T.R.N.C

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MALONDIALDEHYDE AND C-REACTIVE PROTeiN VALUES IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS

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# Tez onayı

(Bu sayfa yerine, başarılı geçen Tez Sınavı sonrası sınav tutanağı ekinde yer alan Tez Onay sayfası gelecektir.)

**THANKS**

Thanks to Allah for planting the soul of patience and faith in me to complete my study.

Cordial thanks go to my supervisor & head of the department of biochemistry Prof.Dr. Güldal Mehmetçik. Iam deeply indebted for her assistance, encouragemet, helpful suggestions and discussions throughout the course of the research.

I would like to thank Prof.Dr. Ihsan Calis, the head of Health and Science Inistitute at Near East University for his help and, my sincere gratitude is also expressed to my Co-advisor Dr. Eda Becer`s assistance in Biochemistry Department in Near East University.

I wish to express my deepest thanks to all staff of thalassemia center in Dr.Burhan Nalbantoğlu Government Hospital especially Ziya Salman for his help in collecting the specimen.

I deeply indebted and owe my sincere gratitude to my all my friend for their support, engcouragement and help.

I would like to thank B.Sc. Sharoukh Hussein for his help and statistical advice

Iam grateful to my beloved family and my wife who gave me their big love, attention and support .

# Index

[Tez onayı 2](#_Toc353790779)

Thanks...............................................................................................................................3

[Index 4](#_Toc353790781)

[TAble Lıst 6](#_Toc353790782)

[fıgure lıst 7](#_Toc353790783)

[symbols/Abbrevıatıons 8](#_Toc353790784)

[abstract 10](#_Toc353790785)

[1. ıntroductıon 11](#_Toc353790787)

[1.1. Thalassemia 11](#_Toc353790788)

[1.1.1. Clinical Classification 11](#_Toc353790789)

[1.1.1.1. Thalassemia Major 12](#_Toc353790790)

[1.1.1.2. Thalassemia Intermedia 13](#_Toc353790791)

[1.1.1.3. Thalassemia Minor 14](#_Toc353790792)

[1.1.2. Types of Thalassemia Diseases 14](#_Toc353790793)

[1.1.2.1. Thalassemia/Hb E disease 14](#_Toc353790794)

[1.1.2.2. Hb H Disease 15](#_Toc353790795)

[1.1.2.3. Homozygous Hb SC 16](#_Toc353790796)

[1.1.2.4. Alpha thalassemia 17](#_Toc353790798)

[1.1.2.5. Beta Thalassemia 18](#_Toc353790799)

[1.1.2.6. Delta ( δ )Thalassemia](#_Toc353790800) 20

[1.1.3. Dominant-thalassemia Trait 21](#_Toc353790801)

[1.1.4. Treatment of thalassemia 21](#_Toc353790802)

[1.1.4.1. Regular blood transfusion 22](#_Toc353790803)

[1.1.4.2. Iron Chelation Therapy 22](#_Toc353790804)

[1.1.4.3. Clinical Effiancy of Iron Chelators 23](#_Toc353790805)

[1.1.4.4. Types of chelating agent 23](#_Toc353790806)

[1.1.4.5. Toxic Effects of Iron Chelators 25](#_Toc353790807)

[1.1.4.6. Transplantation 25](#_Toc353790808)

[1.2. Oxidative stress 26](#_Toc353790809)

[1.2.1. Free Radicles 28](#_Toc353790810)

[1.2.1.1. Reactive Oxygen Species 28](#_Toc353790811)

[1.2.1.2. Reactive nitrogen species (RNS) 29](#_Toc353790812)

[1.2.2. Antioxidants 32](#_Toc353790816)

[1.3. Malondialdehyde (MDA) 33](#_Toc353790822)

[1.4. Inflammation 35](#_Toc353790823)

[1.4.1. CRP 35](#_Toc353790824)

[2. materıals and methods 37](#_Toc353790825)

[2.1. General Laboratory Equipment 37](#_Toc353790826)

[2.2. Disposable Laboratory Equipment 38](#_Toc353790827)

[2.3. Chemicals and Reagents 38](#_Toc353790828)

[2.4. Laboratory analyses 38](#_Toc353790829)

[2.5. Malondialdehyde ( MDA ) Detection 39](#_Toc353790830)

[2.6. C-reactive proteins ( CRP ) detection 39](#_Toc353790831)

[2.7. Statistical analysis 40](#_Toc353790832)

[3. results 41](#_Toc353790833)

[4. Dıscussıon 43](#_Toc353790834)

[references 4](#_Toc353790835)7

[Ethics Commıttee form 49](#_Toc353790836)

# TAble Lıst

Table 1.1: Thalassemia genotypes ………...………………………………………....12

Table 1.1: Available iron-chelating agents for the treatment of iron overload.……….25

**Table 3.1:** Baseline characteristics of studied populations …………………...……...41

Table 3.2: Blood count results of patients and the healthy subjects …...………….….42

# fıgure lıst

Figure 1.1: Carrier Frequencies for Common Hemoglobin Disorders………………...17

Figure 1.2: Clinical Classification of β-thalassemia………………………………… 20

Figure 1.3: Mechanism of oxidative stress……..………………………………...……27

Figure 1.4: Electron structures of common reactive oxygen species…..…..…...……..29

Figure 1.5: Conversion of Nitrate to nitrite by the action of Nitrate Reductase………30

Figure 1.6: Schematic illustration of the activation of NADPH OxidaseSignal Transduction…………………………………………………………………..31

**Figure 3.1:** Showing differences in serum level of MDA and CRP between control and β-thalassemic patients……………………………………………………………...42

# symbols/Abbrevıatıons

%: Percentage

°C: Degree celsius

µg: Micro gram

µl: Microlitre

µM : Micro molar

1H: Proton

1O2: Singlet oxygen

AlCl3: Aluminium chloride

ArO: Aroxylradica

ATP: Adenosine triphosphate

C H O: Carbon Hrdogen Oxygen

CO2 : Carbon dioxide

CoA : Co-enzyme A

CRP: C-reactive protein

DNA: Deoxyribonucleic acid

FBS : Fetal bovine serum

Fe2+: Ferrous ion

Fe3+: Ferric ion

FeCl3: Ferric chloride

h : Hour

H+: Hydrogen ion

H2O : Water

Hb : Hemoglobin

HClO : Hypochlorous acid

IMA: Ischemia modified albumin

Kg: Kilogram

L: Lipid radical

LOO: Lipid peroxy radical

LOOH: Lipid hydroperoxide

LPS: Lipopolysaccharide

MDA: malondialdehyde

min: Minute(s)

ml: Millilitre

n: Number

NADH: Nicotinamide adenine dinucleotide

NADPH: Nicotinamide adenine dinucleotide phosphate

NaNO2: Sodium nitrite

NO: Nitric Oxide

NO: Nitric oxide radical

NOS: Nitric oxide synthase

O2: Oxygen molecule

O2-: Superoxide anion radical

O3: Ozone

OH: Hydroxyl

OH: Hydroxyl radical

OPI: organophosphorus insecticide

RBC: Red blood cells

RO: Alkoxyl radicals

RO2- : Peroxyl anion

ROO: Peroxyl radicals

ROOH: Alkyl peroxide

ROS: Reactive oxygen species

S: Singlet

SD: Standard deviation

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

TCA: Tricholoroacetic acid

Vitamin C: Ascorbic acid

Vitamin E: Tocopherol

WBC: white blood cells

WHO: World Health Organization

α: alpha

β: beta

# abstract

**Ab.Jabar A., Malondialdehyde and C-reactive protein values in transfusion dependent β-thalassemia patients. Near East University, Institute of Health Science, Biochemistry program, Master Thesis, Nicosia, 2013.**

Thalassemia is an inherited autosomal blood disorder that mainly originates in the Mediterranean countries. In thalassemia, the disease is caused by the excessive destruction or degradation of red blood cells due to formation of abnormal hemoglobin molecules, because of a defect through a genetic mutation or deletion. Blood transfusion is life saving for beta-thalassemic patients but sometimes iron overload occure as a result of chelation therapy secondary to blood transfusion which may lead to oxidative stress and inflammation.This may cause further complications like growth retardation, cardiopulmonary complications including pericadits, cardiac failure, hepatobiliary disease and hepatitis. The study group consisted of 24 thallassemic patients and 24 control groups. The blood samples of thalassemic patients were collected in Thalassemia Center in Dr.Burhan Nalbantoğlu Government Hospital. After centrifugation,aliquots of serum and plasma will be stored. In these thesis the aim are to determines C-reactive proteins as biomarker of inflammation, serum ferritin as biomarker of iron overload, MDA as biomarker of oxidative stress, CBC and all biochemical parameters in both transfusion dependent thalassemic patients and normal volunteers, the estimation of CRP, MDA and other biomarkers of inflammation and oxidation which may gives some helpeful knowledges that may be usefull for preventing futher complication of beta-thalassemia disorders and also for better management of the sepatients.

Keywords: Malondialdehyde, thalassemia and C-reactive protein.

# ıntroductıon

## Thalassemia

The thalassemia is a group of inherited disorders of hemoglobin (Hb) synthesis. In thalassemia the absence of chain synthesis results from several causes such as a complete block at transcription or RNA processing, leading to lacking of the globin mRNA production. In some cases, it is caused by point mutation in the DNA sequence, for instance a nonsense mutation providing a production of an incomplete globin chain (Pranee Winichagoon, 2002). Clinical severity varies widely, ranging from asymptomatic forms to severe or even fatal entities (Table 2.1).

Normally, hemoglobin is composed of four protein chains, two α and two β-globin chains arranged into a heterotetramer. In thalassemia, patients have defects in either the α or β globin chain (unlike sickle-cell disease, which produces a specific mutant form of β globin), causing production of abnormal red blood cells.The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α thalassemias, production of the α globin chain is affected, while in β-thalassemia production of the β globin chain is affected.

### Clinical Classification

Thalassemia is classified into three clinical groups:

1- Severe thalassemia (thalassemia major)

2- Thalassemia intermedia

3- Asymptomatic thalassemia (thalassemia minor)

Table 1.1: Thalassemia genotypes , ( Giardina PJ,2009 ).

|  |  |  |
| --- | --- | --- |
|  | **Genetics** | **Genotype** |
| α-Thalassemia | Normal | αα/αα |
|  | Silent Carrier | -aα/αα |
|  | Minor | -aα/-α , --/αα |
|  | Hb H disease | --/,-α , --/αcsα |
|  | Barts Hydrops Fetalis | --/-- |
|  | | |
| β-Thalassemia | Normal | β/β |
|  | Minor | β/β+ , β/β0 |
|  | Intermedia | Typically β+/β+; also β+/β0, β0/β0, α |
|  | Major | >β0/β0 ; also β+/β0, β+/β+ |
|  | | |
| Hemoglobin E Thalassemia | HbE trait | βE/β |
|  | Hemoglobin E homozygous | βE/βE |
|  | HbE-Beta-thalassemia | βE/β+, βE/β0 |
|  | Compound heterozygous (SCD) | βE/βS |
|  |  |  |

#### Thalassemia Major

This consists of thalassemic diseases with severe anemia and associated symptoms. Hemoglobin levels of the patients with severe thalassemia are usually 6 g/dl or lower. Untreated, the patients die early, at birth or before in the case of Hb Bart's hydrops fetalis or in the first two decades of life. Severe thalassemia consists mainly of two categories, i.e. homozygous -thal 1 and numerous -thalassemic diseases. The severity of the α thalassemia is correlated with the number of affected α globin genes: the greater, the more severe will be the manifestations of the disease.In case of α.thalassemia , there are 2 severe forms,when 3 alleles missing in out of 4, the condition is called Hemoglobin H disease. Two unstable hemoglobins are present in the blood: Hemoglobin Barts (tetrameric γ chains) and Hemoglobin H (tetrameric β chains). Both of these unstable hemoglobins have a higher affinity for oxygen than normal hemoglobin, resulting in poor oxygen delivery to tissues. There is a microcytic hypochromic anemia with target cells and Heinz bodies (precipitated HbH) on the peripheral blood smear, as well as splenomegaly. The disease may first be noticed in childhood or in early adult life, when anemia and splenomegaly are noted and when 4 alleles missing The fetus cannot live once outside the uterus and may not survive gestation: most such infants are dead at birth with hydrops fetalis, and those who are born alive die shortly after birth. They are edematous and have little circulating hemoglobin, and the hemoglobin that is present is all tetrameric γ chains (hemoglobin Barts) (Steensma DP et al. , 2005). While in β-thalassemia major or severe also called aniemia, in which both alleles have thalassemia mutations.This is a severe microcytic, hypochromic anemia.When untreated, it causes anemia, splenomegaly, and severe bone deformities. It progresses to death before age 20.

General Types of severe Thalassemia :

Type I: Included patients with the most severe clinical manifestations, in which diagnosis was made very early in life and treatment started at the age of 3-24 months because of low hemoglobin.

Type II: Included patients with rather milder clinical manifestations; hemoglobin was preserved at satisfactory levels (> 8 g/dl) during the first two years of life, but later deteriorated and transfusions were started at the age of 3-5 years.

#### Thalassemia Intermedia

The term “β-thalassemia intermedia” (TI) was ﬁrst suggested to describe patients who had clinical manifestations that are to severe to be termed “β-thalassemia minor” yet too mild to be termed “β-thalassemia major”(TM). Patients with TI usually present to medical attention in later childhood or even adulthood. They show mild to moderate anemia and a hemoglobin level ranging between 7 and 10 g/dL, which is sustainable without the need for regular transfusion therapy (Khaled M. Musallam et al. , 2012) Unfortunately, many patients with TI are set on a life of unnecessary regular transfusions, that is, similar to patients with TM, particularly if they present during a period of intercurrent infection requiring a few transfusions. It is essential to evaluate the patient carefully over the ﬁrst few months after the genetic diagnosis of β-thalassemia is established and not to embark on any treatment modality, especially regular transfusion therapy, too hastily. (Steinberg et al. 2009; Taher et al. 2011).

#### Thalassemia Minor

This is a thalassemic diseases with mild to moderate anemia with hemoglobin levels of 7 g/dl or higher at steady state. Generally the patients have very mild or are free of symptoms, not requiring blood transfusions.

All the heterozygotes or thalassemia traits are asymptomatic, but many homozygous and double heterozygous states are also symptoms free. These are homozygous -thal 2, homozygous Hb E, some homozygous Hb CS, double heterozygosity between one of the - thal genes with either -thal or Hb E, for examples. In Thalassemia minor, the hemoglobin genes are inherited during conception, one from the mother (ovum) and one from the father (sperm). People with a Thalassemia trait in one gene is known as carrier or is said to have thalassemia minor. The only way to know if you carry the Thalassemia trait is to have a special blood test called hemoglobin electrophoresis which can identify the gene. The carriers of thalassemia minor become anemic or slightly anemic (Tha.Fau.Canada, 2011).

### Types of Thalassemia Diseases

#### Thalassemia/Hb E disease

Thalassemia/Hb E disease is common in Southeast Asia. Because of increased population migration, its frequency is rapidly increasing in countries outside Southeast Asia and it has become a worldwide health problem. Despise having similar genetic background, Although deﬁnition of the two clinical extremes (mild andsevere courses) is simple, assigning a clear separating cutoff point can be problem, which showed that all patients carrying β+ thalassemia interacting with Hb E and 90% of patient who have a-thalassemia coinheritance were among the mild group (Orapan Sripichai ,et. al. 2008).

About half of the Hb E patients manifest as thalassemia intermedia, while the other half have severe thalassemic disease. Hemoglobin E disease results when the offspring inherits the gene for HbE from both parents. At birth, babies homozygous for the hemoglobin E allele do not have symptoms due to HbF (fetal hemoglobin) they still have. In the first month of life, fetal hemoglobin disappears and the amount of hemoglobin E increases, so the subjects start to have a mild β thalassemia. People who are heterozygote for hemoglobin E (one normal allele and one abnormal allele) do not show any symptoms (there is usually no anemia or hemolysis). Subjects homozygous for the hemoglobin E allele (two abnormal alleles) have a mild hemolytic anemia and mild splenomegaly. People who have hemoglobin E/β thalassemia have inherited one gene for hemoglobin E from one parent and one gene for β-thalassemia from the other parent. Hemoglobin E/β thalassemia is a severe disease, and it still has no universal cure. It affects more than a million people in the world (Vichinsky E., 2007). The consequences of hemoglobin E/β thalassemia when it is not treated can be heart failure, the enlargement of the liver, problems in the bones, etc.

There is a variety of genotypes depending on the interaction of HbE and α thalassemia. The presence of the α thalassemia reduces the amount of HbE usually found in HbE heterozygotes. In other cases, in combination with certain thalassemia mutations, it provides an increased resistance to malaria (P. falciparum).(Bachir D and Galacteros F,2004).

#### Hb H Disease

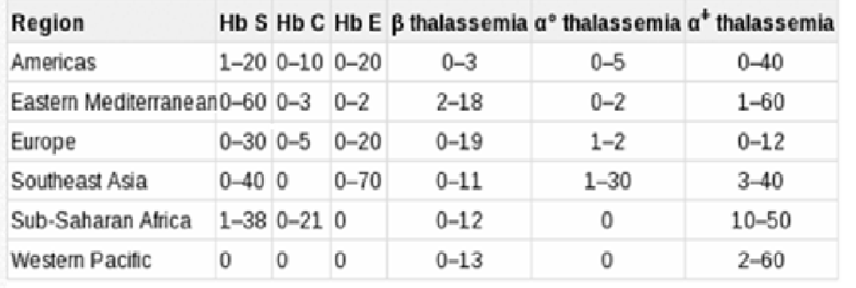
Hemoglobin H (Hb H) disease is the most common form of thalassemia intermedia and has many features that require careful consideration in management. In the majority of cases, Hb H disease results from double heterozygosity for alpha(0)-thalassemia due to deletions that remove both linked alpha-globin genes on chromosome 16, and deletional alpha(+)-thalassemia from single alpha-globin gene deletions (--/-alpha). However, Hb H disease may occur from interactions between alpha(0)-thalassemia with non-deletional mutations (alpha(T)alpha or alpha(T)) or with abnormal hemoglobins such as Hb Constant Spring, Hb Paksé, Hb Quong Sze, and Hb Pak Num Po. In a steady state, patients with Hb H diseases have hemoglobin levels around 9 to 10 g/dL; however, during hemolytic crisis, which frequently develops in or after acute infections with high fever, the hemoglobin level may drop significantly and patients can develop shock or renal shutdown. Even though splenectomy leads to significant elevation of hemoglobin levels, it is not recommended because the majority of patients do well with said steady-state hemoglobin levels. Patients with non-deletional Hb H disease are usually more anemic with significant splenomegaly, and some may require regular blood transfusions and be even as severe as "Hb H hydrops fetalis." However, there is no clear genotype-phenotype correlation associated with this severe clinical syndrome since patients with identical genotypes do not necessary show the same severity. This suggests that other genetic and environmental factors play a role in modifying the degree of clinical severity in patients with non-deletional Hb H disease (Fucharoen S, 2009). Practically all cases of the common Hb H disease, either of the -thal 1/ -thal 2 or -thal 1/Hb CS genotypes, manifests as thalassemia intermedia.

#### Homozygous Hb SC

Hemoglobin C disease is an autosomal recessive disorder that results from the biparental inheritance of the gene that encodes for hemoglobin C. It causes mildhemolytic anemia. Considered one of the benign hemoglobinopathies, hemoglobin C disease may not be diagnosed until adulthood. Patients with hemoglobin C disease require multispecialty care. Hemoglobin C comprises 2 normal alpha chains and 2 variant beta chains in which lysine has replaced glutamic acid at position 6. This unstable hemoglobin precipitates in red blood cells (RBCs) to form crystals (see the image below). These intracellular crystals lead to a decrease in RBC deformability and an increase in the viscosity of the blood. The spleen effectively removes these crystal-containing cells.

Individuals with sickle cell–hemoglobin C (HbSC), have the gene for HbS inherited from one parent and the gene for HbC is inherited from the other parent: they are "heterozygous". Since HbC does not polymerize as readily as HbS, there is less sickling (fewer sickle cells). The peripheral smear demonstrates mostly target cells and only a few sickle cells. There are fewer acute vaso-occlusive events. However, persons with hemoglobin SC disease (HbSC) have more significant retinopathy, ischemic necrosis of bone, and priapism than those with pure SS disease (Nagel LR et. al., 2009). The gene for Hb S is distributed throughout Sub-Saharan Africa, the Indian subcontinent, and the Middle East, where carrier frequencies range from 5 to 40 percent or more. Hb E is found in the eastern half of the Indian subcontinent and throughout Southeast Asia, where carrier rates may exceed 60 percent.(Weatherall and Clegg 2001b ) (Figure 2.1).

Figure 1.1.Carrier Frequencies for Common Hemoglobin Disorders, by World Health Organization Region, 2001.



#### 

#### 1.1.2.3. Alpha thalassemia

When the body has a problem producing alpha globin. Some children with alpha thalassemia have no symptoms and may require no treatment. Others with more severe cases need regular blood transfusions to treat anemia and other symptoms. A child can only get alpha thalassemia by inheriting it from his or her parents. Genes are "building blocks" that play an important role in determining physical traits and many other things about human body. In alpha thalassemia, there is a mutation p arm in chromosome 16. Alpha globin is coded on chromosome 16. So, if there is any mutation on the 16th chromosome alpha globin will be missing or mutated and less alpha globin will be made. This affects hemoglobin and decreases the ability of red blood cells to transport oxygen around the body.Its distribution is heterogeneous. In Thailand, the overall frequency of a thalassemia is 20-30%. The frequency of thalassemia 1 is higher in Northern than in the Southern part; 10% in Chiengmai and 3.5% in Bangkok whereas thalassemia 2 is between 16-20%.(Pranee Winichagoon, 2002).

#### 1.1.2.4. Beta Thalassemia

Beta-thalassemia is dicoverd in 1925 by Thomas cooley & Lee,which is characterized by low hemoglobin and red blood cells than normal .It is a disease commonly seen in Mediterranean region and nearly 3% of the world population are carriers of beta thalassemia,with the highest levels been recorded in South Asia (Maldives) in which 16% of its population are carriers. In india 3 of 1000 children are born with beta-thalassemia annually.This disease is also seen in Europe (Greece), coastal region of Turkey and Mediterranean Islands, eg. Sardinia, Malta and Cyprus (Olivieri NF, 1999).

Beta thalassemia occurs when the gene that controls the production of beta globin is defective and a child can only get beta thalassemia by inheriting it from his or her parents. The homozygous or compound heterozygous states for thalassemia also run a variable course, although without transfusion, death usually occurs in the first few years (Weatherall and Clegg 2001).When someone has beta thalassemia, there is a mutation in chromosome 11. Beta globin is made on chromosome 11 (beta globin, along with alpha globin, is one of the proteins that makes up hemoglobin). So, if one of the genes that tells chromosome 11 to produce beta globin is altered, less beta globin is made. Identified mutations include single base pair changes that lead to frameshift mutations or changes in canonical sequences that affect mRNA stability and processing (Chin, Joanna Y. , et. al. , 2008). This affects hemoglobin and decreases the ability of red blood cells to transport oxygen around the body (Robin Miller, 2012).

Some of the important mutations having effect on β-globin chain synthesis are as below:

a)Transcription defect: Mutation affecting transcriptional promoter sequence causes reduced synthesis of β-globin chain. Hence the result is partially preserved synthesis i.e. β+ thalassemia.

b)Translation defect: Mutation is in the coding sequence causing stop codon (chain termination) interrupting β-globin messenger RNA. This would result in no synthesis of β-globin chain and hence β0 thalassemia.

c)mRNA splicing defect: Mutation leads to defective mRNA that is degraded in the nucleus. Depending upon whether part of splice-site remains intact or is totally degraded, it may result in β+thalassemia or β0 thalassemia.

Depending on the extent of reduction in β-chain synthesis, there are three types of β-thalassemia ( JPharm Bioallied, 2012 ) :

Homozygous form: β-thalassemia major:

It is the most severe form of congenital hemolytic anemia. Individuals with thalassemia major usually present within the first two years of life with severe anemia, which requires regular red blood cell (RBC) transfusions. Number of complications can occure in untreated or poorly transfused individuals with thalassemia major, wich can be seen in some developing countries, includes growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow. Regular transfusion therapy leads to iron overload-related complications including endocrine complication (growth retardation, failure of sexual maturation, diabetes mellitus, and insufficiency of the parathyroid, thyroid, pituitary, and less commonly, adrenal glands), dilated myocardiopathy, liver fibrosis andcirrhosis) (Galanello,Renzo;Origa , et. al., 2010).

The other two types of thalassemia are:

β0 thalassemia major characterized by complete absence of β chain synthesis.

β+ thalassemia major having incomplete suppression of β chain synthesis.

β-Thalassemia intermedia.

It is β-thalassemia of intermediate degree of severity that does not require regular blood transfusion. These cases are genetically heterozygous (β0/β or β0/β).

β-Thalassemia minor (trait):

It is a mild asymptomatic condition in which there is moderate suppression of β-chain synthesis.

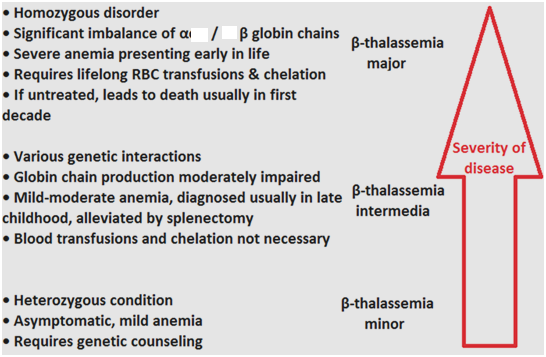


Figure1.2: Clinical Classification of β-thalassemia ( Taher A, et al. 2006 ).

#### Delta ( δ )Thalassemia

The human and Tge globine gene encodes the β-likeT subunit (δ globin chain) of the hemoglobin (Hb) A,the minor fraction of adult Hb. The δ globin gene lies on chromosome 11 within the P-like globin gene cluster. Reduced (δ ') or absent (δ ") production of the δ -globin chains is the hallmark of a group of genetic disorders referred to as δ -thalassemias (δ -thal).' The clinical relevance of δ -thal is related to the fact that, when coinherited either in cis or in trans with P-thal, the resulting phenotype is characterized by normal HbA, levels, thus confusing the identification of the P-thal carrier state. The molecular bases for δ -thal have so far been elucidated solely in a few cases in individuals of Italian and Belgian origin. Deletion, frameshift, and messenger RNA (mRNA) processing mutations have been characterized. In Japanese individuals with δ "-thal, & globin gene analysis showed only a T -+ C change at position -77 5' to the cap site, which is very close to the CCAAC box This gene, however, showed a normal function on transient expression assay (P Moi, et al., 1992).

### Dominant-thalassemia Trait

All the -thal and -thal heterozygotes are asymptomatic. Some very rare chain mutants result in either unstable hemoglobins or undigested shortened versions of globin chain that precipitate in the red blood cells leading to premature destruction. Heterozygotes of such mutants then have symptoms of thalassemia intermedia.

There are three types of thalassaemia trait:

- Alpha plus thalassaemia trait which has one missing alpha haemoglobin gene. (Normally there are four of these genes.) This trait can only cause a problem if your partner has alpha zero thalassaemia trait - in which case your children might inherit Hb H disease (explained below). Apart from that situation, it will not affect you or your children.

- Alpha zero thalassaemia trait which is a condition with two missing alpha haemoglobin genes (out of the normal four alpha genes). If both of the partners have alpha zero thalassaemia trait, the child might inherit a severe condition called Hb Barts (explained below). Or, if one of the partners has alpha plus thalassaemia trait, then the child might inherit Hb H disease.

- Beta thalassaemia trait is a state with one abnormal beta haemoglobin gene (out of the normal two beta genes).

### Treatment of thalassemia

Most patients with β-thalassemia major are severely anaemic and many of distressing symptoms of the condition are directly realted to the anaemia. The companesatory mechanisms recruited by the body to improve the production of viable red cells may also cause symptoms and in some patients are more troublesome than the anaemia itself (Propper R.D. 1983). The expanding hyperplastic bone marrow leads to gross skeletal changes particularly affecting the face, masses of extramedullary erythroid tissue compress vital structures and increased iron absorption causes deposition of iron in parenchymal tissues (Wolman J. 1999).

#### Regular blood transfusion

Regular blood transfusions are essential support therapy for patients with transfusion-dependent aneamias such as thalassemia and sickle cell disease. However , since humans lack a mechanism for the active exretion of excess iron, these patients invariably develop iron overload acquired from chronic blood transfusions (Olivieri N,F, 1999: J.B. 2001). Blood transfusion is a solution for anaemia but ineffective erythropoiesis also result in iron overload. Liver, heart, and endocrine glands are among the most affected organs in these forms of systemic iron overload. Iron overloading with repeated transfusion, results in the saturation of transferrin and than results in labile iron pool (LIP) (iron within cells, not bound to dedicated proteins) selectively in some organs (Pak J Physiol, 2009). Iron overload is clearly associated with an increased cancer risk. Hepatocellular carcinoma, common in hereditary hemochromatosis, is also frequently seen in thalassemia (Bonassi,et. al.2000).

#### Iron Chelation Therapy

Iron chelation therapy is the removal of excess iron from the body with special drugs. Chelate is from the Greek word "claw".Chelation therapy has a long history of use in clinical toxicology. Iron overload occurs when the intake of iron is increased over a prolonged period of time and is commonly seen in patients with hereditary or refractory anemias (e.g. β-thalassaemia major, sickle cell anemia and myelodysplastic syndromes) who receive frequent blood transfusions. The iron excess is initially stored in the reticuloendotelial system, which has a capacity of about 10–15 g, and then in all parechymas, resulting in life-threatening complications, namely cardiopathy, liver and endocrine dysfunction and reduced patient’s survival (Porter JB, 2001 , Modell B, 1977 , Modell B,et. al., 1984). The goal of iron chelation therapy is to prevent iron-mediated injury to cells. Iron ions in aqueous solution exist either in the ferrous (Fe2+) state or the ferric (Fe3+) state. The shift of electrons between iron and donor molecules is the basis of energy production by controlled oxidation of carbohydrates, proteins, and lipids. Iron is a key element in most of the cytochrome enzymes involved in the oxidative phosphorylation of the Krebs cycle.

#### Clinical Effiancy of Iron Chelators

No ideal chelator exists to treat patients with transfusional iron overload. The characteristics of such a compound can be extrapolated from the clinical requirements:

1.Oral administration.

2.Good tissue penetration

3.Easy mobilization of the iron-chelator complex

4.Inexpensive

5.Non-toxic

6.Hexidentate binding of iron ions

#### Types of chelating agent

1. Deferoxamine:

Deferoxamine (also known as desferrioxamine B, desferoxamine B, DFO-B, DFOA, DFB or desferal) is a bacterial siderophore produced by the actinobacteria Streptomyces pilosus. It has medical applications as a chelating agent used to remove excess iron from the body (Mellar, Marvin J, 1989). Deferoxamine acts by binding free iron in the bloodstream and enhancing its elimination in the urine. By removing excess iron, the agent reduces the damage done to various organs and tissues, such as the liver. A recent study also shows that it speeds healing of nerve damage (and minimizes the extent of recent nerve trauma) (Lee HJ et al., 2007).

2.Deferasirox

Deferasirox (Exjade) is iron chelating medication that comes in a tablet form. It is dissolved in juice or water and taken (by mouth) once a day. Most patients tolerate it very well, but side effects can include nausea, diarrhea, rash, and more serious effects such as kidney or liver injury. Once the body gets used to the drug, side effects usually go away.The doctor should monitor the liver and kidneys for potentially serious side effects while he is taking deferasirox.

3. Deferiprone

Deferiprone (DFP, Ferriprox™, Kelfer™, L1, CP20) is one of a series of hydroxypyridinone iron chelators synthesized by Dr. Kontoghiorghes in the early to mid 1980s in the laboratory of Professor R. Hider at the University of Essex in London (Kontoghiorghes 1985). The medicinal chemists in this laboratory were researching a molecule that could be taken orally, bind iron in conditions of iron overload, such as thalassemia, and excrete it from the body. When screening techniques revealed efficacy in 59Fe-labeled liver macrophages and leukemic cell lines, they tested this chelator in iron-loaded mice, rats, and rabbits and found that it was absorbed into the body and did excrete excess iron (Hoffbrand, 2005).

4. Combination Therapy

Therapy with either deferiprone (DFP) or deferoxamine (DFO) is inadequate in achieving negative iron balance in many patients with thalassemia. There are mounting theoretical, experimental, and clinical evidences of increased efficacy when therapy includes both chelating agents. DFP and DFO chelate excess iron in different ways without affecting each other's metabolism. When both chelators are administered simultaneously, they interact either in an additive or synergistic manner, probably through "shuttling" iron from DFP to DFO. Iron-balance studies have shown that the use of both agents on the same day can induce negative iron balance in all patients. Long-term combined therapy with DFO with DFP results in considerable reduction of both ferritin levels and liver iron concentration as well as significant improvement in cardiac siderosis and function. This therapeutic regimen is well tolerated and safe, even though it may be related to a small increase in the incidence of agranulocytosis compared with DFP monotherapy. Combining the available iron chelators offers many therapeutic options that can be tailored to each patient individually. It is an exciting advance in treating hemosiderosis in thalassemic patients (Ann N Y Acad Sci. 2005).

Table 1.2: Available iron-chelating agents for the treatment of iron overload(Misbet-Brown E. et al.2003).

|  |  |  |
| --- | --- | --- |
| Agent | Pharmacology | Route of Administration |
| Deferasirox | Tridentate molecule:  2:1 stoichiometry for iron | Oral |
| Deferipron | Bidentate molecule  3:1 stoichiometry for iron | Oral |
| Deferoxamine | Hexadentate molecule  1:1 stoichiometry for iron | IV, IM, s,c |

IV= intravenous ; IM = intramuscular; s.c = subcutaneous

#### Toxic Effects of Iron Chelators

Designing an ideal iron chelator is a difficult challenge because of iron paradox, Iron is an essential elenment for many important metabolic functions (oxygen transportation and utilization, DNA synthesis, electron transport and many other biological processes), but it becomes toxic when accumulated. A chelator should remove only excess iron. Thus, in addition to possible direct toxic effects, iron chelators may alter iron homeostasis(absorption, distribution and utilization) or interfere with iron-dependent enzymes (ribonucleotide reductase, lipoxygenase) or remove other metals such as zinc, copper and caliciun from metabolic pools(Cohen A.R. et al 2000).

#### Transplantation

Untreated thalassemia major eventually leads to death usually by heart failure therefore birth screening is very important. Bone marrow transplantation is the only cure for thalassemia, and is indicated for patients with severe thalassemia major. Transplantation can eliminate a patient's dependence on transfusions. If there is no matching donor for a child with thalassemia, a savior sibling can be conceived by preimplantation genetic diagnosis (PGD) to be free of the disease as well as match the recipient's human leucocyte antigen (HLA) type in order to be a donor for the sick child(Maggio et al. , 2002).

## Oxidative stress

Oxidative stress is caused by an imbalance between the production of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA . short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis. Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Reactive oxygen species are also used in cell signaling. This is dubbed redox signaling. In chemical terms, oxidative stress is a large rise (becoming less negative) in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis.A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage. The major portion of long term effects is inflicted by damage on DNA.

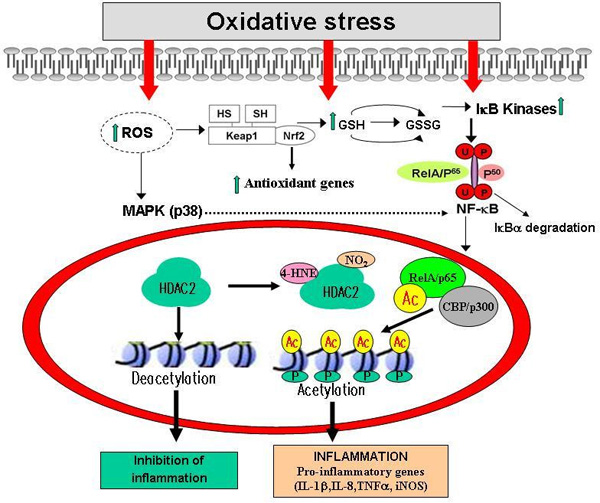


Figure 1.2Mechanism of oxidative stress. (Fiers, W., et al., 1999 , Nicholls, D.G., and Budd, S.L.,2000 , Hayes, J.D., et al., 1999).

Oxidative stress is imposed on cells as a result of one of three factors: 1) an increase in oxidant generation, 2) a decrease in antioxidant protection, or 3) a failure to repair oxidative damage. Cell damage is induced by reactive oxygen species (ROS). ROS are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them. Examples are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. The main source of ROS in vivo is aerobic respiration, although ROS are also produced by peroxisomal b-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. Under normal conditions, ROS are cleared from the cell by the action of superoxide dismutase (SOD), catalase, or glutathione (GSH) peroxidase. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Additionally, oxidative stress and ROS have been implicated in disease states, such as Alzheimer's disease, Parkinson's disease, cancer, and aging.(Fiers, W., et al., 1999 , Nicholls, D.G., and Budd, S.L.,2000 , Hayes, J.D., et al., 1999).

### Free Radicles

#### Reactive Oxygen Species

Reactive oxygen species (ROS) resulting from conditions such as ischemia, hypoxia, acidosis, free radicals, and free iron can decrease the ability of the N-terminus to bind with transition metals (Bar-Or D, 2000, Bar-Or D, 2001 ,Chan B., 1995, Roy D 2006). Also defind as molecules or ions formed by the incomplete one-electron reduction of oxygen.They contribute to the microbicidal activity of phagocytes, regulation of signal transduction and gene expression, and the oxidative damage to nucleic acids; proteins; and lipids (MeSH ,12- 2012).

ROSs include superoxide anion radical, hydrogen peroxide, singlet molecular oxygen, hypochlorous acid, and hydroxyl radical; recently, the role of nitric oxide radicals as been appreciated . These ROSs are generated in increased amounts in thalassemic red blood cells (RBCs) because the deposition of excess unmatched globin chains (Alpha in Alpha thalassemia and Beta in Beta thalassemia) contain free iron, nonheme iron, and hemichromes.These compounds can generate ROS by several mechanisms, including action as a Fenton reagent. The failureof therapeutic trials with agents like vitamin E is not surprising given newer information on the highly specific actions of the different free radicals, which in turn produce equally specific alterations in membrane lipids, intracellular hemoglobin, and membrane proteins like band 3 (Celedon G, et al. 2001 , Kattamis C et al. 2001 ,Stanley L Sciere 2002).

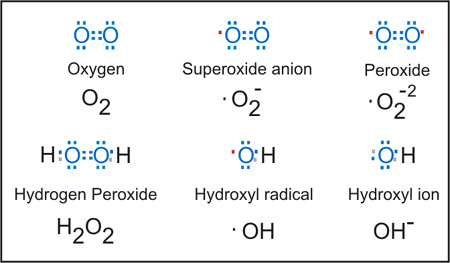


Figure 1.3: Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. The designates an unpaired electron (PaulHeld ,2010).

#### Reactive nitrogen species (RNS)

#### The free radical nitric oxide (•NO) is produced by a number of different cell types with a variety of biological functions. Nitric oxide is a product of the oxidation of L-arginine to L-citrulline in a two step process catalyzed by the enzyme nitric oxide synthase (NOS). Two major isoforms of nitric oxide synthase have been identified. The constitutive isoform found in neurons and endothelial cells, produces very low amounts of nitric oxide in a calcium- and calmodulin-dependent fashion. •NO activates soluble guanlyate cyclase in target cells, resulting in increased levels of cGMP, which in turn facilitates neuronal transmission and vascular relaxation, and inhibits platelet aggregation (Misko et. al. ,1993).

#### The inducible isoform, found in macrophages, fibroblasts, and hepatocytes, produces •NO in relatively large amounts in response to inflammatory or mitogenic stimuli and acts in a host defensive role through its oxidative toxicity (Nathan, 1992). Regardless of the source or role, the free radical •NO has a very short half life (t½= 4 seconds), reacting with several different molecules normally present to form either nitrate (NO3-) or nitrite (NO2-). A commonly used method for the indirect determination of •NO is the determination of its composition products nitrate and nitrite colorimetrically. This reaction requires that nitrate (NO3) first be reduced to nitrite (NO2), typically by the action of nitrate reductase.

#### Açıklama: image description

Figure 1.4: Conversion of Nitrate to nitrite by the action of Nitrate Reductase. (Nathan, 1992).

Reactive oxygen species have a role in a number of cellular processes. High levels of ROS, which can lead to cellular damage, oxidative stress and DNA damage, can elicit either cell survival or apoptosis mechanisms depending on severity and duration of exposure. Nitric oxide (•NO) has been shown to serve as a cell-to-cell messenger, being responsible for such effects as decreasing blood pressure (Hou Y.C., et. al. ,1999). Intra-cellularly, ROS species, in conjunction with antioxidant enzymes, are believed to play a role in turning enzymes on and off by redox signaling in a manner akin to that of the cAMP second messenger system (HouY.C., et. al. ,1999). Examples include superoxide anion, hydrogen peroxide.

The steady state level of •O2- is estimated to be so low, however that its activity is spatially limited. Hydrogen peroxide (H2O2) is normally unreactive with thiols in the absence of catalyzing agents (e.g. enzymes,” multivalent metals etc.), it does react with thiolate anion (S-), to form sulfenic acid, which in turn ionizes to form sulfenate (SO-). This intermediate can be reversed by the action of glutathione (Forman et. a. ,2002).

Mitogenic signaling begins at the cell surface with the ligand-dependent activation of receptor tyrosine kinases, which activate important MAP kinase cascades necessary for proliferation. These cascades lead to the generation of H2O2 from several enzyme catalysts, including the NADPH oxidases (Park et. al. ,2006). It has been estimated that the production of H2O2 at nanomolar levels is required for proliferation in response to growth factors (Burch et. al. ,2005). Hydrogen peroxide interacts with both the SOS-Ras-Raf-ERK and PI3K/Akt pathways through several mechanisms and in a does-dependant manner. It has been suggested that small increases of H2O2, as a result of Nox1 expression result in increased reentry into the cell cycle, while sustained high levels of H2O2 lead to cell arrest and eventual apoptosis after prolonged arrest.

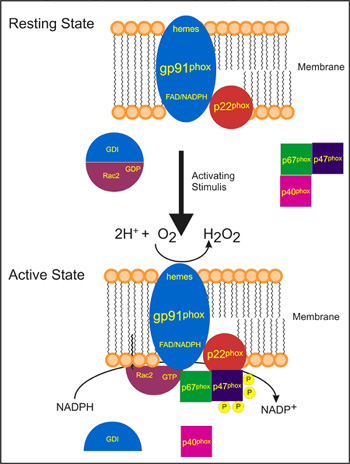


Figure 1 .5: Schematic illustration of the activation of NADPH Oxidase.

Signal Transduction (Bokoch, et. al. 2002 ).

### Antioxidants

### Antioxidant is any substance that when present at low concentrations compared with that of an oxidizable substrate significantly delays or inhibits oxidation of that substrate (Sies H. 1993). Antioxidants are important in maintaining cellular integrity in normal subjects by inhibiting oxidation reaction (Radak Z. et al. 1995), and human body has several mechanisms for defense are complementary to one another because they act on different oxidants in different cellular compartments (Sies H. 1991).

### There are two types of antioxidants in human body: enzymatic antioxidants and non-enzymatic antioxidants (Pierce J.D. et al. 2004; Van Langendonck A. et al. 2002).

### None-enzymatic antioxidants: The body`s complex antioxidant system is influenced by dietary intake of antioxidant vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, copper taurine hypotaurine, glutathione,beta carotene, and carotene (Agarwal A. et al. 2003). Vitamin C helps recycle oxidized vitamin E and glutathione (Chan A.C. 1993).

### Enzymatic antioxidants: These are superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, which also can cause reduction of hydrogen peroxide to water and alcohol. The enzymatic system contains mitochondrial (Mn-SOD), cytosolic (Cu,Zn-SOD and extra-cellular co) to convert reactive superoxide to less powerful hydrogen peroxide, Glutathione peroxidase (GPX). This enzyme consists of the amino acid Glutathion and the trace mineral selenium which are important in the reduction of hydroperoxides, catalase decompose hydrogen peroxide to water. Metal reductase is a unique enzyme that has demonstrated an ability to remain extraordinary restricted (Rae T.D. et al. 1999). As a result, the delivery of copper to specific pathways within the cell is mediated by a family of proteins termed metallochaperones that function to provide copper directly to target pathways while protecting this metal from intracellular scavenging (O Halloran T.V. 2000; Huffman D.L. and O Halloran T.V. 2001).

### Zinc is necessary for the functioning of over 300 enzymes and plays a vital role in enormaous number of biological processes. Zinc is acofactor for the antioxidant enzyme superoxide dismutase (SOD) and is in a number of enzymatic reaction involved in carbohydrate and protein metabolism (Aydemir T.B. et al. 2006).

## Malondialdehyde (MDA)

MDA is a marker for [oxidative stress](http://en.wikipedia.org/wiki/Oxidative_stress) and is an endogenous genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation whose adducts are known to exist in DNA isolated from healthy human beings**.** It is the [organic compound](http://en.wikipedia.org/wiki/Organic_compound) with the [formula](http://en.wikipedia.org/wiki/Chemical_formula) CH2(CHO)2. (Laura J. Niedernhofer,et al. 2002).

Superoxide dismutase (SOD), whose substrate is a free radical (superoxide anion; O2·-) catalyzes dismutation reaction resulting in the generation of hydrogen peroxide (H2O2). This H2O2 is decomposed to water and molecular oxygen by the action of catalase. When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage/death. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing malondialdehyde (MDA) as the end product.

In view of the possible oxidative stress involved in OPI poisoning, it has been decided to estimate the levels of SOD and MDA as an index of antioxidant status and oxidative stress respectively, and to see whether any difference existed in these parameters before and after therapy with atropine sulfate plus pralidoxime (PAM). (Vidyasagar J, et al. , 2004).

Malondialdehyde (MDA) was found to react with normal hemoglobin A (Hb A), forming a number of less cationic components which were detected by cellulose acetate electrophoresis and gel electrofocusing. All the modified components moved down the cation-exchange resin at a quicker rate than Hb A, and this chromatographic behavior of the modified components was similar to that of glycosylated Hb A. Some of these modified components were intermolecularly crosslinked, and showed fluorescence with an excitation maximum at 390 nm and an emission maximum at 460 nm. It is likely that MDA reacts nonspecifically with the epsilon-amino groups of lysine and N-terminal amino groups to produce aminoacrolein, crosslinks, and strongly fluorescent 1,4-dihydropyridine-3,5-dicarbaldehyde. Oxygen affinity of the modified hemoglobins was increased. The modified hemoglobins were more readily oxidized into met-form. Mechanical stability of Hb A was also decreased by the modification. These results suggest that a considerable conformational change in Hb A was induced by the treatment with MDA. Since MDA is generated in erythrocytes as a consequence of liquid peroxidation, MDA may react with intracellular Hb A and influence the function and the stability of hemoglobin. ([Kikugawa K](http://www.ncbi.nlm.nih.gov/pubmed?term=Kikugawa%20K%5BAuthor%5D&cauthor=true&cauthor_uid=6703702), et. al. 1984).

ROS can attack double bonds in polyunsaturated fatty acids, and thus induces lipid peroxidation; peroxidation (auto-oxidation) of lipids can be a result of deteriorated food which causes damage to tissues in vivo and may be a cause of cancer, inflammatory diseases, atherosclerosis, and aging. The deleterious effects are considered to be caused by free radicals (ROO ˖ ,RO ˖ , OH˖) produced during peroxide formation from fatty acides containing methylene-interrupted double bonds, i.e., those found in the naturally occurring polyunsaturated fatty acides (Mayes P and Botham K 2006). ROS mediated oxidation of cell membrane lipid leads to the formation of lipid peroxidation products, such as MDA (Halliwell B. 1991).

The whole process can be depicted as follows:

1. Initiation:

ROOH+ Metal n+ ROO˖ + Metal (n-1) +H+

X˖ + RH R˖ + XH

1. Propagation:

R˖ +O2 ROO˖

ROO˖ + RH ROOH + R˖

3- Termination

ROO˖ + ROO˖ ROOR + O2

ROO˖+ R˖ ROOR

R˖ + R˖ RR

\

## Inflammation

Inflammation is the body's response to injury or infection which can be classified as either acute or chronic. Acute inflammation is the initial inflammatory response .It occurs almost immediately after minor injuries like burns and cuts as well as major trauma such as myocardial infarction (MI). Acute inflammation results in the healing of the tissue when the injury or infection is removed. Symptoms include redness, swelling, heat, pain and stiffness in the affected area. Chronic inflammation or prolonged inflammation may follow acute inflammation or exist independently. Chronic inflammation is a continuous process for example tissue breakdown and repair attempts that often results in scarring and tissue destruction. Chronic inflammation can arise after bacterial infection or as a result of an autoimmune disease. In autoimmune diseases, the inflammatory response is triggered when there are no stimuli and the immune system attacks itself.

Extra protein is often released from the site of inflammation and these proteins can be readily detected in the bloodstream and are therefore referred to as inflammatory markers. The most commonly used marker of inflammation is C-reactive protein (CRP).

### CRP

C-reactive protein was discovered in 1930. In 2005, two studies published in the January 6, 2005, issue of The New England Journal of Medicine provide has given the best evidence to date that the C-reactive protein level in a person's blood is an important and highly accurate predictor of future heart disease.

C-reactive protein (CRP) is a sign of inflammation in the walls of arteries. Its genes composed of 34 kilo base genomic DNA segments, located in first chromosome (1q21\_q23) is a 224 residue protein with annular pentameric disc in shape. The CRP gene structures are typical in all eukaryotic genes and there is an interon located between axon containing signal peptide & exon. Studies have shown that most effective stimulator for CRP synthesis by hepatic liver is interleukin 6 & also it have been detected that physiological role of CRP is to bind to phosphocholines expressed on surface of infectious cells inorder to activate complement system by assisting complement binding to foreign & infectious cells enhancing phagocytosis by macrophages thus it has important role in innate immune system. (Deron, Scott J. .2003 , Fleming et al. 2004)

The researches showed that intensive inflammation can sometimes have adverse effects on the blood vessels which transport oxygen and nutrients throughout the body. Atherosclerosis, which involves the formation of fatty deposits or plaques in the inner walls of the arteries, is now considered in many ways an inflammatory disorder of the blood vessels, similar to the way arthritis can be considered an inflammatory disorder of the bones and joints. Inflammation affects the atherosclerotic phase of heart disease and can cause plaques to rupture, which produces a clot and interfere with blood flow, causing a heart attack or stroke.

There is an association between elevated levels of inflammatory markers (including CRP) and the future development of heart disease. This correlation applies even to apparently healthy men and women who have normal cholesterol levels. CRP level can be used by physicians as part of the assessment of a patient's risk for heart disease because it is a stable molecule and can be easily measured with a simple blood test. In patients already suffering from heart disease, doctors can use CRP levels to determine which patients are at high risk for recurring coronary events. (Deron, Scott J. .2003 , Fleming et al. 2004).

# materıals and methods

This prospective study examines patients who attended the outpatients clinic of Thalassemia Department in Dr. Burhan Nalbantoğlu Goverment Hospital in Cyprus between October 2012 to June 2013. The study population consisted of 50 subjects divided into two groups: 24 Thalassemia patients and 26 healthy control subjects. The control group consisted of healthy patients without any history of thalassemia disease. All subjects provided written informed consent before the study, and the study was approved by our Local Research Ethic Committee. General health characteristics such as age, sex, smoking status, menopausal status and alcohol consumption were investigated by a self administened questionaire.

The height (m), weight (kg), and waist circumference (cm) of each subject were recorded and body mass index (BMI) was calculated (kg / m2).

Blood samples were drawn from the antecubital vein, after overnight fasting and centrifuged at 4000 rpm for 10 minutes and seperated. The serum samples were stored at -20°C until they were analyzed for MDA and CRP.

## General Laboratory Equipment

* Centrifuge
* Automated spectrophotometer
* Automated chemistry analyzer
* Immunoassay analyzer
* Hot plate withrrer
* Vortex
* Sensitive Electronic balance
* Water bath
* Micropipettes: (50-200) µl, (100-1000)µl
* Refrigerator
* Beakers: 100 ml, 250 ml
* Dark bottle: 250 ml
* Volumetric Flask: 100 ml

## Disposable Laboratory Equipment

* Absorbent paper.
* Test tubes.
* Distilled water.
* Gloves
* Parafilm
* Plain tubes
* Syringes, 10 ml

## Chemicals and Reagents

* Antagonistic Liquid.
* Trichloroacetic acid (TCA), Catalog Number: 91070 Fluka, Switzerland.
* Thiobarbituric acid, Catalog Number : 88481, Fluka, Switzerland.

## Laboratory analyses

The level of serum glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), ferritin, ALT, AST, uric acid and low-density lipoprotein cholesterol (LDL-C) were determined using a fully automated clinical chemistry analyzer ( Abbott Architect C8000).

WBC, RBC, LYM, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, PCT and PDW were measured using a routine automated method.

## Malondialdehyde ( MDA ) Detection

Serum MDA was measured according to the method of Buege and Aust (1978) and was expressed as nmol/l.

Buege Reagent

%100 TCA 15ml

375 mg TBA

2.1 ml HCl

\* Volume complete up to 100 ml.

|  |  |
| --- | --- |
| **Reagents** | **Quantity** |
| Serum sample | 500 ul |
| Buege reagent | 2 ml |
| NaCl %0.9 | 500ul |
| Mix well by vortex and boil in water bath for 15 minutes. | |
| Centrifuge at 2000 rpm for 10 minutes, take out the supernatant, and the absorbance(A) was taken at 535 nm.  Calculation: 38.46 x Absorbance. C-reactive proteins ( CRP ) detection The level of CRP is determind in serum samples by manually CRP agglutination test, in which drops of serum is mixed with CRP latex to see degree of agglutination by which level of CRP in patients and control groups are determined. | |

Procedure :

- After separating serum from blood samples ,they are stored in the refrigerator at -20 Ċ.

- Drops of serum sample are mixed with the CRP latex on agglutination paper.

-The drops are mixed with a stirrer,spreading them over the entire surface of the circle.The slide is placed on a mechanical rotator for ( 2) minutes.

- The presence or absence of visible agglutination is examined macroscopically.

-The presence of agglutination indicates a CRP concentration equal or greater than 6 mg/L.

-Calculation:

• If agglutination occure only from first mixture of serum & CRP latex, the CRP level is (0.6 gm/dl) .

• If agglutination occure only from first mixture of serum & CRP latex & after one dilution, the CRP level is (1.2 gm/dl).

• If agglutination occure only from first mixture of serum & CRP latex & after two dilutions, the CRP level is (2.4 gm/dl).

• If agglutination occure only from first mixture of serum & CRP latex & after three dilutions, the CRP level is (4.8 gm/dl).

• If agglutination occure only from first mixture of serum & CRP latex & four one dilutions, the CRP level is (9.6 gm/dl).

## Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 15.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All results were expressed as mean ± standard deviation. The laboratory characteristics in both groups were compared by Student’s t-test. Differences were considered significant when P < 0.05.

# results

Descriptive statistics of metabolic characteristics of the study population are presented in Table 4.1. There was no significant differences between the mean value of fasting glucose,triglycerides, urea and creatinine in both β-thalassemia and control groups. Thalassemia patients had significantly higherAST (p<0.001), ALT (p= 0.007), uric acid(p<0.001), lactate dehydrogenase(p<0.001), ferritin (p=0.016), C-reactive protein(p<0.001) and MDA (p<0.001) levels compared to control groups. Total cholesterol (p<0.001)and HDL-cholesterol levels(p<0.001) were significantly higher in control subjects.

Table 3.1: Baseline characteristics of studied populations :

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Controls** | **Thalassemia patients** | **p-value** |
| Fasting glucose (mg/dl) | 92.54 ± 9.14 | 92.25 ± 34.92 | 0.76 |
| Urea | 13.91 ± 3.05 | 15.00 ± 2.96 | 0.160 |
| Total cholesterol (mg/dl) | 206.92 ± 21.64 | 128.08 ± 18.46 | < 0.001 |
| HDL-cholesterol (mg/dl) | 56.00 ± 11.99 | 28.12 ± 7.26 | < 0.001 |
| Triglycerides (mg/dl) | 107.67 ± 60.04 | 132.12 ± 66.11 | 0.205 |
| AST | 12.20 ± 3.76 | 29.16 ± 17.83 | < 0.001 |
| ALT | 14.91 ± 4.44 | 38.04 ± 36.88 | 0.007 |
| Uric acid | 3.28 ± 0.41 | 5.07 ± 1.51 | < 0.001 |
| Creatinine | 0.63 ± 0.22 | 0.55 ± 0.096 | 0.096 |
| Lactate dehydrogenase | 133.25 ± 7.88 | 244.58 ± 108.37 | < 0.001 |
| Ferritin | 433.82 ± 228.61 | 617.92 ± 238.63 | 0.016 |
| C-reactive protein | 0.325 ± 0.398 | 1.350 ± 1.142 | < 0.001 |
| MDA | 5.638 ± 1.219 | 7.734 ± 1.557 | < 0.001 |

Data are expressed as means ± SD and were compared by t-test.

Blood count results of patients and control subjects are peresent in Table 4.2. No significant differences in MCV, MCH, PDW and MCHC levels were detected betweenβ-thalassemia and control groups (p>0.05).WBC (p<0.001), RBC (p<0.001), LYM (p<0.001), HGB (p<0.001), HCT(p<0.001),RDW(p<0.001), PCT (p=0.012) and PLT(p<0.001) levels were significantly higher in patients with β-thalassemic as compared to control group.

Table 3.2: Blood count results of patients and the healthy subjects.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Controls** | **Thalassemia patients** | **p-value** |
| WBC | 6.40 ± 1.25 | 13.98 ± 5.12 | < 0.001 |
| RBC | 5.37 ± 0.499 | 3.66 ± 0.531 | < 0.001 |
| LYM | 1.99 ± 0.88 | 6.81 ± 3.61 | < 0.001 |
| HGB | 13.46 ± 1.232 | 10.42 ± 1.422 | < 0.001 |
| HCT | 40.99 ± 3.421 | 30.65 ± 4.588 | < 0.001 |
| MCV | 82.50 ± 4.32 | 83.70± 3.64 | 0.286 |
| MCH | 28.58 ± 1.526 | 27.39 ± 5.402 | 0.314 |
| MCHC | 33.67 ± 1.260 | 34.07 ± 0.829 | 0.245 |
| RDW | 14.037 ± 1.402 | 18.654 ± 4.059 | < 0.001 |
| PLT | 303.62 ± 33.538 | 494.62 ± 244.43 | < 0.001 |
| PCT | 0.354 ± 0.106 | 0.511 ± 0.230 | 0.012 |
| PDW | 18.654 ± 4.919 | 16.987 ± 0.953 | 0.121 |

MDA and c-reactive protein levels were significantly higher in β-thalassemia patients than control subjects. MDA and c-reactive protein levels of patients and control subjects are peresent in figure 4.1.

**Figure 3.1:** Showing differences in serum level of MDA and CRP between control and β-thalassemic patients.

# Dıscussıon

In beta-thalassemia major, impaired biosynthesis of beta globin leads to accumulation of unpaired alpha globin chain. An iron overload, usually observed, generates oxygen-free radicals and peroxidative tissue injury. This study takes us through the mechanics and tests that need to be done for better managing β-thalassemic patients and yet for preventing further complications that may also occur in those patients. For this purpose in the study malondialdehyde (MDA), a marker of lipid per oxidation was measured as a biomarker of oxidative stress, CRP as a biomarker of inflammation and several hematological parameters are taken and compared between β-thalassemic patients and control groups.

MDA level was higher in β-thalassemic patients (5.638 ± 1.219) than in control groups (7.734 ± 1.557) in our study. Rukhsana Jokhio et al. found that MDA can serve as a marker of oxidation to circulating proteins have been found increased in patients with β-thalassaemia with iron overload. Mona Ramadan Nasr et al. showed that the oxidative capacity of blood and along with excess serum iron and ferritin may underly the highly significant increase of MDA in patients with β-thalassemia. Associated study have been done by Patrick B. Walter, et al. who showed that mean plasma malondialdehyde concentration levels of the two treatment groups (56 nmol/L) was significantly higher than that of the control group and showed that MDA levels can be controlled by both deferoxamine and deferasirox and MDA level can be reduced by chelation. Also similar results were obtained by Simsek et al. who found significantly increased levels of serum ferritin and MDA in β-thalassemia.

The value in the control group was in the range found for healthy controls in other studies. There is extensive of in vivo oxidative damage as well as enhanced sensitivity to exogenous oxidant stress in red cells of β-thalassemia (Kattamis C.and KattamisA.C., 2001). Clemens has been postulated that the biochemical and metabolic changes of β-thalassemia red blood cells are associated with a constant oxidative stress within the cells caused by the precipitation of excess alpha-globin chains, iron decompartimentalization, and release of free iron. Several studies reported that plasma MDA is increased in β-thalassemia ( Meral A and Surmen-Gur E.2000; Tesoriero L.D. et al 2001; Livrea M.A. et al. 1998 )

Samir M. Awadallah et al. concluded that MDA, a product of lipid peroxidation, significantly is elevated in thalassemic patients through different mechanisms including excess amount of iron binding to erythrocytes and free α-globin chain precipitation and the consequent generation of intracellular ROS. It has also been suggested that increased liver lipid peroxidation as a result of ferritin accumulation could raise the rate of leakageof MDA into the circulation .The levels of IMA (Ischemia modified albumin) are significantly higher in thalassemic patients as compared with healthy controls. High levels of IMA in thalassemic patients significantly correlate with ferritin, MDA, and ferroxidase. While ferritin was found to be the only predictor of MDA status in thalassemic patients, both ferritin and ferroxidase were found to be the predictors of the IMA status in such patients.

MDA is a measure oxidative damage, and it has been found regularly transfused thalassemia major patients (Cighetti G et al. 2002). Multifactorial theories have postulated to explain the high levels of serum MDA in β-thalassemic patients.

We used CRP as a biomarker of various inflammatory conditions. Our study showed increased CRP level in β-thalassemic patients (1.350 ± 1.142) than in control groups (0.325 ± 0.398) which are in accordance with the results of similar studies.

In 2009 a research done in Pakistan by Rukhsana Jokhio et.al. concluded that CRP can serve as a biomarker of inflammatory conditions, progression of cardiovascular diseases and as indicator of morbidity and mortality. High C-reactive proteins in these patients indicate on going iron overload toxicity related damage in these patients.

Archararit N et al. has found that interestingly there was a trend towards increasing C-reactive protein levels in beta thal/HbE postsplenec patients with higher platelet count, although no correlation was observed. Besides the inflammatory process, platelet and/or factor(s) that control(s) thrombopoiesis seem(s) to play a role in the high serum C-reactive protein levels in the studied population.

C-reactive proteins and cytokines have been used by a number of workers as biomarker of inflammation in thalassaemia patients as well for other disease as pyogenic infection including pneumonia, infective pulmonary exacerbation in cystic fibrosis, diabetes, hepatitis and as marker for the development of cardiovascular diseases which is a major morbid complication of thalassaemia.

Ino Kanavak, et al. in 2009 they found that all endothelial adhesion molecules and CRP were significantly increased in β-thalassemia intermedia patients (p<0.001) and not influenced by treatment. These results agree with the study published by Archararit et al., where CRP levels were found elevated in β-thalassemia patients compared to healthy individuals, especially in splenectomized patients.

Additionally they observed no significant correlation between CRP and endothelial adhesion molecules. Probably, despite the fact that elevated levels of CRP reflect the overall alteration of the biochemical profile of these patients, there is no direct interaction between inflammation markers and endothelial adhesion molecules.

Through out the world, several regions have initiated universal prenatal screening programs to address homozygous α-thalassemia. Although, the prognosis for thalassemia disorders is improving, but still prenatal diagnosis and neonatal screenings are needed. Comprehensive services that address language and social barriers as well as access to Hb F-enhancing agents and transfusions are needed.

Further studies need to be done for more provable evidence showing that hydroxyurea therapy may be helpful for some patients with transfusion dependent β-thalassemia and may reduce the need for blood transfusions. Because until now the potential usefulness of hydroxyurea in the thalassemia syndromesis considerably less clear. The standard therapeutic approach to β-thalassemia major still relies on regular blood transfusions and the use of iron chelators. In patients with β-thalassemia intermedia, the hematologic response to hydroxyurea alone or in combination with recombinant human erythropoietin is controversial, and little is known about the effectiveness of long-term therapy.

# REFFERENCES

[Alain J. Marengo-Row](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Marengo-Rowe%20AJ%5Bauth%5D) (2007). The thalassemias and related disorders. Proc (Bayl Univ Med Cent),1, 27–31.

Andrews NC (1999). Disorders of iron metabolism N Engl J Med., 341, 498-511.

Archararit N, Chuncharunee S, Pornvoranunt A, Atamasirikul K, Rachakom B and Atichartakarn V. (2000). Serum C-reactive protein level in postsplenectomized thalassemic patients. J Med Assoc Thai., 1, 63-9.

Babior, B.M., R.S. Kipnes, and J.T. Curnutte (1973). The Productions by leukocytes of superoxide; a Potential Bactericidal Agent. J. Clin. Invest., 52, 741.

Bachir D and Galacteros F. (2004). Hemoglobin E disease. J Pediatr Hematol Oncol , 24, 421.

Bar-Or D, Curtis G, Rao N, Bampos N, Lau E. (2001). Characterization of the Co (2+) and Ni (2+). Binding amino-acid residues of the N-terminus of human albumin. [Eur J Biochem.](http://www.ncbi.nlm.nih.gov/pubmed/11121100), 268, 42-7.

Bar-Or D, Lau E, Winkler JV. (2000). Novel assay for cobalt–albumin binding and its potential as a marker for myocardial ischemia a preliminary report. J Emerg Med.. 19, 311–315.

[Bernard Chan](http://academic.research.microsoft.com/Author/56147352/bernard-p-chan), [Neil Dodsworth](http://academic.research.microsoft.com/Author/53040206/neil-dodsworth), John Woodrow, [Alan Tucker](http://academic.research.microsoft.com/Author/10738740/alan-tucker) and  [Roy Harris](http://academic.research.microsoft.com/Author/28641967/r-v-harris) (1995). auto-degradation of human serum albumin. [European Journal Of Biochemistry](http://academic.research.microsoft.com/Journal/1585/eur-j-biochem-european-journal-of-biochemistry), 227, 524-528.

Bokoch, G.m. and B.D. Diebold (2002). Current Molecular Models for NADPH Oxidase Regulation by Rac GTPase. Blood. 100, 2692-2696.

Bonassi,S., Hagmar,L., Stromberg,U., Montagud,A.H., Tinnerberg,H., Forni, et.al (2000). Chromosomal aberration sinlymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res, 60,1619–1625.

Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. (2004). Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica, 89, 1187-93.

[Boros LG](http://www.ncbi.nlm.nih.gov/pubmed?term=Boros%20LG%5BAuthor%5D&cauthor=true&cauthor_uid=16126993), [Nichelatti M](http://www.ncbi.nlm.nih.gov/pubmed?term=Nichelatti%20M%5BAuthor%5D&cauthor=true&cauthor_uid=16126993) and [Shoenfeld Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Shoenfeld%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=16126993). [(2005). Fermented wheat germ extract (Avemar) in the treatment of cancer and autoimmune diseases.](http://www.ncbi.nlm.nih.gov/pubmed/16339663" \o "Annals of the New York Academy of Sciences.) [Ann N Y Acad Sci.](http://www.ncbi.nlm.nih.gov/pubmed/10911951), 1051, 529-42.

Bouva MJ, Harteveld CL, van Delft P, Giordano PC (January 2006). ["Known and new delta globin gene mutations and their diagnostic significance"](http://www.haematologica.org/cgi/pmidlookup?view=long&pmid=16434382).  Haematologica, 91, 129–32.

Breiterman-White R..(2006). C-reactive protein and anemia: implications for patients on dialysis. Nephrol Nurs J., 33, 555-8.

Brittenham Dr. Gary M, Alan R. Cohen, Christine E. McLaren,  Marie B. Martin, Patricia M. Griffith,  Arthur W. Nienhuis,(1993). Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. [Am J Hematol.](http://www.ncbi.nlm.nih.gov/pubmed/8416302)  , 42, 81-5.

Burch, P.M. and H.H. Heintz (2005). Redox Regulation of Cell-cycle Re-entry: Cyclin D1 as a Primary Target for the Mitogenic Effects of Reactive Oxygen and Nitrogen Species. Antioxidants & redox Signaling, 7, 741-751.

Burhans, W. and N. Heintz (2009).  The Cell Cycle is a Redox Cycle: Linking phase-specific targets to cell fate. Free Radical Biology and Medicine., 47, 1282-1294.

Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, Aydinok Y, et.al (2006). A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. [Blood](http://www.ncbi.nlm.nih.gov/pubmed/16352812), 107, 3455-62.

Celedon G, Rodriguez I, Espana J, et al. (2001). Contribution of hemoglobin and membrane constituents modification to human erythrocyte damage promoted by peroxyl radicals of different charge and hydrophobicity. Free Radic Res, 17, 31-34.

Chan B, Dodsworth N, Woodrow J, Tucker A, Harris R. Site-specific N-termin Chin, et al. (2008). [Correction of a splice-site mutation in the beta-globin gene stimulated by triplex-forming peptide nucleic acids](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2533221/). Proceedings of the National Academy of Sciences,105,13514–9.

Choi, M.H., I. K. Lee, G.W. Kim, B.U. Kim, Y.H. Han, D.Y. Yu, et. al (2005). Regulation of PDGF Signalling and Vascular Remodelling by Peroxiredoxin II. Nature, 19435, 347-53.

Ci Yun-Xiang and F. Wang (1991). Catalytic effects of peroxidase-like metaloporphoryns on the fluorescence reaction of homovanillic acid with hydrogen peroxide. Fresenius’ Journal of Analytical Chemistry, 3390, 46-49.

[Cighetti G](http://www.ncbi.nlm.nih.gov/pubmed?term=Cighetti%20G%5BAuthor%5D&cauthor=true&cauthor_uid=11886433), [Duca L](http://www.ncbi.nlm.nih.gov/pubmed?term=Duca%20L%5BAuthor%5D&cauthor=true&cauthor_uid=11886433), [Bortone L](http://www.ncbi.nlm.nih.gov/pubmed?term=Bortone%20L%5BAuthor%5D&cauthor=true&cauthor_uid=11886433), [Sala S](http://www.ncbi.nlm.nih.gov/pubmed?term=Sala%20S%5BAuthor%5D&cauthor=true&cauthor_uid=11886433), [Nava I](http://www.ncbi.nlm.nih.gov/pubmed?term=Nava%20I%5BAuthor%5D&cauthor=true&cauthor_uid=11886433), [Fiorelli G](http://www.ncbi.nlm.nih.gov/pubmed?term=Fiorelli%20G%5BAuthor%5D&cauthor=true&cauthor_uid=11886433),and [Cappellini MD](http://www.ncbi.nlm.nih.gov/pubmed?term=Cappellini%20MD%5BAuthor%5D&cauthor=true&cauthor_uid=11886433) (2002). Oxidative status and malondialdehyde in beta-thalassaemia patients. [Eur J Clin Invest.](http://www.ncbi.nlm.nih.gov/pubmed/11886433), 1, 55-60.

David Weatherall, Olu Akinyanju, Suthat Fucharoen, Nancy Olivieri, David Weatherall, Olu Akinyanju, et.al (2001). Inherited Disorders of Hemoglobin. Disease Control Priorities in Developing Countries. 2nd edition. Chapter 34, 663-666.

Donovan JM, Plone M, Dagher R, Bree M, Marquis J (2005). Preclinical and clinical development of deferitrin, a novel, orally available iron chelator. Ann N Y Acad Sci., 1054, 492-4.

Deron Scott J. (2003). (C-Reactive Protein)C-Reactive Protein: text book of C-Reactive Protein. 1st edition, 1-19.

[E. D. Wills](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Wills%20ED%5Bauth%5D) (1971). Effects of lipid peroxidation on membrane-bound enzymes of the endoplasmic reticulum, Biochem J., 123, 983–991.

Elisa Ferro a, GiuseppaVisalli, RosaCiva, MariaAngelaLaRosa, GaetanoRandazzoPapa c, Barbara Baluce, and Angela DiPietro, (2012). Oxidative damage and genotoxicity biomarkers in transfused and untransfused thalassemic subjects. [Free Radic Biol Med.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Elisa+Ferro+a%2C+GiuseppaVisalli%2C+RosaCiva%2C+MariaAngelaLaRosa%2C+GaetanoRandazzoPapa+c%2C+Barbara+Baluce%2C+and+Angela+DiPietro.(2012)+Oxidative+damage+and+genotoxicity+biomarkers+in+transfused+and+untransfused+thalassemic+subjects.), 53, 1829-37.

Etsuo Niki, Yasukazu Yoshida, Yoshiro Saito, Noriko Noguchi, (2005). Lipid peroxidation: Mechanisms, inhibition, and biological eﬀects. Biochemical and Biophysical Research Communications, 338, 668–676.

Fiers, W., Fiers, W., Beyaert, R., Declercq, W., and Vandenabeele (1999). More than one way to die: apoptosis, necrosis and reactive oxygen damage. Oncogene, 18, 7719-7730.

Fleming, Richard M., and Tom Monte. (2006). Stop Inflammation Now!: A Step-by-Step Plan to Prevent, Treat, and Reverse Inflammation-The Leading Cause of Heart Disease and Related Conditions. text book of Stop Inflammation Now!:, 3rd ed., 36-43, ISBN: 9780399151118.

Forman, H.J. and M. Torres (2002). Reactive Oxygen Species and Cell Signaling, Respiratory Burst in Macrophage Signaling, Am. J. Respir. Crit. Care Med., 166, 4-8.

[Fucharoen S](http://www.ncbi.nlm.nih.gov/pubmed?term=Fucharoen%20S%5BAuthor%5D&cauthor=true&cauthor_uid=20008179), [Viprakasit V](http://www.ncbi.nlm.nih.gov/pubmed?term=Viprakasit%20V%5BAuthor%5D&cauthor=true&cauthor_uid=20008179)., (2009). Hb H disease: clinical course and disease modifiers. [Hematology Am Soc Hematol Educ Program](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fucharoen+S%2C+Viprakasit+V.%2CHb+H+disease%3A+clinical+course+and+disease+modifiers.2009%2C), 2009, 26-34.

Gabutti V, Piga A., (1996). Results of long-term iron-chelating therapy. [Acta Haematol.](http://www.ncbi.nlm.nih.gov/pubmed/8604584), 95, 26-36.

Galanello R, Piga A, Forni GL, Bertrand Y, Foschini ML, Bordone E, Leoni G and Lavagetto A, et.al (2010). [Beta-thalassemia](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2893117/). Orphanet Journal of Rare Diseases, 2010 5, 11.

[Galanello R](http://www.ncbi.nlm.nih.gov/pubmed?term=Galanello%20R%5BAuthor%5D&cauthor=true&cauthor_uid=17018383), [Piga A](http://www.ncbi.nlm.nih.gov/pubmed?term=Piga%20A%5BAuthor%5D&cauthor=true&cauthor_uid=17018383), [Forni GL](http://www.ncbi.nlm.nih.gov/pubmed?term=Forni%20GL%5BAuthor%5D&cauthor=true&cauthor_uid=17018383), [Bertrand Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Bertrand%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=17018383), [Foschini ML](http://www.ncbi.nlm.nih.gov/pubmed?term=Foschini%20ML%5BAuthor%5D&cauthor=true&cauthor_uid=17018383), and [Bordone E](http://www.ncbi.nlm.nih.gov/pubmed?term=Bordone%20E%5BAuthor%5D&cauthor=true&cauthor_uid=17018383) et. al (2006). Phase II clinical evaluation of deferasirox, a once-daily oral chelating agent, in pediatric patients with beta-thalassemia major. Haematologica,. 91, 1343-51.

Giardina PJ, and Forget BG. Thalassemia Syndromes. In: Hoffman R, Benz EJ, Shattil SJ, et al, (2009). Hematology Basic Principles and Practice. 5th ed. Philadelphia, PA: Churchill Livingstone, 535-563.

Greece C. Kattamis, A. Metaxotou-Mavromati, V. Ladis, H. Tsiarta, S. Laskari, and E. Kanavakis, (1982). The Clinical Phenotype of fl and Thalassemias in Thalassemia Unit, [European Journal of Pediatrics](http://link.springer.com/journal/431)., 139, 135-138.

Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok, and S.R. Tannenbaum (1982). Analysis of Nitrate, Nitrite, and [15N] Nitrate in Biological Fluids, Analytical Biochemistry, 126, 131-138.

[Haferlach T](http://www.ncbi.nlm.nih.gov/pubmed?term=Haferlach%20T%5BAuthor%5D&cauthor=true&cauthor_uid=17938925), [Bacher U](http://www.ncbi.nlm.nih.gov/pubmed?term=Bacher%20U%5BAuthor%5D&cauthor=true&cauthor_uid=17938925), [Kern W](http://www.ncbi.nlm.nih.gov/pubmed?term=Kern%20W%5BAuthor%5D&cauthor=true&cauthor_uid=17938925), [Schnittger S](http://www.ncbi.nlm.nih.gov/pubmed?term=Schnittger%20S%5BAuthor%5D&cauthor=true&cauthor_uid=17938925) and  [Haferlach C](http://www.ncbi.nlm.nih.gov/pubmed?term=Haferlach%20C%5BAuthor%5D&cauthor=true&cauthor_uid=17938925). [(2008). The diagnosis of BCR/ABL-negative chronic myeloproliferative diseases (CMPD). Ann Hematol., 87, 1-10.](http://www.ncbi.nlm.nih.gov/pubmed/18351337)

Hancock, J.T., R. Desikan, S.J. Neill, (2001). Role of Reactive Oxygen Species in Cell Signaling Pathways. [Biochem Soc Trans.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hancock%2C+J.T.%2C+R.+Desikan%2C+S.J.+Neill%2C+(2001)+Role+of+Reactive+Oxygen+Species+in+Cell+Signaling+Pathways.+Biochemical+and+Biomedical+Aspects+of+Oxidative+Modification%2C+29(2)%3A345-350.), 29, 345-50.

Hancock, J.T., R. Desikan, S.J. Neill, (2001). Role of Reactive Oxygen Species in Cell Signaling Pathways. Biochemical and Biomedical Aspects of Oxidative Modification, 29, 345-350.

Havens, C.G., A. Ho, N. Yoshioka, and S.F. Dowdy (2006). Regulation of Late G1/S Phase Transition and APCCdh1 by Reactive Oxygen Species. Molecular and Cellular Biology; 26, 4701-11.

Hayes, J.D., et al., (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defense against oxidative stress. Free Radic. Res., [Free Radic Res.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hayes%2C+J.D.%2C+et+al.%2C+Glutathione+and+glutathione-dependent+enzymes+represent+a+co-ordinately+regulated+defense+against+oxidative+stress.+Free+Radic.+Res.%2C+31%2C+273-300+(1999).),  31, 273-300.

Hinkle, p.c., Butow r.a., Racker e., and Chance b. (1967). partial resolution of the enzymes catalyzing oxidative phosphorylation.  Xv reverse electron transfer in the flavin-cytochrome beta region of the respiratory chain of beef heart. [J Biol Chem.](http://www.ncbi.nlm.nih.gov/pubmed/4294331),   242, :5169-73.

Hoffman, A, L.M. Spetner, and M. Burke (2008). Ramifications of a Redox Switch within a Normal Cell; its Absence in a Cancer cell. Free Radical Biology and Medicine, 45, 265-8.

Hou Y.C., Janczuk A. and Wang P.G. (1999). Current trends in the development of nitric oxide donors. Curr. Pharm. Des., 5, 417-41.

Huang,X., (2003). Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. Mutat. Res. 533, 153–71.

Iarmarcovai,G. Ceppi M., Botta, A., Orsi\_ere T., Bonassi S, Micronuclei (2008). Frequency in peripheral blood lymphocytesof cancer patients: ameta- analysis. Mutat. Res., 659, 274–283.

Ino Kanavaki, Periklis Makrythanasis, Christina Lazaropoulou, Maria Tsironi, and Antonis Kattamis, et. al (2009). Soluble endothelial adhesion molecules and inflammation markers in patients with β-thalassemia intermedia. [Blood Cells Mol Dis.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ino+Kanavaki%2C+Periklis+Makrythanasis%2C+Christina+Lazaropoulou%2C+Maria+Tsironi%2C+Antonis+Kattamis%2C+Ioannis+Rombos%2C+Ioannis+Papassotiriou%2C+Soluble+endothelial+adhesion+molecules+and+inflammation+markers+in+patients+with+%CE%B2-thalassemia+intermedia%2C+2009.), 43, 230-4.

J. Vidyasagar, N. Karunakar M. S., Reddy, K. Rajnarayana, T. Surender, and D. R. Krishna, (2004). oxidative stress and antioxidant status in acute organophosphorus insictiside poisoning, Indian J Pharmacol, 36, 76-79.

K. M. Schaich, (2005). Lipid Oxidation Theoretical Aspects,  In book: Bailey's Industrial Oil and Fat Products, ISBN: 9780471678496 DOI:10.1002/047167849X.bio067.

Kanitta Srinoun, Saovaros Svasti and Pranee Winichagoon (2009). Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice. Haematologica, 94, 1211-1219.

[Kanner J](http://www.ncbi.nlm.nih.gov/pubmed?term=Kanner%20J%5BAuthor%5D&cauthor=true&cauthor_uid=3304843), [German JB](http://www.ncbi.nlm.nih.gov/pubmed?term=German%20JB%5BAuthor%5D&cauthor=true&cauthor_uid=3304843), and [Kinsella JE](http://www.ncbi.nlm.nih.gov/pubmed?term=Kinsella%20JE%5BAuthor%5D&cauthor=true&cauthor_uid=3304843)., (1987). Initiation of lipid peroxidation in biological systems. [Crit Rev Food Sci Nutr.](http://www.ncbi.nlm.nih.gov/pubmed/3304843), 25, 317-64.

[Kassab-Chekir A](http://www.ncbi.nlm.nih.gov/pubmed?term=Kassab-Chekir%20A%5BAuthor%5D&cauthor=true&cauthor_uid=14637270), [Laradi S](http://www.ncbi.nlm.nih.gov/pubmed?term=Laradi%20S%5BAuthor%5D&cauthor=true&cauthor_uid=14637270), [Ferchichi S](http://www.ncbi.nlm.nih.gov/pubmed?term=Ferchichi%20S%5BAuthor%5D&cauthor=true&cauthor_uid=14637270), [Haj Khelil A](http://www.ncbi.nlm.nih.gov/pubmed?term=Haj%20Khelil%20A%5BAuthor%5D&cauthor=true&cauthor_uid=14637270), [Feki M](http://www.ncbi.nlm.nih.gov/pubmed?term=Feki%20M%5BAuthor%5D&cauthor=true&cauthor_uid=14637270), and [Amri F](http://www.ncbi.nlm.nih.gov/pubmed?term=Amri%20F%5BAuthor%5D&cauthor=true&cauthor_uid=14637270), (2003).  Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. [Clin Chim Acta.](http://www.ncbi.nlm.nih.gov/pubmed/14637270), 38, 79-86.

Khaled M. Musallam, Ali T. Taher, and Eliezer A. Rachmilewitz,(2012). β-Thalassemia Intermedia. A Clinical Perspective.Cold Spring Harb Prespective, 2, 13482.

[Kikugawa K](http://www.ncbi.nlm.nih.gov/pubmed?term=Kikugawa%20K%5BAuthor%5D&cauthor=true&cauthor_uid=6703702), [Kosugi H](http://www.ncbi.nlm.nih.gov/pubmed?term=Kosugi%20H%5BAuthor%5D&cauthor=true&cauthor_uid=6703702), and [Asakura T](http://www.ncbi.nlm.nih.gov/pubmed?term=Asakura%20T%5BAuthor%5D&cauthor=true&cauthor_uid=6703702)., (1984). Effect of malondialdehyde, a product of lipid peroxidation, on the function and stability of hemoglobin. [Arch Biochem Biophys](http://www.ncbi.nlm.nih.gov/pubmed/6703702), 229, 7-14.

Kontoghiorghes GJ (1985). New orally active iron chelators. Lancet. 1, 817.

Kontoghiorghes GJ, Aldouri MA, Sheppard L, and Hoffbrand AV (1987). 1,2-Dimethyl-3-hydroxypyrid-4-one, an orally active chelator for treatment of iron overload. [Lancet.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kontoghiorghes+GJ%2C+Aldouri+MA%2C+Sheppard+L%2C+Hoffbrand+AV+Lancet.++1%2C2-Dimethyl-3-hydroxypyrid-4-one%2C+an+orally+active+chelator+for+treatment+of+iron+overload.%3B+1(8545)%3A1294-5.), 1, 1294-5.

Kundu, K. S.F. Knight, N. Willet, S. Lee, R. Taylor, and N. Murthy (2009). Hydrocyanines: A class of fluorescent sensors that can image reactive oxygen species in cell culture, tissue, and in vivo. Angew. Chem. Int. Ed., 48, 299-303.

 Ladis V, et al. Ann N Y Acad Sci. (2005). Effects of Iron Overload inTransfusion-Dependent Thalassemia. New York Academy of Sciences., 1054, 445-50

[Ladis V](http://www.ncbi.nlm.nih.gov/pubmed?term=Ladis%20V%5BAuthor%5D&cauthor=true&cauthor_uid=16339695), [Chouliaras G](http://www.ncbi.nlm.nih.gov/pubmed?term=Chouliaras%20G%5BAuthor%5D&cauthor=true&cauthor_uid=16339695), [Berdousi H](http://www.ncbi.nlm.nih.gov/pubmed?term=Berdousi%20H%5BAuthor%5D&cauthor=true&cauthor_uid=16339695), [Kanavakis E](http://www.ncbi.nlm.nih.gov/pubmed?term=Kanavakis%20E%5BAuthor%5D&cauthor=true&cauthor_uid=16339695), and [Kattamis C](http://www.ncbi.nlm.nih.gov/pubmed?term=Kattamis%20C%5BAuthor%5D&cauthor=true&cauthor_uid=16339695).(2005). Longitudinal study of survival and causes of death in patients with thalassemia major in Greece. [Ann N Y Acad Sci](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kuypers%2C+F.A.%3BAmes%2CB.N.+Measuring+chromosome+breaks+in+patients+with+Ladis+V%2C+et+al.+Ann+N+Y+Acad+Sci.+2005%3B1054%3A445-50.), 1054, 445-50.

Lambeth, J, G. Cheng, R. Arnold, and W. Edens (2000). Novel Homologs of gp91phox. TIBS, 25, 459-461.

Latella, L.; A. Sacco., D. Pajalunga, M. Tianen, D. Macera, M. D’Angelo, A. Felici, A. Sacchi, and M. Crescenzi. (2001). Reconstitution of Cyclin D1-associated Kinase Activity Drives Terminally Differentiated Cells into the Cell Cycle. Molecular and Cellular Biology, 21, 5631-5643.

Laura J. Niedernhofer, J. Scott Daniels, Carol A. Rouzer, Rachel E. Greene, and Lawrence J. Marnett (2003). Malondialdehyde, a Product of Lipid Peroxidation, Is Mutagenic in Human Cells. [J Biol Chem](http://www.ncbi.nlm.nih.gov/pubmed/?term=Laura+J.+Niedernhofer%2C+J.+Scott+Daniels%2C+Carol+A.+Rouzer%2C+Rachel+E.+Greene%2C+++++++++++++++++Lawrence+J.+Marnett%2C+Malondialdehyde%2C+a+Product+of+Lipid+Peroxidation%2C+Is+Mutagenic+in+Human+Cells%2C+December+9%2C+2002.), 278, 1426-33.

Lee HJ, Lee J, Lee SK, Lee SK, and Kim EC. (2007). Differential regulation of iron chelator-induced IL-8 synthesis via MAP kinase and NF-kappaB in immortalized and malignant oral keratinocytes. BMC Cancer, 13, 7, 176.

Ley TJ, Griffith P, Nienhuis AW. (1982). Transfusion haemosiderosis and chelation therapy. Clin Haematol, 11, 437-445.

[Livrea MA](http://www.ncbi.nlm.nih.gov/pubmed?term=Livrea%20MA%5BAuthor%5D&cauthor=true&cauthor_uid=8896430), [Tesoriere L](http://www.ncbi.nlm.nih.gov/pubmed?term=Tesoriere%20L%5BAuthor%5D&cauthor=true&cauthor_uid=8896430), [Pintaudi AM](http://www.ncbi.nlm.nih.gov/pubmed?term=Pintaudi%20AM%5BAuthor%5D&cauthor=true&cauthor_uid=8896430), [Calabrese A](http://www.ncbi.nlm.nih.gov/pubmed?term=Calabrese%20A%5BAuthor%5D&cauthor=true&cauthor_uid=8896430), [Maggio A](http://www.ncbi.nlm.nih.gov/pubmed?term=Maggio%20A%5BAuthor%5D&cauthor=true&cauthor_uid=8896430), [Freisleben HJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Freisleben%20HJ%5BAuthor%5D&cauthor=true&cauthor_uid=8896430), et al. (1996). Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. [Blood](http://www.ncbi.nlm.nih.gov/pubmed?term=Oxidative%20stress%20and%20antioxidant%20status%20in%20beta-thalassemia%20major%3A%20iron%20overload%20and%20depletion%20of%20lipid-soluble%20antioxidants.), 88. 3608-14.

M Uguccioni, R Meliconi, E Lalli, S Nesci, C Delfini, G Lucarelli, et al. (1992). Serum amyloid A protein concentration in bone marrow transplantation for β- thalassaemia. [J Clin Pathol](http://www.ncbi.nlm.nih.gov/pubmed/?term=M+Uguccioni%2C+R+Meliconi%2C+E+Lalli%2C+S+Nesci%2C+C+Delfini%2C+G+Lucarelli%2C+G+Gasbarrini%2CA+Facchini%2C+Serum+amyloid+A+protein+concentration+in+bone+marrow+transplantation+for+B+thalassaemia%2C+2009.), 45, 348-51.

Maggio A, D'Amico G, Morabito A, et al. (2002). Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. Blood Cells Mol Dis., 28, 196-208.

Maggio, Aurelio; d'Amico, Gennaro; Morabito, Alberto; Capra, et al. (2002). "Deferiprone versus Deferoxamine in Patients with Thalassemia Major: A Randomized Clinical Trial". Blood Cells, Molecules, and Diseases, 28, 196.

[Mark B. Pepys](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Pepys%20MB%5Bauth%5D) and [Gideon M. Hirschfield](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Hirschfield%20GM%5Bauth%5D), (2003). C-reactive protein: a critical update. ,J Clin Invest, 111, 1805-12.

McCord, J.M. and I. Fidovich (1968). The Reduction of Cytochrome C  by Milk Xanthine Oxidase. J. Biol. Chem., 243, 5733-5760.

[Meral A](http://www.ncbi.nlm.nih.gov/pubmed?term=Meral%20A%5BAuthor%5D&cauthor=true&cauthor_uid=11127401), [Tuncel P](http://www.ncbi.nlm.nih.gov/pubmed?term=Tuncel%20P%5BAuthor%5D&cauthor=true&cauthor_uid=11127401), [Sürmen-Gür E](http://www.ncbi.nlm.nih.gov/pubmed?term=S%C3%BCrmen-G%C3%BCr%20E%5BAuthor%5D&cauthor=true&cauthor_uid=11127401), [Ozbek R](http://www.ncbi.nlm.nih.gov/pubmed?term=Ozbek%20R%5BAuthor%5D&cauthor=true&cauthor_uid=11127401), [Oztürk E](http://www.ncbi.nlm.nih.gov/pubmed?term=Ozt%C3%BCrk%20E%5BAuthor%5D&cauthor=true&cauthor_uid=11127401), and  [Günay U](http://www.ncbi.nlm.nih.gov/pubmed?term=G%C3%BCnay%20U%5BAuthor%5D&cauthor=true&cauthor_uid=11127401). (2000). Lipid peroxidation and antioxidant status in beta-thalassemia. [Pediatr Hematol Oncol.](http://www.ncbi.nlm.nih.gov/pubmed/11127401), 17, 687-93.

Miller, E.V., O. Tulyathan, E.Y. Isacoff, and C.J. Chang (2007).  Molecular imaging of hydrogen peroxide produced for cell signaling. Nature Chemical Biology, 3, 263-267.

Miller, Marvin J. (1989). "Syntheses and therapeutic potential of hydroxamic acid based siderophores and analogs". Chemical Review, 89, 1563–1579.

Misko, T.P., R.J. Schilling, D. Salvemini, W.M. Moore, and M.G. Currie (1993). A Fluorometric Assay for the Measurement of Nitrite in Biological Samples. Analytical Biochemistry, 214, 11-16.

Nagel RL, Fabry ME, Steinberg MH (2003). [The paradox of hemoglobin SC disease](http://linkinghub.elsevier.com/retrieve/pii/S0268960X03000031) . Blood Rev., 17, 167–78.

[Naithani R](http://www.ncbi.nlm.nih.gov/pubmed?term=Naithani%20R%5BAuthor%5D&cauthor=true&cauthor_uid=16317757), [Chandra J](http://www.ncbi.nlm.nih.gov/pubmed?term=Chandra%20J%5BAuthor%5D&cauthor=true&cauthor_uid=16317757), [Bhattacharjee J](http://www.ncbi.nlm.nih.gov/pubmed?term=Bhattacharjee%20J%5BAuthor%5D&cauthor=true&cauthor_uid=16317757), [Verma P](http://www.ncbi.nlm.nih.gov/pubmed?term=Verma%20P%5BAuthor%5D&cauthor=true&cauthor_uid=16317757), [Narayan S](http://www.ncbi.nlm.nih.gov/pubmed?term=Narayan%20S%5BAuthor%5D&cauthor=true&cauthor_uid=16317757) (2006). Peroxidative stress and antioxidant enzymes in children with beta-thalassemiamajor. [Pediatr Blood Cancer](http://www.ncbi.nlm.nih.gov/pubmed/16317757), 46, 780-5.

Nakano, H. A. Nakajima, S. Sakon-Komazawa, J-H. Piao, X. Xue, and K. Okumura (2006). Reactive Oxygen Species Mediate Crosstalk between NF-kB and JNK. Cell Death and Diff, 13, 730-737.

Nathan, C. (1992). Nitric oxide as a secretory product of mammalian cells. FASEB Journal, 6, 3051-3064.

Nicholls, D.G., and Budd, S.L. (2000). Mitochondria and neuronal survival. Physiol. Rev., 80, 315-360.

OfferT., Bhagat A., Lal A ., Atamna W., Singer S.T., Vichinsky E.P., Olivieri NF, et.al (1997). beta-thalassemia. Human Mutation, [9,](http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1098-1004(1997)9:4%3C%3E1.0.CO;2-W/issuetoc)344–347.

Orapan Sripichai, Wattanan Makarasara, Thongperm Munkongdee, Chutima et.al (2008). A scoring system for the classification of b-thalassemia/HbE disease severity. Am.J. Hematol., 83, 482–484.

Otsuka S, Maruyama H, Listowsky I. (1981). Structure, assembly, conformation, and immunological properties of the two subunit classes of ferritin. Biochemistry, 20, 5226-5232.

P Moi, G Loudianos, J Lavinha, S Murru, P Cossu, et al. (1992). Delta-thalassemia due to a mutation in an erythroid-specific bindingprotein sequence 3' to the delta-globin gene. Blood, 79, 512-516.

[Patrick B Walter](http://walter.pb.lib.bioinfo.pl/auth:Walter,PB), [Eric A Macklin](http://macklin.ea.lib.bioinfo.pl/auth:Macklin,EA), [John Porter](http://porter.j.lib.bioinfo.pl/auth:Porter,J), [Patricia Evans](http://evans.p.lib.bioinfo.pl/auth:Evans,P), Ellis J. Neufeld,Thomas Nancy Olivieri, et. al (2008). Inflammation and oxidant-stress in β-thalassemia patientstreated with iron chelators deferasirox (ICL670) or deferoxamine , [Haematologica., 93, 817-25](http://lib.bioinfo.pl/pmid/journal/Haematologica).

Park, H.S., D. Park, and Y.S. Bae (2006) Molecular Interaction of NADPH Oxidase 1 with betaPix and Nox Organizer.  Biochemical and Biophysical Research Communications, 339, 985-990.

[Pérez Encinas MM](http://www.ncbi.nlm.nih.gov/pubmed?term=P%C3%A9rez%20Encinas%20MM%5BAuthor%5D&cauthor=true&cauthor_uid=7739278), [Bello López JL](http://www.ncbi.nlm.nih.gov/pubmed?term=Bello%20L%C3%B3pez%20JL%5BAuthor%5D&cauthor=true&cauthor_uid=7739278), [Pérez Crespo S](http://www.ncbi.nlm.nih.gov/pubmed?term=P%C3%A9rez%20Crespo%20S%5BAuthor%5D&cauthor=true&cauthor_uid=7739278) and [Lete Achirica I](http://www.ncbi.nlm.nih.gov/pubmed?term=Lete%20Achirica%20I%5BAuthor%5D&cauthor=true&cauthor_uid=7739278). (1995). C-reactive protein in differential diagnosis of primary thrombocytosis. [Med Clin (Barc)](http://www.ncbi.nlm.nih.gov/pubmed/7739278) , 104, 441-3.

[Peter C.Y. Tong](http://care.diabetesjournals.org/search?author1=Peter+C.Y.+Tong&sortspec=date&submit=Submit), PHD[1](http://care.diabetesjournals.org/content/25/8/1480.full#aff-1),  [Maggie C.Y. Ng](http://care.diabetesjournals.org/search?author1=Maggie+C.Y.+Ng&sortspec=date&submit=Submit), [Wing Y. So](http://care.diabetesjournals.org/search?author1=Wing+Y.+So&sortspec=date&submit=Submit), MRCP[1](http://care.diabetesjournals.org/content/25/8/1480.full#aff-1), et al. (2002). MD C-Reactive Protein and Insulin Resistance in Subjects With +Thalassemia Minor and a Family History of Diabetes, Diabetes care, 25, 1480-1.

Pippard MJ, et al. (1979). [Iron Physiology and Pathophysiology in Humans](http://books.google.com/books?id=wtmeZX2Ku6AC&pg=PA339&lpg=PA339&dq=Pippard+MJ,+et+al.+Lancet.+1979;2:819-21.&source=bl&ots=sIcjhXG_T1&sig=jGo9rCGktPkMyMTqhRl3Ilk3KTU&hl=en&sa=X&ei=KtOyUZOmMdG1hAf3ioDQAg&ved=0CC8Q6AEwAA). Lancet. 2, 819-21.

Porter JB. (1997). A risk-benefit assessment of iron-chelation therapy. Drug Saf., 17, 407-421.

Porter JB. (2001). Practical management of iron overload. Br J Haematol, 115, 239–252.

[Rachmilewitz EA](http://www.ncbi.nlm.nih.gov/pubmed?term=Rachmilewitz%20EA%5BAuthor%5D&cauthor=true&cauthor_uid=1252619), [Shohet SB](http://www.ncbi.nlm.nih.gov/pubmed?term=Shohet%20SB%5BAuthor%5D&cauthor=true&cauthor_uid=1252619) and [Lubin BH](http://www.ncbi.nlm.nih.gov/pubmed?term=Lubin%20BH%5BAuthor%5D&cauthor=true&cauthor_uid=1252619), (1976). Lipid membrane peroxidation in beta-thalassemia major. [Blood.](http://www.ncbi.nlm.nih.gov/pubmed/1252619), 47, 495-505.

Rahim F, Keikhaei B, Zandian K, Soltani A (2008). **Diagnosis And Treatment Of Cord Compression Secondary To Extramedullary Hematopoiesis In Patients With Beta-Thalassemia Intermedia. jornal of clinical and diagnostic center, 2, 643-747.**

[Raja JV](http://www.ncbi.nlm.nih.gov/pubmed?term=Raja%20JV%5BAuthor%5D&cauthor=true&cauthor_uid=22923960), [Rachchh MA](http://www.ncbi.nlm.nih.gov/pubmed?term=Rachchh%20MA%5BAuthor%5D&cauthor=true&cauthor_uid=22923960), and [Gokani RH](http://www.ncbi.nlm.nih.gov/pubmed?term=Gokani%20RH%5BAuthor%5D&cauthor=true&cauthor_uid=22923960), (2012). Recent advancesing enetherapy for thalassemia. J Pharm Bioallied Sci., 4, 194–201.

Roy D, Quiles J, Gaze DC, Collinson P, Kaski JC, Baxter GF. (2006). Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modifiedalbumin. Heart, 92, 113–4.

Reszka, K.J., B.A. Wagner, C.P. Burns, and B.E. Britigan  (2005). Effects of peroxidase substrates on the Amplex red/peroxidase assay: Antioxidant properties of anthracyclines. Anal. Biochem., 342, 327-337.

Rund D, Rachmilewitz E N (2005). Review Beta-thalassemia. Engl J Med., 353, 1135-46.

Richmond J, Kiss J.(2009). Anemia in COPD: the role of blood transfusion. Transfusion Medicine Respir J, 2007, 29:923.

Ridker PM, Cushman M, Stampfer MJ, Tracy RP and Hennekens CH. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med, 336, 973-9.

Ridker PM, Glynn RJ, Hennekens CH. (1998). C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation, 26, 97, 2007-11.

Roth, G. and G. Valet (1990). Flow cytometric analysis of respiratory  burst activity in phagocytes with hydroethidine and 2’,7’-dichlorofluorescein. J. Leukoc. Biol., 47, 440-411.

Ruchaneekorn W. Kalpravidh1, Angkana Wichit1, Noppadol Siritanaratkul and Suthat Fucharoen (2005). Effect of Co-enzyme Q10 as an antioxidant in beta-thalassemic patients. [Biofactors.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ruchaneekorn+W.+Kalpravidh1%2C+Angkana+Wichit1%2C+Noppadol+Siritanaratkul2%2C+Suthat+Fucharoen%2C+Effect+of+Co-enzyme+Q10+as+an+antioxidant+in+beta-thalassemic+patients.%2C2004.), 25, 225-34.

Rukhsana Jokhio, Yaqoub Khan, Latafat Ali Chughtai, and Zaib-un-Nisa Mughal (2009). C-reactive proteins in transfusion dependent thalassemic patients. Pak J Physiol, 5, 2.

Samir M. Awadallah, Manar F. Atoum, Nisreen A. Nimer and Suleiman A. Saleh (2012). Ischemia modified albumin: An oxidative stress marker in β-thalassemia major., Clinica Chemica Acta, [413,](http://www.sciencedirect.com/science/journal/00098981/413/9) 907–910.

[Schrier SL](http://www.ncbi.nlm.nih.gov/pubmed?term=Schrier%20SL%5BAuthor%5D&cauthor=true&cauthor_uid=11844995). (2002). Pathophysiology of thalassemia. [Curr Opin Hematol](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pathophysiology+of+thalassemia+Stanley+L.+Schrier%2C+MD%2C2002+.), 9, 123-6.

Suthat Fucharoen, Pranee Winichagoon (2002). Thalassemia and Abnormal Hemoglobin. International Journal of Hematology, 76, Supplement II.

Steensma DP, Gibbons RJ and Higgs DR (2005). [Acquired alpha-thalassemia in association with myelodysplastic syndrome and other hematologic malignancies](http://www.bloodjournal.org/cgi/pmidlookup?view=long&pmid=15358626) . Blood, 105, 443–52.

Taher A, et al. (2006). [Pathophysiology and Management of Thalassaemia Intermedia](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CDQQFjAA&url=http%3A%2F%2Fwww.thalassaemia.org.cy%2Fconference_presentations%2FWorkshop%25204%2FAli%2520Taher%2520TIF%2520-%2520TI%2520Pathophysiology%2520final.ppt&ei=X8-yUcnUHsaThgebsoGoCQ&usg=AFQjCNEEFs2wAAB6g7xpN_jIdvoOD6WXKw&sig2=kbDU9NZUftfbdkkV8AoWXA). Blood Cells Mol Dis,, 37, 12-20.

Tarpley, M.M., C.R.  White, E. Suarez, G. Richardson, R. Radi, and B.A. Freeman. (1999). Chemiluminescence detection of oxidants in vascular tissue: Lucigenin but not coelenterazine enhances superoxide formation. Circulation Research, 84, 1203-1211.

Tarpley m.m., Wink .a.d, and Grisham m.b. (2004). Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am . J. Physiol Regul Integr Comp Physiol, 286, 431-444.

Tal Offer ,  Amrita Bhagat, Ashutosh Lal,  Wafa Atamna, Sylvia T. Singer, Et Al.  (2005). Measuring Chromosome Breaks in Patients with Thalassemia. Thalassemia. Ann. N.Y.Acad.Sci., 1054, 439–444.

Thompson D, Pepys MB, Wood SP (1999). The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure,  7, 169–77.

Uggeri, J., R. Gatti, S. Belletti, R. Scandroglio, R. Corradini, B.M. Rotoli, and G. Orlandini (2004). Calcein-AM is a detector of Intracellular Oxidative Activity. Histochem Cell Biol., 122, 499-505.

Vichinsky E. (2007). Hemoglobin E Syndromes. ASH Education Book, 1, 79-83.

Victor Hoffbrand (2005). AReview Deferiprone therapy for transfusional iron overload. Best Pract Res Clin Haematol, 18, 299-317.

Vidyasagar J, Karunakar N, Reddy MS, Rajnarayana K, Surender T and Krishna DR, (2004). Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Indian Journal of Pharmacology, 36, 76-79 .

[Von Zglinicki T](http://www.ncbi.nlm.nih.gov/pubmed?term=von%20Zglinicki%20T%5BAuthor%5D&cauthor=true&cauthor_uid=10911951) (2000). Role of oxidative stress in telomere length regulation and replicative senescence. [Ann N Y Acad Sci.](http://www.ncbi.nlm.nih.gov/pubmed/10911951), 908, 99-110.

[Yuzbasioglu Ariyurek S](http://www.ncbi.nlm.nih.gov/pubmed?term=Yuzbasioglu%20Ariyurek%20S%5BAuthor%5D&cauthor=true&cauthor_uid=21870146) and [Aksoy K](http://www.ncbi.nlm.nih.gov/pubmed?term=Aksoy%20K%5BAuthor%5D&cauthor=true&cauthor_uid=21870146)., (2012). Effect of oxidative stress on membrane proteins in thalassemia and iron deficiency anemia. [Indian J Pediatr.](http://www.ncbi.nlm.nih.gov/pubmed/21870146), 79, 755-8.

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