study has been carried out by Jouda who measured this parameter in male and female Iraqi patients with thalassemia (4-30) years old using colorimetric detection kit **(Jouda., 2017)**.

The observed decrease in TAS in serum of β-thalassemia major patients compared to their control group may reflect the role of antioxidants (enzymatic and non-enzymatic) in modulating the severity of this disease **(Hamed and Eimelegy., 2010)**. The present studied patients were under blood transfusion, this lead to iron overload. The iron overload can generate oxygen-free radicals and promote peroxidative damage to cell and organelle membranes in organs that accumulate the excess iron including liver, pituitary gland, pancreas and heart. In such condition, depletion of endogenous antioxidants may be expected **(Farzin *et al*., 2011)**. The accumulation of plasma (non-transferrin bound iron) NTBI in β-thalassemia major correlates with an increase in oxidation products and decrease in antioxidant capacity **(Ghatreh-Samani *et al*., 2016)**.

As far as, FRC measurement is concerned, there is no study in serum of β-thalassemia major patients, or any other types of this disease. Meanwhile, the non-significant increase measured in saliva FRC (Table 1) agree with Rahim *et al* who have measured the salivary oxidative stress in Indian and Chinese with β-thalassemia major patients **(Rahim *et al*., 2016).**

The measured unchanged in TAS and FRC in saliva specimen (Table 1) indicate that a balance exists between the formation of oxidant agents and the antioxidant defense system in this fluid **(Abdalla *et al*., 2011)**. The antioxidant activity of a serum against a free radical does not match its activity in the saliva specimen, because each of these fluid (serum and saliva) has their own different antioxidant defenses mechanisms **(Katalinic *et al*., 2005)**. In serum specimen of β-thalassemia major patients the observed increase in FRC at variance of TAS can be explained as follows: TAS measure all antioxidants present in the fluid in contract to FRC which measure the ability of the fluid to reduce Fe^{+2} to Fe^{+3}, so the variance arise may be because of the [uric acid] increase in β-thalassemia patients **(Bazvand *et al*., 2011),** Also the patients of the present study were taking folic acid as a supplement for red blood cells (RBCs) production. Both uric acid and folic acid act to help in increasing the antioxidant ability of β-thalassemia patients to the level almost similar to healthy controls **(Mohamed *et al*., 2012; Shalaby and Shanab., 2013)**.

Frei and his co-workers reported that the decreased level of antioxidants play important roles in the pathogenesis of anemias. Which indicated that erythrocytes might be expected to be highly susceptible to peroxidation **(Frei *et al*., 1990).** It is well documented that disturbances of oxidant-antioxidant balance occur in hemoglobinopathies **(Kattamis., 2001)**. Furthermore, it has shown that individuals with homozygous thalassemia have a reduced antioxidant capacity through decreasing the activity of individual's antioxidant enzymes and this may reflect the role of antioxidants in modulating the severity of the disease **(Hamed and Eimelegy., 2010)**.

In the current study TOS which give an idea of the total oxidants rather than individual one was measured. The results in the Table (2) shows the comparison between the total oxidant status as well as oxidative stress index in the patients and controls group. The results for the serum specimen shows a highly significant increase ($P<0.001$) in TOS and OSI in all studied patients compared with controls, while for the saliva a significant increase ($P<0.05$) was measured in both TOS and OSI in the patients compared with that of control groups.

Table (٢): Mean values± SD of total oxidant status and oxidative stress index parameters in the serum and saliva of β-thalassemia major patients

Parameters	Controls group (N=40) Means ± standard deviation	Patients group (N=51) Means ± standard deviation	P-Value
Total oxidant status (mmol H_2O_2 Eq/L) in serum	0.0197±0.0058	0.0371±0.0035	0.000**
OSI in serum	0.0403±0.0129	0.09849±0.0370	0.000**
Total oxidant status (mmol H_2O_2 Eq/L) in saliva	0.0096±0.0079	0.0130±0.0071	0.037*
OSI in saliva	0.0147±0.0120	0.02107±0.0132	0.019*

*The difference is significant at the 0.05 level. **The difference is highly significant at the 0.001 level

The result of TOS and OSI in serum agrees with few studies carried out in Turkey that measured individual oxidative stress parameters. Which showed that there was an oxidative stress in sera of their patient's. Such as the study by Selek *et al* who studied Turkish individuals that suffered from β-thalassemia minor **(Selek *et al*., 2007)**. Also, the present results agree with the study that done by Cakmak *et al* who studied serum Turkish patients with thalassemia major **(Cakmak *et al*., 2010)**.

Meanwhile, there are no studies reported in the literature which deals with the measurement of salivary TOS and OSI in β-thalassemia major.

Several causes may have expected lead to oxidative stress in β-thalassemias, among them are the following: In these patients, the ineffective erythropoiesis and premature hemolysis of erythrocyte in the peripheral circulation result leads to patients suffer from anemia **(Şimşek *et al*., 2005)**. impaired, or reduce biosynthesis of β-globin leads to accumulation of unpaired α-globin chain. Such accumulation of these chains was reported to be the primary reason for the cellular oxidative injury in β-thalassemia patients **(Chakraborty and Bhattacharyya., 2001)**. The other possible cause is the iron overload as a result of both high plasma iron and high intracellular non-hemoglobin iron in these patients. Moreover, repeated blood transfusions

received by these patients, leads to accumulation of excess iron in the body tissue **(Livrea *et al*., 1996)**. This secondary iron overload was responsible for pre-oxidative damage **(Khan., 2007).** All these reasons explain the observed elevation in both sera and saliva TOS and OSI (Table 2).

Correlation between serum and saliva of all studied parameters:

Serum / Saliva	Control, R: Personal correlation P: P-Value	Patient, R: Personal correlation P: P-Value
TOS	R: -0.45 P: 0.783	R: 0.005 P: 0.970
TAS	R: -0.424 P: 0.006	R: -0.261 P: 0.064
OSI	R: -0.072 P:0.657	R: -0.098 P:0.492
FRC	R: 0.202 P:0.212	R: 0.358** P:0.01

**Correlation is a highly significant at the 0.01 level.
*Correlation is significant at the 0.05 level.

It is obvious from the above results, no significant correlation ($p>0.05$) is present in all studied parameters except in FRC which is highly significant correlated ($p<0.01$) between sera and saliva of the patients group.

Conclusion

1-These patients have an oxidative stress which due to the decrease in TAS and increase in FRC eventhogh the decrease in TAS is non-significant.

2- Saliva can be used for measurement of FRC in β-thalassemia major patients instead of serum

References

Al-Elq, A. H., & Al-Saeed, H. H. (2004). Endocrinopathies in patients with thalassemias. *Saudi medical journal*, *25*(10), 1347-1351.

Abdalla, M. Y., Fawzi, M., Al-Maloul, S. R., El-Banna, N., Tayyem, R. F., & Ahmad, I. M. (2011). Increased oxidative stress and iron overload in Jordanian β-thalassemic children. *Hemoglobin*, *35*(1), 67-79.

Bazvand, F., Shams, S., Esfahani, M. B., Koochakzadeh, L., Monajemzadeh, M., Ashtiani, M. T. H., & Rezaei, N. (2011). Total antioxidant status in patients with major β-thalassemia. *Iranian journal of pediatrics*, *21*(2), 159.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.

Bazvand, F., Shams, S., Esfahani, M. B., Koochakzadeh, L., Monajemzadeh, M., Ashtiani, M. T. H., & Rezaei, N. (2011). Total antioxidant status in patients with major β-thalassemia. *Iranian journal of pediatrics*, *21*(2), 159.

Borgna-Pignatti, C. (2007). Modern treatment of thalassaemia intermedia. *British journal of haematology*, *138*(3), 291-304.

Cakmak, A., Soker, M., Koc, A., & Aksoy, N. (2010). Prolidase activity and oxidative status in patients with thalassemia major. *Journal of clinical laboratory analysis*, *24*(1), 6-11.

Chakraborty, D., & Bhattacharyya, M. (2001). Antioxidant defense status of red blood cells of patients with β-thalassemia and Eβ-thalassemia. *Clinica chimica acta*, *305*(1-2), 123-129.

Darvishi-Khezri, H., Salehifar, E., Kosaryan, M., Karami, H., Alipour, A., Shaki, F., & Aliasgharian, A. (2017). The impact of silymarin on antioxidant and oxidative status in patients with β-thalassemia major: A crossover, randomized controlled trial. *Complementary therapies in medicine*, *35*, 25-32.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 38(12), pp: 1103-1111.

Erel, O. (2004). A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem*. 37(2), pp: 112-119.

Farzin, L., Sajadi, F., & Kupai, L. (2011). Serum Antioxidant Levels in Children with Beta-Thalassemia Major. *Iranian Journal of Blood and Cancer*, *4*(1), 1-5.

Fragomeno, C., Roccabruna, E., & D'Ascola, D. G. (2015). Effect of deferasirox on iron overload in patients with transfusion-dependent haemoglobinopathies. *Blood Cells, Molecules, and Diseases*, *55*(4), 382-386.

Ghone, R. A., Kumbar, K. M., Suryakar, A. N., Katkam, R. V., & Joshi, N. G. (2008). Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. *Indian Journal of Clinical Biochemistry*, *23*(4), 337-340.

Ghatreh-Samani, M., Esmaeili, N., Soleimani, M., Asadi-Samani, M., Ghatreh-Samani, K., & Shirzad, H. (2016). Oxidative stress and age-related changes in T cells: is thalassemia a model of accelerated immune system aging?. *Central-European journal of immunology*, *41*(1), 116.

Hamed, E. A., & ElMelegy, N. T. (2010). Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. *Italian journal of pediatrics*, *36*(1), 39.

Hasan, H. R., & Mahmoud, H. Q. (2017). Oxidative stress status in sera and saliva relevance to alcoholic and nonalcoholic fatty liver disease in Iraqi patients. *Research journal of pharmaceutical biological and chemical sciences*, *8*(3), 1465-1473.

Jouda, J. (2017). Investigation of Antioxidant Status in Iraqi Patients with Beta-Thalassemia Major. *Journal of Global Pharma Technology*, *9*(7).

Khan, F. U. (2007). Frequency of complications In Beta thalassemia major in DI Khan. *Biomedica*, *23*(6), 31-3.

Karim, M. F., Ismail, M., Hasan, A. M., & Shekhar, H. U. (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*(1), 7.

Kang, H. M., & Saltveit, M. E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry*, *50*(26), 7536-7541.

Karakas, Z., Yilmaz, Y., Celik, D. D., Annayev, A., Demirel, S., & Kuruca, S. E. (2015). Total oxidant and antioxidant capacity in patients with transfusion dependent and nondependent beta thalassemia. Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. Clin Chem 2000;46:1723– 7.

Katalinic, V., Modun, D., Music, I., & Boban, M. (2005). Gender differences in antioxidant capacity of rat tissues determined by 2, 2′-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *140*(1), 47-52.

Kattamis, C., & Kattamis, A. C. (2001). Oxidative stress disturbances in erythrocytes of ß-thalassemia. *Pediatric hematology and oncology*, *18*(2), 85-88.

Livrea, M. A., Tesoriere, L., Pintaudi, A. M., Calabrese, A., Maggio, A., Freisleben, H. J., ... & Bongiorno, A. (1996). Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood*, *88*(9), 3608-3614.

Mohamed, R., Campbell, J. L., Cooper-White, J., Dimeski, G., & Punyadeera, C. (2012). The impact of saliva collection and processing methods on CRP, IgE, and Myoglobin immunoassays. *Clinical and translational medicine*, *1*(1), 19.

Manafikhi, H., Drummen, G., Palmery, M., & Peluso, I. (2017). Total Antioxidant Capacity in beta-thalassemia: A systematic review and meta-analysis of case-control studies. *Critical reviews in oncology/hematology*, *110*, 35-42.

Phumala, N., Porasuphatana, S., Unchern, S., Pootrakul, P., Fucharoen, S., & Chantharaksri, U. (2003). Hemin: a possible cause of oxidative stress in blood circulation of β-thalassemia/hemoglobin E disease. *Free radical research*, *37*(2), 129-135.

Pavlova, L. E., Savov, V. M., Petkov, H. G., & Charova, I. P. (2007). Oxidative stress in patients with beta-thalassemia major. *Prilozi*, *28*(1), 145-154.

Rahim, M. R. A., Tan, J. A. M. A., Mani, R. R., & Kuppusamy, U. R. (2016). Non-invasive Sampling for Assessment of Oxidative Stress and Pro-inflammatory Cytokine Levels in Beta-Thalassaemia Major Patients. *Revista Romana de Medicina de Laborator*, *24*(1), 83-92.

Selek, S., Aslan, M., Horoz, M., Gur, M., & Erel, O. (2007). Oxidative status and serum PON1 activity in beta-thalassemia minor. *Clinical biochemistry*, *40*(5-6), 287-291.

Shalaby, E. A., & Shanab, S. M. (2013). Antioxidant compounds, assays of determination and mode of action. *African journal of pharmacy and pharmacology*, *7*(10), 528-539.

Frei, B., Stocker, R., England, L., & Ames, B. N. (1990). Ascorbate: the most effective antioxidant in human blood plasma. In *Antioxidants in therapy and preventive medicine* (pp. 155-163). Springer, Boston, MA.

Selek, S., Aslan, M., Horoz, M., Gur, M., & Erel, O. (2007). Oxidative status and serum PON1 activity in beta-thalassemia minor. *Clinical biochemistry*, *40*(5-6), 287-291.

Şimşek, F., Öztürk, G., KEMAHLI, S., Erbaş, D., & Hasanoğlu, A. (2005). Oxidant and antioxidant status in beta thalassemia major patients Beta talasemi major hastalarinda oksidan ve antioksidan düzeyleri. *Ankara Üniversitesi Tıp Fakültesi Mecmuası*, *58*(01).

Taher, A. T., Musallam, K. M., & Inati, A. (2009). Iron overload: consequences, assessment, and monitoring. *Hemoglobin*, *33*(sup1), S46-S57.