Arrival Date: 04.11.2023 | Published Date: 28.02.2024 | Vol: 9, Issue: 1 | pp: 136-142 | Doi Number: http://doi.org/10.5281/zenodo.10721666

RECENT DEVELOPMENTS IN PATIENTS WITH THALASSEMIA; COMPARISON OF ANTIOXIDANT AND CYTOKINE LEVELS AND POSSIBLE MEASURES

TALASEMİLİ HASTALARDA SON GELİŞMELER; ANTİOKSİDAN VE SİTOKİN DÜZEYLERİNİN KARŞILAŞTIRILMASI VE OLASI ÖNLEMLER

Naci Omer ALAYUNT [1], Emrah YERLIKAYA [2], Osman OZUDOGRU [3]
[1] Siirt University, Faculty of Medicine, Siirt,Türkiye
[2] Siirt University, Faculty of Health Sciences, Siirt, Türkiye
[3] Erzincan Binali Yildirim University, Faculty of Medicine, Erzincan, Türkiye

ABSTRACT

Objective: It is aimed to investigate the underlying causes of thalassemia, which has widespread and negative effects around the world, and to create solutions. For this purpose, antioxidant and anti-inflammatory cytokine levels of thalassemia patients were investigated in our study.

Methods: In our study, experimental and control groups were formed with 40 people in each group. The experimental group consisted of patients with thalassemia followed up at Siirt Training and Research Hospital, and the control group consisted of healthy individuals who came for routine control. After informing the participants in the research and taking their consent, their blood was taken. In serum samples taken from participants in the research, catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, enzyme activities and malondialdehyde, Vitamins A, E, C, cytokines, total oxidant capacity, and total antioxidant capacity levels were measured. Analyzes were performed on ELISA and HPLC instruments using appropriate kits. The data were analyzed in the IBM SPSS 21.0 statistical package program.

Results: When the results were examined, it was seen that thalassemia patients had lower antioxidant levels and increased anti-inflammatory cytokine levels compared to the control group.

Conclusion: It is possible to say that thalassemia is effective on cytokine and oxidant systems. These harmful effects can be eliminated with food supplements or drugs to be used. We believe that this study will shed light on the preparation of more comprehensive and new treatment protocols in the future.

Keywords: Antioxidant, Cytokine, Enzyme, Hemoglobin, Thalassemia.

ÖZET

Amaç: Dünya çapında yaygın ve olumsuz etkileri olan talasemi hastalığının altında yatan nedenlerinin araştırılması ve çözüm üretilmesi amaçlanmaktadır. Bu amaçla çalışmamızda talasemi hastalarının antioksidan ve antiinflamatuar sitokin düzeyleri araştırıldı.

Gereç ve Yöntem: Çalışmamızda her grupta 40 kişi olacak şekilde deney ve kontrol grupları oluşturuldu. Deney grubu Siirt Eğitim ve Araştırma Hastanesi'nde takip edilen talasemi hastalarından, kontrol grubu ise rutin kontrole gelen sağlıklı bireylerden oluşturuldu. Araştırmaya katılan katılımcılar bilgilendirilip onamları alındıktan sonra kanları alındı. Araştırmaya katılanlardan alınan serum örneklerinde süperoksit dismutaz, katalaz, glutatyon peroksidaz, glutatyon redüktaz enzim aktiviteleri ve malondialdehit, A,E,C Vitaminleri, sitokinler, toplam oksidan kapasite ve toplam antioksidan kapasite seviyeleri ölçüldü. Analizler uygun kitler kullanılarak ELISA ve HPLC cihazlarında yapıldı. Veriler IBM SPSS 21.0 istatistik paket programında analiz edildi.

Bulgular: Sonuçlar incelendiğinde talasemi hastalarının kontrol grubuna göre antioksidan düzeylerinin daha düşük, antiinflamatuar sitokin düzeylerinin ise yüksek olduğu görüldü.

Sonuç: Talaseminin sitokin ve oksidan sistemler üzerinde etkili olduğunu söylemek mümkündür. Bu zararlı etkiler gıda takviyeleri veya kullanılacak ilaçlarla ortadan kaldırılabilir. Bu çalışmanın gelecekte daha kapsamlı ve yeni tedavi protokollerinin hazırlanmasına ışık tutacağına inanıyoruz.

Anahtar Kelimeler: Antioksidan, Enzim, Hemoglobin, Sitokin, Talasemi.

Sorumlu Yazar / Corresponding Author: Emrah YERLIKAYA, Assoc.Prof, Siirt University, Faculty of Health Sciences, Siirt, Türkiye. **E-mail:** emrahyerlikaya@siirt.edu.tr

Bu makaleye atıf yapmak için / Cite this article: Alayunt NO., Yerlıkaya E., & Ozudogru O. (2024). Recent Developments in Patients with Thalassemia; Comparison of Antioxidant and Cytokine Levels and Possible Measures. *Gevher Nesibe Journal of Medical & Health Sciences*, 9(1), 136-142. http://doi.org/10.5281/zenodo.10721666

* *This study was presented* presented *as an abstract at SILK ROAD 2nd International Scientific Research Congress (26.09.2023 -27.09.2023).*

INTRODUCTION

Thalassemias (Thal) lead to chronic anemia and ineffective erythropoiesis and are known as a heterogeneous group of disorders of hemoglobin synthesis. The disease was first documented in countries surrounding the Mediterranean and its prevalence is high in this region. In addition, carrier rates of some varieties of Thal have been reported to be as high as 70%. The incidence is increasing noticeably in India and Southeast Asia (Williams and Weatherall, 2012; Taher *et al.,* 2018). The 2 main categories of Thal are alpha and beta Thal, which correspond to the respective genes. It is known that the presence of Thal mutations in both betaglobin genes will often lead to anemia. In this case, continuous red cell transfusion is inevitable for Beta Thal major patients. Intermediate patients may need intermittent transfusion. Chronic transfusion causes heavy iron deposition in major organs. This often results in multi-organ morbidity (Pinto and Forni, 2020). More than 3% of the world's population and up to 40% in South East Asia carry the highest number of thalassemia genes (Laksmitawati et al, 2003). β-thalassemia major (TM), the most common inherited blood disease, is the most severe type of thalassemia (Baysal *et al.,* 1992). Since TM patients are severely anemic, they require lifelong blood transfusions to survive. Repeated blood transfusion in TM patients leads to iron deposition in various organs (Abdalla *et al.,* 2011; Rahim *et al.,* 2016). Iron overload, which is usually observed, generates oxygen free radicals and peroxidative tissue damage. Oxidative stress is known as the dynamic imbalance between reactive oxygen species (ROS) produced in the body and antioxidants that protect the body against the harmful effects of ROS (Rajendran *et al.,* 2014). If this balance is disrupted, oxidative damage may occur in macromolecules such as nucleic acids, lipids and proteins (Oguzhan *et al.,* 2015). Because transfusions are repeated in TM patients, it causes unnecessary iron accumulation in their bodies. Therefore, constant blood transfusions are required to deal with anemia. Excessive iron load triggers excessive production of oxidative stress. With increasing stress, the routine physiology of organs and cells is disrupted. In TM patients, rapid apoptosis and ineffective erythropoiesis may occur due to oxidative damage to erythrocytes. For this reason, the antioxidant potential of these patients becomes quite important. Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) are the most commonly used variables to determine serum oxidative stress in many studies. Ferritin, a cytoplasmic protein, stores iron as ferric. The main organ where iron is stored in the body is the liver. Many studies on TM patients have revealed that iron overload is first seen in the liver if the amount of ferritin in the serum exceeds 1000 µg/dL. Additionally, as ferritin levels increase, iron accumulation occurs in other organs such as the pancreas, heart and spleen. The most appropriate method to estimate iron reserves in the body is ferritin monitoring (Ikuta *et al.,* 2011). Accumulation of toxic amounts of iron leads to the formation of ROS, which triggers oxidative stress (Tangvarasittichai *et al.,* 2013). Oxidative stress, known as the shift in the balance between oxidant-antioxidant systems, has an important place in the pathology of many diseases (Maryam *et al.,* 2018; Ozdem *et al.,* 2008). Increased apoptosis along with oxidative stress may cause shortening of erythrocyte lifespan (Bhagat *et al.,* 2013). Increased iron load and decreased hemoglobin (HbA1C) levels in adults cause oxidative damage, especially in TM patients (Dhawan *et al.,* 2005; Nassima *et al.,* 2015). In patients with thalassemia, erythrocytes consistently produce higher amounts of pro-oxidants than normal cells. Keeping the reserves of antioxidants alive, which will eliminate the negative effects of oxidative stress, will provide convenience to minimize the amount of ROS, which is the basis of all chronic diseases.

Our study aimed to evaluate antioxidant and cytokine levels in thalassemia patients by comparing them with healthy individuals.

MATERIAL AND METHOD

Before the study was conducted, the necessary approval was obtained from the Siirt University Non-Interventional Clinical Research Ethics Committee (2020/01-02). In this study, experimental and control groups were formed with 40 people in each group. The experimental group consisted of thalassemia patients followed at Siirt Training and Research Hospital. The other group consisted of healthy people who came for routine check-ups. After informing the participants in the research and taking their consent, their blood was taken. The blood samples of the participants in the study were taken as 5 cc in flat biochemistry tubes containing gel without anticoagulant after 12 hours of fasting. After the blood coagulated in the tube, the serums were separated by centrifugation at 4000 rpm for 10 minutes. It was stored at -20 °C until the working day. When the serums from all groups were

completed, the analysis phase of the study was started.In serum samples taken from participants in the research, SOD, CAT, GSH-Px, GSH-Rd enzyme activities and malondialdehyde, Vitamins A,E,C, Cytokines (TNF-α, IL-1 β, IL-6), TOS and TAS levels were measured. Analyzes were performed on ELISA and HPLC instruments using appropriate kits. IBM SPSS 21.0 statistical package program was preferred for statistical evaluation in comparing the data obtained from patient documents and the hospital's information technology.

Statistical Assessment

For statistical evaluation of the information obtained, SPSS 21.0 package program was preferred. Data were expressed as median (min-max value), qualitative data as percentage, and non-normally distributed data as Median (IQR, Inter Quntifier Ratio, 25%-75%). In evaluating the information and determining the distribution of continuous variables, Kolmogorov-Smirnov and Shapiro-Wilk normality tests were preferred. When the information was not distributed normally, the Mann-Whitney U test was preferred to reveal the relationship between pairs. Frequency distributions, number, median, minimum and maximum values were given in descriptive statistics. The value considered significant was $p<0.05$.

RESULT

In serum samples taken from patients with thalassemia, total antioxidant capacity, total oxidant capacity, Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and cytokine levels were measured in the ELISA device using appropriate kits. In addition, malondialdehyde and vitamins (A, E, C) were analyzed in HPLC device using appropriate kits and column. When the results were examined, it was seen that thalassemia patients had lower antioxidant levels and increased anti-inflammatory cytokine levels compared to the control group (Table 1, 2 and 3).

Table 1. Comparison of the Parameters of the Thalassemias and Control Groups

Descriptives		**N**	**Median**	**Min-Max**	**IQR**	**P value**
Vit A (mg/L)	Thalassemia	40	0.61	0.51-0.75	0.08	**p < 0.05**
	Control	40	0.75	0.65-0.86	0.11	
Vit E (mg/L)	Thalassemia	40	10.87	8.89-13.41	1.52	**p < 0.05**
	Control	40	12.88	10.31-14.12	1.39	
Vit C (mg/L)	Thalassemia	40	9.35	8.14-10.54	1.18	**p < 0.05**
	Control	40	11.14	9.14-13.24	1.65	
MDA (mg/L)	Thalassemia	40	0.64	0.38-0.83	0.11	**p < 0.05**
	Control	40	0.48	0.33-0.63	0.14	
CAT (ng/mL)	Thalassemia	40	43.79	10.00-80.52	27.86	**p < 0.05**
	Control	40	55.17	16.57-202.70	55.99	
SOD (ng/mL)	Thalassemia	40	64.85	27.07-281.41	55.47	**p < 0.05**
	Control	40	100.15	2.00-282.6	81.06	
GSH-PX (ng/mL)	Thalassemia	40	22.55	8.61-51.97	13.03	**p < 0.05**
	Control	40	34.19	15.35-58.56	19.42	
GSH (µmol/L)	Thalassemia	40	2.49	1.63-8.29	1.79	**p < 0.05**
	Control	40	3.21	1.63-8.42	1.87	

When Vit A, Vit C and Vit E values in patients with thalassemia are compared with the control group, it is seen that the values decrease significantly. According to the table 1, the median value of the Vit A in patients with thalassemia was 0.61 mg/L, and the median value of the control group was 0.75 mg/L. Same way, the median value of the Vit E in patients with thalassemia was 10.87 mg/L, and the median value of the control group was 12.88 mg/L. Although the values are closely related to nutrition, they are low in patients with thalassemia. Low vitamin values reduce body resistance in thalassemia patients. However, MDA values were found to be higher in patients with thalassemia, in contrast to Vit A and Vit E. The median value of the MDA in patients with thalassemia was 0.64 mg/L, and the median value of the control group was 0.48 mg/L.

As with the Vit A and Vit E values, a decrease is observed in the protective enzyme systems in the patient group as well. Protective enzymes such as CAT, SOD, GSH-Px and GSH were detected at

lower levels in patients with thalassemia compared to the control group. The median values of CAT, SOD, GSH-Px and GSH enzymes in the thalassemia patient group were 43.79 ng/mL, 64.85 ng/mL, 22.55 ng/mL and 2.49 µmol/L, respectively. The median values of CAT, SOD, GSH-Px and GSH enzymes in the control group were 55.17 ng/mL, 100.15 ng/mL, 34.19 ng/mL and 3.21 µmol/L, respectively (Table 1).

Table 2. Comparison of the Parameters of the Thalassemias and Control Groups

Descriptives		**N**	**Median**	**Min-Max**	**IQR**	**P value**
TNF-α (ng/mL)	Thalassemia	40	125.07	55.91-276.34	79.74	**p < 0.05**
	Control	40	98.43	64.16-226.35	40.40	
IL-1 β (ng/mL)	Thalassemia	40	1.04	0.30-1.79	0.53	**p < 0.05**
	Control	40	0.99	0.20-2.28	0.50	
IL-6 (ng/mL)	Thalassemia	40	93.70	47.10-225.16	61.87	**p < 0.05**
	Control	40	76.04	43.92-166.008	54.05	

On the contrary, it was determined that cytokine levels were increased in patients with thalassemia compared to the control group. The median values of TNF-α, IL-1 β and IL-6 cytokines in the thalassemia patient group were 125.07, 1.04 and 93.7 ng/mL, which was statistically significant, respectively. The median values of TNF-α, IL-1 β and IL-6 cytokines in the control group were 98.43, 0.99 and 76.04 ng/mL, respectively (Table 2).

Table 3. Comparison of the Parameters of the Thalassemias and Control Groups

Descriptives		**N**	**Median**	**Min-Max**	**IQR**	**P value**
TAS (U/mL)	Thalassemia	40	23.14	2.13-212.80	21.03	**p < 0.05**
	Control	40	34.83	11.09-175.41	48. 88	
TOS (U/mL)	Thalassemia	40	51.01	37.97-4410.00	22.94	**p < 0.05**
	Control	40	55.99	32.41-91.10	19.06	

While the median TOS value of the control group was 55.99 U/mL, the median TOS value was found to be 51.01 U/mL in the thalassemia patient group. While the median TAS value of the control group was 34.83 U/mL, the median TAS value was found to be 23.14 U/mL in the thalassemia patient group.

DISCUSSION

Approximately 60,000 children with beta thalassemia are born worldwide each year. Carriers are estimated to number approximately 90 million people. This corresponds to 1.5% of the global population (Origa, 2017). In the last century, the most severe forms led to premature death due to severe anemia. Regular red cell transfusions 63 years ago made transfusion-induced thalassemia no longer a fatal childhood disease. It turned it into a chronic disease (Lal, 2020). In the last 45-50 years, the survival rate of beta-thalassemia patients has increased significantly, mostly thanks to regular blood transfusions and chelation treatments (Tartaglione *et al.,* 2022). Researching the underlying causes of this common and adverse disease and creating solutions maintain its importance today. In our study, antioxidant and anti-inflammatory cytokine levels of thalassemia patients were investigated. In the study of Goldberg *et al*., it was observed that Thal patients were mostly malnourished in terms of vitamins A, C, D, selenium and zinc. In the same study, the prevalence of nutritional deficiency was positively correlated with age and iron overload. Evidence supporting the role of vitamin D and zinc in bone health has been observed, and zinc has also been found to improve glucose metabolism (Goldberg *et al.,* 2022). In our study, the vitamins A, E and C values of the thalassemia patient group were found to be significantly lower than the healthy control group. Thalassemia does not only affect patients physiologically. At the same time, it has a negative effect on patients psychologically. In the study by Jaafari Z. et al., the prevalence of depression in the pool was found to be 45% and 39%, respectively, in medium and high quality studies. The high prevalence of depression in patients with thalassemia detected as a result of the research is remarkable (Jaafari *et al.,* 2022). Many clinical studies are carried out for the early diagnosis and treatment of thalassemia. One of them is the study

by Zhang J *et al.* By developing a machine learning model based on MALDI-TOF mass spectrometry quantification of hemoglobin chains in blood, they contributed to the rapid screening of thalassemia in large populations (Zhanga *et al.*, 2022). MDA is the end product of lipid peroxidation. To evaluate tissue damage due to lipid peroxidation; Precursors of lipid hydroperoxides such as conjugated diene and peroxy radical or lipid hydroperoxides can be measured or secondary products such as alkane and aldehyde breakdown products, fluorescent schiff-base compounds can be determined (Sinclair *et al.*, 1990; Kilic *et al.*, 1988). MDA is one of these degradation products and is a three-carbon dialdehyde. MDA, which is free or in complex with tissue contents, causes cross-linking in lipids. It is also known as carcinogenic and mutagenic. It is possible to show lipid peroxidation with MDA determination (Kilic *et al.*, 1988). In our study, MDA values were determined to be higher in thalassemia patients compared to the control group. It is thought that the decrease in oxygen utilization capacity in patients with thalassemia may play a role in finding high MDA levels. In another study, oxidant and antioxidant properties were investigated in pediatric beta thalassemia major patients who received regular transfusion and chelation therapy. While MDA levels were found to be higher than the control group, Vit E levels were also found to be lower than the control group (Simsek *et al.*, 2005). These results support our research.

Antioxidant defense systems have enzymatic and non-enzymatic complex systems. For this reason, there are first, second, and third antioxidant defense mechanisms in the cell. The first line of defense is the antioxidant defense systems of SOD, CAT and GSH-Px, which suppress the formation of free radicals (Niki, 1993). In our research, SOD, CAT and GSH-Px values of thalassemia patients were found to be significantly lower than the control group. Decreased in SOD, CAT and GSH-Px levels increase the formation of free radicals. Increasing the daily SOD, CAT and GSH-Px support of patients with thalassemia protects the immune system and reduces the risk of getting diseases and the aging process. Cytokines are produced by cells in response to complex stimuli. They also serve as key mediators of the host response to different infections, inflammatory and immunological challenges. They mostly have low molecular weight (Opal and DePalo, 2000; Nicod, 1993; Elias *et al.*, 1990). In our study, cytokine levels were determined to be higher in thalassemia patients compared to the control group. The study conducted by Yenisey *et al.* to evaluate the diagnostic use of cytokines has similar results. When the patient and control groups were compared, the difference between IL-1β, IL-6 and TNF-α levels between exudative serum and pleural fluid samples was found to be statistically significant (Yenisey *et al.*, 2006). In the study conducted to investigate the total antioxidant capacity in beta thalassemia patients, the total antioxidant capacity of the patients was found to be significantly higher than that of healthy individuals (2.75 vs. 2.10 mmol/L; p=0.01). In the same study, total bilirubin level was found to be significantly higher in non-transfusion-dependent patients than in transfusion-dependent patients (5.7±3.3 vs.1.9±1.4; p<0.001) (Karakas *et al.*, 2020). On the contrary, in our study, TAS values of the control group were higher than the patient group.

CONCLUSION

Oxidative stress is one of the most important risk factors for many diseases. Increasing oxidative stress and the resulting decrease in antioxidant capacity require strengthening of the antioxidant system in the treatment of diseases. In addition, it is possible to say that thalassemia is effective on cytokine and oxidant systems. These harmful effects can be eliminated with food supplements or drugs to be used. We believe that this study will shed light on the preparation of more comprehensive and new treatment protocols in the future.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author Contributions

Concept: NOA, **Literature Search:** NOA, EY. **Data Collection and Processing:** NOA, OO. **Analysis or Interpretation:** NOA, EY. **Written:** NOA, EY.

Funding

Financial support was provided by Siirt University Scientific Research Coordinator, Project code: 2020-SİÜTIP-011.

REFERENCES

Abdalla, M., Fawzi, M., Al-Maloul, S., El-Banna, N., et al. (2011). Increased oxidative stress and iron over load in Jordanian β-thalassemic children, Hemoglobin, 35, 67-79.

Baysal, E., Indrak, K.G., Bokurt, G.A., Erkalp, A., et al. (1992). The beta-thalassaemia mutations in the population of Cyprus. Br. J. Haematol., 81, 607-609.

Bhagat, S.S., Sarkar, P.D., Suryakar, A.N., Padalkar, R.K., et al. (2013). Attenuation of serum ferritin and iron burden by intake of antioxidants in beta thalassemia majör. Indian J. Physiol. Pharmacol., 57, 189-194.

Dhawan, V., Kumar, K., Marwaha, R.K., Ganguly, N.K. (2005). Antioxidant status in children with homozygous thalassemia. Indian Pediatr., 42, 1141-1145.

Elias, J.A., Freundlich, B., Kern, J.A, et al. (1990). Cytokine networks in the regulation of inflammation and fibrosis in the lung. Chest, 97, 1439-1445.

Goldberg, E.K., Ashutosh, L., Fung, E.B. (2022). Nutrition in Thalassemia: A Systematic Review of Deficiency, Relations to Morbidity, and Supplementation Recommendations. J Pediatr Hematol Oncol. 44(1), 1-11.

Ikuta, K., Kohgo, Y., Ohtake, T., Torimoto, Y., et al. (2011). Body iron metabolism and pathophysiology of ironoverload, Int. J. Hematol., 88, 7-15.

Jaafari, Z., Sadidi, N., Zahra Abdolahinia, Z. and Shahesmaeili, A. (2022). Prevalence of Depression among Iranian Patients with Beta-Thalassemia Major: A Systematic Review and Meta-analysis. Iran J Med Sci., 47(1), 15-24.

Karakas, Z., Yilmaz, Y., Celik, D.D., Annayev, A. et al. (2020). The total antioxidant capacity may not be related to bilirubin and uric acid level in patients with beta thalassemia. J Ist Faculty Med, 83(4), 373-377.

Kilic, N., Malhatun, E., Elmali, E., Altan, N. (1988). An investigation into the Effects of the Sulfonylurea Glyburide on Glutathione peroxidase activivity in Streptozotocin-Induced Diabetic Rat Muscle Tissue, Gen Pharmacol, 30, 399-401.

Laksmitawati, D.R., Handayani, S., Udyaningsih-Freisleben, S.K., Kurniati, V., et al. (2003). Iron status and oxidative stress in beta-thalassemia patients in Jakarta, Biofactors, 199, 53-62.

Lal, A. (2020). Challenges in Chronic Transfusion for Patients with Thalassemia. Hematol. Am. Soc. Hematol. Educ. Program, 1, 160–166.

Maryam, M., Hamid, Y., Saeed, S., Ali, A., et al. (2018). Increased levels of advanced glycation end products positively correlate with iron overload and oxidative stress markers in patients with β-thalassemia majör. Ann. Hematol., 97, 679-684.

Nassima, B., Malika, I., Khadidja, B., Nassima, N., et al. (2015). Oxidative status and plasma lipid profile in b-thalassemia patients, Hemoglobin, 39, 36-41.

Nicod, L.P. (1993). Cytokines: Overview. Thorax, 48, 660-667.

Niki, E. (1993). Antioxidant defenses in eukaryotic cells. In: Poli G, Albano E, Dianzani MU, editors. Free radicals: From basic science to medicine. Basel, Switzerland: Birkhauser Verlag, 365–73.

Oguzhan, O., Huseyin, E., Gokhan, C., Zafer, Y. (2015). Oxidative stress and its impacts on intracellular lipids, proteins and DNA. Journal of Clinicaland Experimental Investigations, 6, 331-336.

Opal, S.M., DePalo, V.A. (2000). Anti-inflammatory cytokines. Chest, 117, 1162-1172.

Origa, R. (2017). Thalassemia. Genet. Med., 19, 609–619.

Ozdem, S., Kupesiz, A., Yesilipek, A. (2008). Plasma homocysteine levels in patients with beta-thalassaemia majör. Scand. J. Clin. Lab. Invest., 68, 134-139.

Pinto, V.M., Forni, G.L. (2020). Management of iron overload in betathalassemia patients: clinical practice update based on case series, Int J Mol Sci, 21, 877-897.

Rahim, A., Tan, A., Mani, R., Kuppusamy, R. (2016). Non-invasive sampling for assessment of oxidative stress and pro-inflammatory cytokine levels in beta-thalassaemia major patients. Revista Română de Medicină de Laborator, 24, 83-92.

Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R., et al. (2014). Antioxidants and human diseases. Clin. Chim. Acta, 25, 332-347.

Simsek, F., Ozturk, G., Kemahlı, S., Erbas, D. et al.(2005). Oxidant and antioxidant status in beta thalassemia major patients. Journal of Ankara University Faculty of Medicine, 58(1), 34-38.

Sinclair, A.J., Barnett, A.H., Lunec, J. (1990), Free radicals and antioxidant systems in health and diseases. J Hosp Med.,43, 334-44.

Taher, A.T., Weatherall, D.J., Cappellini, M.D. (2018). Thalassaemia, Lancet, 391, 155-167.

Tangvarasittichai, S., Pimanprom, A., Choowet, A., Tangvarasittichai, O. (2013). Association of iron overload and oxidative stress with insulin resistance in transfusion-dependent beta-thalassemia major and beta-thalassemia/HbE patients, Clin. Lab., 59, 861-868.

Tartaglione, I., Carfora, R., Brotto, D., Barillari, M.R., Costa, G., Perrotta, S., Manara, R. (2022). Hearing Loss in Beta-Thalassemia: Systematic Review. J. Clin. Med. 11, 102. https://doi.org/10.3390/jcm11010102.

Williams, T.N., Weatherall, D.J.(2012). World distribution, population genetics, and health burden of the hemoglobinopathies, Cold Spring Harb Perspect Med, .2, a011692.

Yenisey, C., Aktogu, S., Kalenci, S., Erer, F.O. (2006). Proinflammatory cytokines: Are they usefull in differential diagnosis of pleural effusions?. Aegean Medical Journal. 45(1), 19-24.

Zhanga, J., Liub, Z., Chenc, R., Mad, Q., Lyud, Q., Fud, S., Hed, Y., Xiaoa, Z., Luoa, Z., Luoe, J., Wangf, X., Liug, X., Anh, P. and Suna, W. A. (2022). MALDI-TOF mass spectrometry-based haemoglobin chain quantification method for rapid screen of thalassaemia. Annals of Medicine, 54(1), 293-301.