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Evaluation of Serum Level of Catalase Enzyme Activity in Patients with Beta-Thalassemia

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Annotation: Beta-thalassemia is a hereditary blood disorder associated with chronic anemia and oxidative stress due to frequent blood transfusions and iron overload. Although oxidative stress is known to contribute to disease complications, limited studies have evaluated specific antioxidant enzyme levels in thalassemia patients. This study aimed to assess the serum activity of catalase (CAT), a key antioxidant enzyme, in beta-thalassemia patients compared to healthy controls. Blood samples were collected from 25 beta-thalassemia patients and 25 age-matched healthy individuals. CAT activity was measured using a colorimetric assay based on hydrogen peroxide degradation. The results revealed a significant decrease in CAT activity in thalassemia patients (8.46 U/ml) compared to the control group (24.64 U/ml), indicating impaired antioxidant defense. These findings suggest that reduced catalase activity may serve as a useful biochemical marker for monitoring oxidative stress and managing complications in beta-thalassemia, with implications for antioxidant-based

therapeutic interventions.

Keywords: Beta-thalassemia, catalase activity, oxidative stress, antioxidant enzyme, hydrogen peroxide, biomarker.

1.1 Introduction

Beta-Thalassemia major considers one of most common single gene inherited hemoglobinopathy results from about 200 beta globin genes mutation (Alizadeh et al.,2014). The prevalence of β-thalassemia major about 3% in the world (Menon et al.,2022). It characterized by the diminished or absent of β-globin chains synthesis that result in excessive α chains production and impaired hemoglobin tetramer formation leading to ineffective erythropoiesis (Thein and Rees,2015). The newly formed RBCs became more fragile with short life span leading to sever anemia which require lifelong blood transfusions to maintain normal life (Osaro et al.,2018). Thalassemia is an inherited blood disorder in which hemoglobin (Hb) and red blood cells (RBC) production are disrupted. Hemoglobin is an oxygen-carrying molecule in erythrocytes, and consists of two types of globin chains (alpha and beta). In patients with thalassemia, the production of these subunits is disrupted, leading to abnormal erythropoiesis and anemia which begins during childhood and lasts throughout life (Koury,2014).Patients with thalassemia suffer from several complications including skeletal, cardiac, and endocrine problems. Some of these complications are caused by the disease itself, whereas others are due to either regular blood transfusions or the consumption of medications such as hydroxyurea. Regular blood transfusions cause iron accumulation and oxidative stress in the body. In addition, hemolytic anemia caused by the destruction of abnormal erythrocytes and their precursors in the bone marrow and spleen leads to bone deformation and spleen enlargement. Liver dysfunction, diabetes, zinc deficiency, hypothyroidism, gallstones, congestive heart failure, osteoporosis, osteopenia, viral infections, and hepatocellular carcinoma are other complications caused by regular blood transfusion and subsequent hemosiderosis (Daraghmeh, 2016). Thalassemia is generally diagnosed during childhood after the emergence of severe microcytic hypochromic anemia associated with elevated HbF and HbA2. The disease is managed with regular blood transfusions to maintain patients Hb level above 9 g/dL. Iron chelators are also used to prevent the effects of iron deposition in the tissues. There are alpha thalassemia and beta thalassemia. Each type divided into several subtypes with their own severity. The most severe type of alpha thalassemia is Hydrops fetalis which is lethal in fetus, while in beta thalassemia is thalassemia major Thalassemia was first recognized in 1925 in United States of America and Italy by Dr. Thomas Cooley. It was first called as Cooley's anemia. Then it changed into thalassemia which contains of two Greek terms, Thalassa (sea) and Emia (blood). Thus thalassemia was anemia happens in Mediterranean. Nowadays Cooley's anemia is also known as thalassemia major. Thalassemia major is the severe form of beta thalassemia. Thalassemia do not happen only to the Mediterranean area now, but also in Africa, Middle East, and Southeast Asia (Kattamis et al.,2020). One proposed theory involves the suppression of oxidative damage in patients. However, there exists some controversy in this regard. Oxidative stress is caused by an imbalance between oxidants and antioxidants in the body. Antioxidants prevent the adverse effects of naturally produced free radicals in the human body during biological processes. Although our body is naturally capable of maintaining a balance in the reactive oxygen species (with antioxidant defenses), oxidative stress occurs when the production of free radicals exceeds the body's capacity to removing them (Chaudhary et al.,2023). Multiple prooxidant and antioxidant markers are commonly used to assess oxidative stress levels. The aim of study is to estimate the medical role of antioxidant as biomarker of Beta-Thalassemia, this aim was achieved through the following objectives: Blood collection from Beta-Thalassemia and control groups Estimation of Catalase enzyme activity.

1.2. Literature Review

1.2.1. Structure of Hemoglobin

Hemoglobin was in tetramer form contains of heme as a non-protein substance and globin as a protein substance. Heme contains iron and protoporphyrin. Globin chain consists of combination of α, β, δ, and γ chain. In adult, adult hemoglobin (HbA) made the most proportion of all hemoglobin by 96–98%, then followed by small quantities of two other hemoglobins (HbF and HbA2). HbA consists of two pairs of polypeptide chains consist of two β globin chains (β2) and two α globin chains (α2) (α2β2). HbF contains of two α globin chains and two γ globin chains (α2γ2). HbA2 contains of two α globin chains and two δ globin chains (α2δ2). HbF is found with the highest percentage during fetal life, then after birth it is then switched into adult hemoglobin (α2β2) (Gavulic *et al.*,2021).Chromosome 16 is responsible for controlling the synthesis of α globin gene, specifically in the cytogenetic location of 16p13.3, which located in the position of 13.3 in the short (p) arm of chromosome 16. It's molecularly located in base pair 176,651 to 177,522 on chromosome 16, while the synthesis of β globin chain take part in chromosome 11 with the exact location of 11p15.4, meanings it is located in position 15.4 in the short (p) arm of chromosome 11. It is molecularly located in base pair 5,225,466 to 5,227,071 on chromosome 11. There are four elements which regulate the α-locus known as MCSR1 to MCSR4. There are five elements which regulate the β-locus known as locus control region (LCR). During the developmental periods, there are several hemoglobin types expressed. In embryonic development stage Hb Portland (ζ2γ2), Hb Gower-I (ζ2ε2), and Hb Gower-II (α2ε2) are expressed. In fetal stage HbF (α2γ2) are expressed. In adult stage HbA2 (α2δ2) and HbA (α2β2) are expressed. In fetal life, HbF has bigger role, then it switch to HbA after birth (İnce *et al.*,2013).

1.2.2 Types of Thalassemia

1.2.2.1 Alpha Thalassemia

Human α-globin cluster (5 -ζ-α2-α1-3). MPG, NPLR3, and Luc7L are the expressed genes surrounding the cluster. MCSRs are below these genes. The regions of duplication are X, Y, and Z boxes that play a part in generating the common α-thalassemia (Putri et al.,2022). Alpha thalassemia is mainly caused by a removal/deletion of one or two alpha genes at the molecular level. Alpha globin gene, which located in chromosome 16, consist of two fragments (α1 and α2) positioned sequentially. Every human has two alpha genes in each chromosome 16, or as usually mentioned as 4 copies (αα/αα) α-globin genes are expressed mostly with the role of MCS-R2 known as HS-40. MCSR2 is placed in the region of 25–65 kb upstream of the α-globin genes, and is one of four MCS (Multispecies Conserved Sequences) (Putri et al.,2022). These four MCSs (MCSR1-MCSR4) know to take part in regulating the α-like globin genes. X, Y, and Z boxes are the region of duplication which embedded the duplicated α-globin genes (αα). α-gene may be decreased or not synthesized at all and give several different outcomes. The more missing genes, the more severe the outcome may become. The number of genes involved influenced the severity of the outcome. According to the number of α-genes involved, alpha thalassemia is classified into several types (Chapin and Giardina,2017):

1. Alpha thalassemia silence carrier: This type is cause by the deletion of one α-gene (-α/αα). Clinically and hematologically is unseen in patient, meaning it is asymptomatic. Patients only have very little possibility of showing anemic features. Red blood cells abnormality in morphology is not found in this type.
2. Alpha thalassemia minor: This type is caused by the deletion of two α- gene. This type is shown as heterozygous αthalassemia (--/αα) or homozygous α-thalassemia (-α/-α). This type shows mild anemia findings. Clinically it is asymptomatic and does not need transfusion for its management.
3. Hemoglobin H disease: This type is caused by deletion of three α-gene (--/-α). The decreased of α-globin expression causes a moderate anemia with microcytosis, hypochromia and red

blood cell fragmentation. Only one α-globin gives the supposed function, causing a turbulent imbalance of globin chain synthesis making some surplus β-globin chain synthesis.

4. Hydrops Fetalis: This type is caused by deletion of four α-gene (--/--). The most intense form of αthalassemia is Hydrops fetalis, and mostly take place in infants whose both parents possess α-thalassemia syndrome.

1.2.2.2 Beta Thalassemia

β-thalassemias are most common in the Mediterranean region, Southeast Asia including China, Indonesia, the Middle East, India. Unlike α-globin gene, β-globin gene is not duplicated and located on chromosome 11 with the exact location of 11p15.5. Thus, each cell only contains one β-globin gene. β-thalassemia is mainly happened because of point mutation causing the reduction of β-globin gene. Most of the mutation are minor nucleotide substitution within the cluster, but sometimes deletion may also happen and caused thalassemia. These mutation might cause the alleviation in the synthesis of βglobin gene (β+-thalassemia) or even the absence of the synthesis of β-globin chain (β0-thalassemia) (Das and Sharma,2016).).Beta thalassemia are defined into 3 types according to the clinical and laboratory findings. These types are :

1. Beta thalassemia minor (β/β+; β/βo) These types also known as carrier of thalassemia. It is important to differ this type of thalassemia with iron deficiency anemia. Patients with beta thalassemia have the Mentzer index of 13, iron deficiency is more common. Hb A2 is usually increased in beta thalassemia patients, (RBC) are usually higher.

2. Beta thalassemia intermedia (β+/β+; β+/βo): Patients with this type of thalassemia have Hb of 7 g/dL without transfusion. Clinically, they have symptoms between the utmost of thalassemia minor and major.

3. Beta Thalassemia Major (βo/βo): This type is also known as Cooley's anemia. Clinical findings are mostly seen between the age of six and twenty-four months. Beta thalassemia happened by the reduced production of β-globin gene chains or complete absence of β-globin gene production.

1.2.3. Oxidative stress

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress. In patients with beta thalassemia major where frequent blood transfusions are required due to severe anemia, oxidative stress occurs as a result of increased levels of lipid peroxides and free-radical intermediates, as well as the decrease in total antioxidant capacity. Use of iron chelatory agents in combination with antioxidants can be helpful in the regulation of the antioxidant status in patients with beta thalassemia major. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major has been studied extensively (Shazia *et al.*,2012).Seventytwo children with beta thalassemia major on iron chelation therapy and 72 age-matched healthy controls irrespective of sex were included in the study. They found a significant increase in the levels of lipid peroxide and iron and significant decrease in levels of vit E and total antioxidant capacity. Serum zinc was significantly increased while copper levels decreased and there is a nonsignificant increase in erythrocyte superoxide dismutase. The results suggested that the oxidative stress and decreased antioxidant defence mechanism play an important role in the pathogenesis of beta thalassemia major. It is concluded that repeated blood transfusions in beta thalassemia major patients causes secondary iron overload and this makes erythrocytes vulnerable to peroxidative injury (Fibach and Dana,2019).Iron overload leads to peroxidative damage in betathalassemia major and antioxidant systems try to reduce tissue damage by lowering lipid peroxidation. They found that the markers of lipid peroxide damage such as MDA, SOD, and NO levels were significantly raised in thalassemia major children while mean glutathione peroxidase (GPx) levels were reduced in patients as compared to controls. These markers significantly correlated with serum ferritin levels.

1.2.4. CAT enzyme

Catalase is a common antioxidant enzyme with the highest turnover rate. It is present in living tissues and is a key clinical enzyme involved in the breakdown of hydrogen peroxide to water and molecular oxygen (Chelikani et al.,2004). Catalase can be divided into three classes namely monofunctional catalase or typical catalase, catalase-peroxidase, and pseudocatalase or Mn-catalase. For instance, it is found in erythrocytes and its exogenous sources are from plants include cotton, sunflower and pumpkin. The mechanism of action of catalase involves two steps. During the enzymatic reaction leading to H2O2 dissociation, catalase is first oxidized to a hypervalent iron intermediate, known as compound I, which is then reduced back to the resting state by a second H2O2 molecule. Catalase is an important biomarker for oxidative stress and for the pathogenesis of many diseases and infections. It helps to prevent the onset of neurological and inflammatory diseases, carcinogenesis and metabolic syndrome. It has also been reported to be used as a therapy for cancer, diabetic retinopathy and cardiac patients (Mohamad et al.,2022). A study revealed increased levels of antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase in red blood cells of beta thalassemia minor and near normal values of these enzymes in red blood cells of beta thalassemia major patients. They concluded that the red cells in beta thalassemia minor react to increased oxidant threat with augmented antioxidant enzyme activities while in beta thalassemia major patients normal antioxidant enzyme levels are due to presence of normal red cells because of to multiple blood transfusions (Shekhar *et al.*,2007).

2. Materials and Methods

2. 1. Study Population

The study subjects comprised from 25 patients selected from Babylon Maternity and Children's with age range (15-30) years. The control group study included 25 people apparently healthy with age range (15-30) years. All subjects in this study were taken written consent before participation in this study.

2.2. Blood Samples

About Three milliliters of venous blood sample was collected from each subject in the study and used to separate the serum by centrifugation at 3000 rpm for 15 min then kept in eppendorf tubes at -20 ºC until used.

2.3. CAT activity

Serum catalase activity was measured by an assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate (Goth , 1991). Optimal conditions for the assay were as follows: 0.2 ml serum was incubated in 1.0 ml substrate (65 pmol \ ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 25C for 60 s. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate ((NH4)6 Mo,O,, .4 H,O) and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against blank.

2.4. Statistical analysis

All the statistical analyses were done with the SPSS statistical software (version 23; SPSS Inc., Chicago, IL).$p<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Catalase activity

The results of the present study was showed significant differences ($P<0.05$) between patients and control groups in some biochemical markers, the catalase activity in control group were (24.64) U/ml while its activity in the patients group were significantly decreased to (8.46) U/ml as shown in (figure 1).

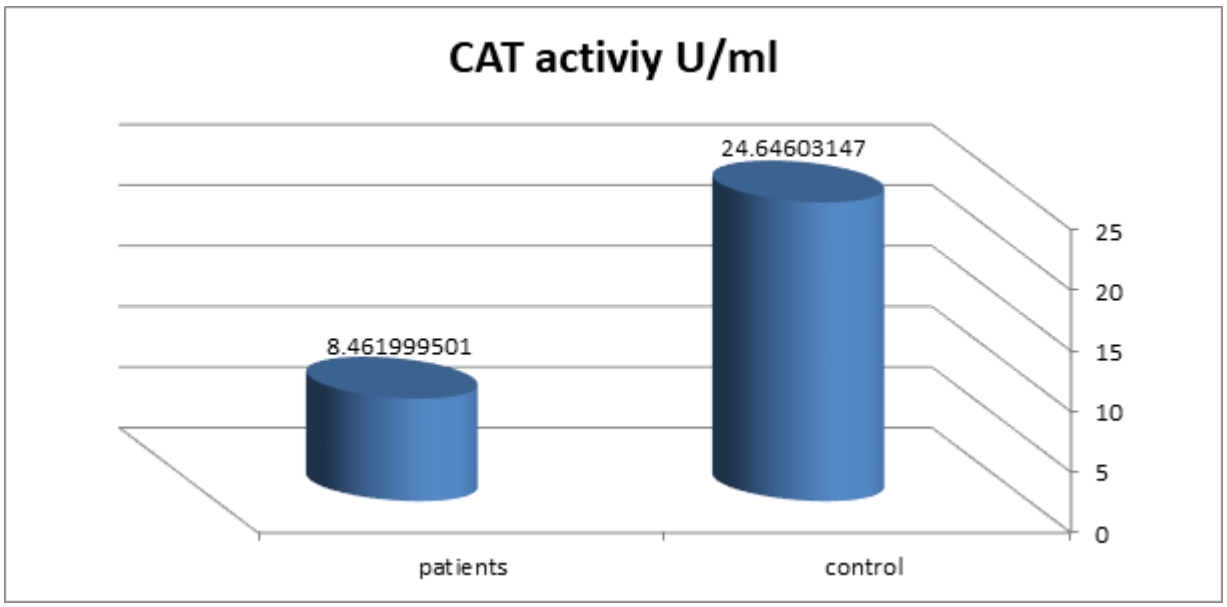

Figure (1): CAT activity in patients and control groups.

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body, due to the excess production of peroxides and free radicals. During the course of metabolism, superoxide anion is converted to H2O2 by ubiquitous enzyme SOD. Normally H2O2 is converted to innocuous compounds by the action of catalase and peroxidase. But if free iron is available, it reacts with H2O2 to form hydroxyl radicals which are extremely reactive species leading to depolymerisation of polysaccharide, DNA strand breakage, inactivation of functional proteins, and other events (Şimşek et al.,2005). Therefore, this imbalance will cause damage to cellular components and tissues in the body leading to OxS, and catalase has a role in it.

Kósa et al (2012) revealed a significant decrease in catalase activity of 43 β-thalassemia carriers and attributed it to catalase protein damage by increased free radicals and H2O2. Another study reported increased levels of antioxidant enzymes like SOD, catalase, and GPx in RBCs of β-thalassemia minor individuals and near normal values of these enzymes in RBCs of β-thalassemia major patients (Gerli et al.,1981). Our study shows a significant decrease in the CAT activity of β-thalassemic patients when compared with control. This finding is by a study done by Nahla and HANAN (2020), who found that serum CAT in the β-thalassemic group were significantly decreased. The decrease in TAOC, low levels of vitamin E and other antioxidant enzymes in thalassemic patients might be due to its consumption of antioxidant defence mechanisms for counterbalancing the effects of excess generation ROS caused by iron overload and suppressingtheir harmful oxidative stress effects and protecting against oxidative Hemolysis (Tsamesidis et al.,2017). Recent study a significant decrease ($p<0.05$) in the efficacy of catalase and a significant increase ($p<0.05$) in the level of the MDA and level ferritin in beta-thalassemia major patients compared to control group (Shakir and Al-Husseini, 2021). High levels of antioxidant enzyme SOD with decrease of antioxidant enzyme catalase, were associated with thalasemic patients compared to controls suggesting that assessment of hematological parameters and serumenzymes are valuable tools to predict thalassemia in Iraqi population (Abdulla, 2018).

4. **Conclusions**: Biochemical parameter such as catalase (CAT) are good indicator for monitoring Beta-Thalassemia, controlling the health condition and preventing secondary diseases associated with the disease.

References

1. **Abdulla,** A. A. (2018). Evaluation of serum antioxidant enzymes in β-thalassemia major patients. International Journal of ChemTech Research, 11(7), 323-328.

2. **Alizadeh,** S., Bavarsad, M. S., Dorgalaleh, A., Khatib, Z. K., Dargahi, H., Nassiri, N., ... & Saki, N. (2014). Frequency of beta-thalassemia or beta-hemoglobinopathy carriers simultaneously affected with alpha-thalassemia in Iran. Clin Lab, 60(6), 941-9.

3. **Chapin,** J. Giardina, P. J.(2017). Thalassemia Syndromes. Chapter 40 -, Editor(s): Ronald Hoffman, Edward J. Benz, Leslie E. Silberstein, Helen E. Heslop, Jeffrey I. Weitz, John Anastasi, Mohamed E. Salama, Syed Ali Abutalib, Hematology (Seventh Edition), Elsevier, Pages 546-570.e10,

4. **Chaudhary,** P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., ... & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in chemistry, 11, 1158198.

5. **Chelikani P, Fita I, Loewen PC (2004)**. "Diversity of structures and properties among catalases". *Cellular and Molecular Life Sciences*. **61** (2): 192–208.

6. **Daraghmeh,** N. M. (2016). Management and complications of thalassemic patients in Palestine: retrospective study (Doctoral dissertation).

7. **Das,** R., & Sharma, P. (2016). Molecular Genetics of Thalassemia Syndromes. Biota Publishing.

8. **Fibach,** E., & Dana, M. (2019). Oxidative stress in β-thalassemia. Molecular diagnosis & therapy, 23, 245-261.

9. **Gavulic,** A. E., Dougherty, D., Li, S. H., Carver, A. R., Bermick, J. R., Mychaliska, G. B., ... & Perrone, E. E. (2021). Fetal hemoglobin levels in premature newborns. Should we reconsider transfusion of adult donor blood?. Journal of pediatric surgery, 56(11), 1944-1948.

10. **Gerli, G. C., Beretta, L., Bianchi, M., Pellegatta, A., & Agostoni, A. (1981).** Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in β-thalassaemia (major and minor). Scandinavian Journal of Haematology, 25(1), 87-92.

11. **HANAN, F., & NAHLA, A. M. (2020).** Evaluation of oxidative stress and antioxidant status in beta thalassemia major patients: A single-center study. The Medical Journal of Cairo University, 88(December), 2147-2155.

12. **İnce,** B., Guloksuz, S., Altınbaş, K., Oral, E. T., Alpkan, L. R., & Altinoz, M. A. (2013). Minor hemoglobins HbA2 and HbF associate with disease severity in bipolar disorder with a likely protective role of HbA2 against post-partum episodes. Journal of affective disorders, 151(1), 405-408.

13. **Kattamis,** A., Forni, G. L., Aydinok, Y., & Viprakasit, V. (2020). Changing patterns in the epidemiology of β-thalassemia. European Journal of Haematology, 105(6), 692-703.

14. **Kósa, Z., Nagy, T., Nagy, E., Fazakas, F., & Góth, L. (2012).** Decreased blood catalase activity is not related to specific beta-thalassemia mutations in Hungary. International Journal of Laboratory Hematology, 34(2), 172-178.

15. **Koury,** M. J. (2014). Abnormal erythropoiesis and the pathophysiology of chronic anemia. Blood reviews, 28(2), 49-66.

16. **Mohamad Fawzi Mahomoodally, Daphne Désiré A.-L., Elodie Rosette M. A.-L., (2022).** Chapter2.2 - Catalase, Editor(s): Seyed Mohammad Nabavi, Ana Sanches Silva, Antioxidants Effects in Health, Elsevier,Pages 81-90,ISBN 9780128190968

17. **Osaro,** E., Lukman, H., Abiodun, E., Charles, A. T., Frank, U., Momodu, I., ... & Festus, A. (2018). A review of the pathophysiology and consequences of red cell storage-fresh versus stored red cells-implication for optimum use of scarce allogenic blood. American Association for Science and Technology Journal of Medicine, 4(2), 32-50.

18. **Putri,** I. M., Gultom, F. P., & Auerkari, E. I. (2022, December). Genetics and Epigenetics Aspects of Thalassemia. In 4th International Conference on Life Sciences and Biotechnology (ICOLIB 2021) (pp. 288-296). Atlantis Press.

19. **Shakir, A.A. and Al-Husseini, A.M. (2021).**Measurement the Levels of Catalase Activity, Malondialdehyde and Ferritin in Beta-thalassemia Major Patient.. Medico Legal Update, 21(2), 22-26.

20. **Shazia,** Q., Mohammad, Z. H., Rahman, T., & Shekhar, H. U. (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.

21. **Şimşek, F., Öztürk, G., Kemahlı, S., Erbaş, D., & Hasanoğlu, A. (2005).** Oxidant and antioxidant status in beta thalassemia major patients. Journal of Ankara University Faculty of Medicine, 58(1), 34-8.

22. **Thein,** S. L., & Rees, D. (2015). Haemoglobin and the inherited disorders of globin synthesis. Postgraduate haematology, 72-97.

23. **Tsamesidis, I., Fozza, C., Vagdatli, E., Kalpaka, A., Cirotto, C., & Pau, M. C. (2017).** Total antioxidant capacity in Mediterranean β-thalassemic patients. Adv Clin Exp Med.; 26 (5): 789-93.

24. **Yadav,** S. S., Panchal, P., & Menon, K. C. (2022). Prevalence and management of β-thalassemia in India. Hemoglobin, 46(1), 27-32