بسم الله الرحمن الرحيم

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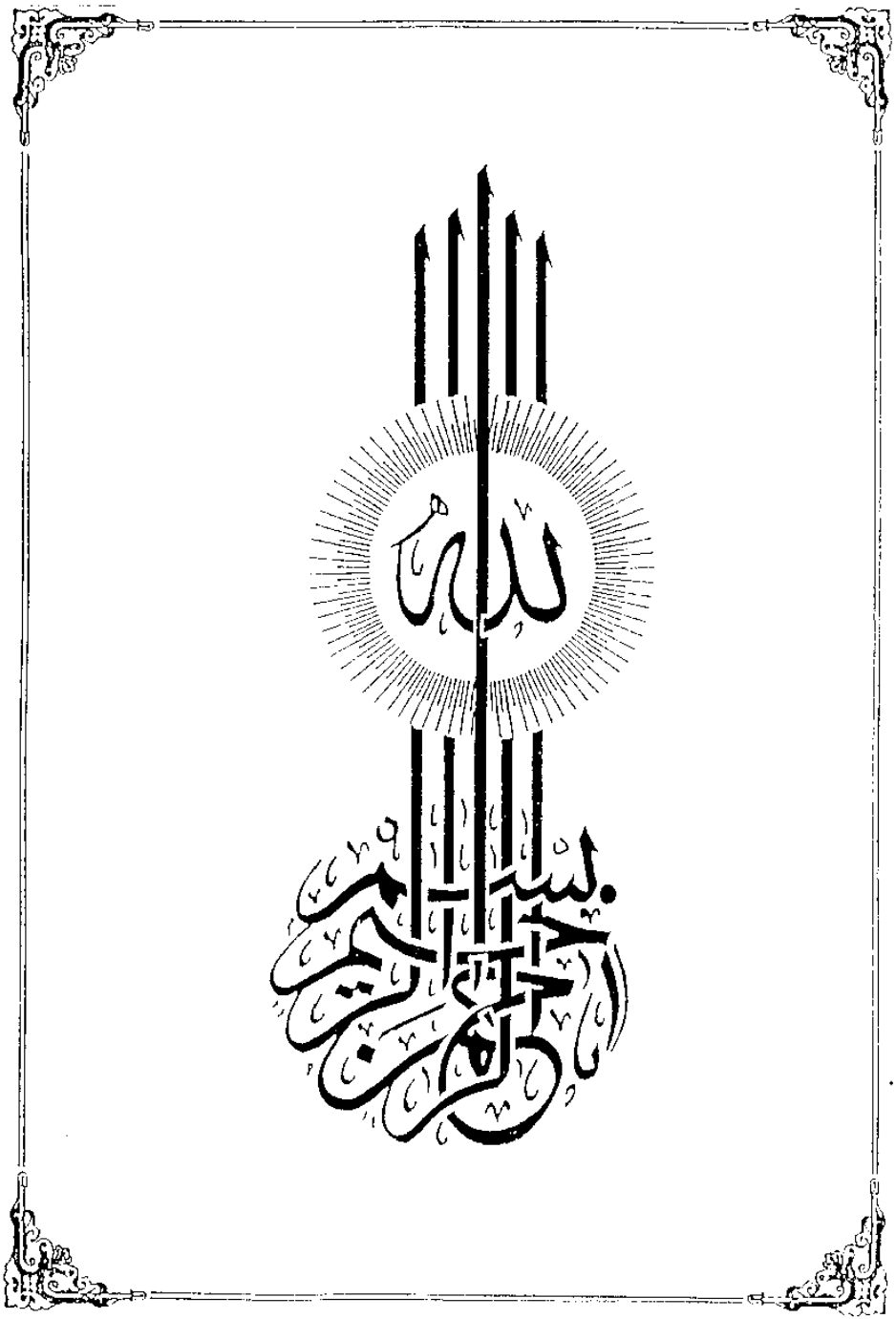

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| $A^o$ | : Angstrom unit |
| AG | : Adenine-Gaunine |
| Anti HBc | : Hepatitis B core antibody |
| Anti HBs | : Hepatitis B surface antibody |
| Baso | : Basophils |
| Chel | : Chelating agent |
| CONTROL | : Control group |
| D.C. | : Direct Current |
| Desfer. | : Desferoxamine |
| Diff. | : Differential |
| dl | : deciliter |
| EOS | : Eosinophils |
| fl | : femtoliter |
| g | : gram |
| gm | : gram |
| GT | : Guanine-Thymidine |
| Hb | : Hemoglobin |
| HBsAg | : Hepatitis B surface Antigen |
| Hct | : Hematocrit |
| HETERO | : Heterozygous group |
| HOMO-NONSPL | : Homozygous nonsplenectomized group |
| HOMO-SPL | : Homozygous splenectomized group |
| $IL_3$ and $IL_6$ | : Interleukin 3 and interleukin 6 |
| I.V.S. | : Intervening sequence |
| L | : Liter |
| Lymph | : Lymphocytes |
| mA | : milliampere |
| MCH | : Mean corpuscular hemoglobin |
| MCHC | : Mean corpuscular hemoglobin concentration |
| MCV | : Mean corpuscular volume |

| | |
|---|---|
| Met-Hb | : Methemoglobin |
| mM | : millimol |
| Mono | : Monocytes |
| N | : Normal |
| nm | : nanometer |
| No. | : Number |
| pg | : picogram |
| Poly | : Polymorphonuclear leucocytes |
| Pts | : Patients |
| RBCs | : Red blood cells |
| V | : Voltage |
| v | : volume |
| WBCs | : White blood cells |

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# Introduction and Aim of The Work

# INTRODUCTION
# AND
# AIM OF THE WORK

The thalassemia syndromes constitute the most prevalent of all known genetic diseases. About 3% of world's population carry β-thalassemia genes *(Lukens, 1993)*.

Each year, 100,000 children throughout the world are born with thalassemia major *(Esposito, 1992)*.

In Egypt, β-thalassemia is the commonest chronic hemolytic anemia *(Sabry, 1973)*.

Along the last 15 years, thalassemia represented 40% of the hematological problems in children attending The Hematology/Oncology Clinic, Children's Hospital, Ain Shams University *(Imam, 1994)*.

To date, well in excess of 100 different thalassemia mutations have been identified *(Huisman, 1992)*.

Although the genetic defects of β-thalassemia, as well as the molecular pathology of these defects have been studied intensively, the reasons leading to premature removal of abnormal RBCs in the marrow

and from peripheral circulation are not clearly understood *(Shinar et al., 1987)*.

The extreme clinical heterogeneity of thalassemia phenotypes reflects an enormous diversity of genetic mutations. Some mutations interact to produce a severe thalassemia phenotype, whereas others result in relatively mild syndromes *(McDonagh and Nienhuis, 1993)*.

The basic pathology in thalassemia is a decrease in either α or β chain synthesis which has several deleterious effects on red cell production and survival. Selective deficiency of one or more polypeptide chains has two immediate consequences: decreased hemoglobin synthesis and imbalance between α and non-α chain production. The absence of complementary globin chains with which to bind, chains whose synthesis is normal form aggregates, precipitate within the cytoplasm, damage cell membranes, and lead to premature cell destruction *(Lukens, 1993)*.

The end product of the precipitated hemoglobin chains is heme, from which eventually iron and globin are liberated. Globin chains have been found to interact and disrupt the RBC membrane, leading to damage of cytoskeleton. Excess of iron is known to be a catalyst of peroxidation, causing damage to the various RBC membrane components. The role of heme has not yet been studied in detail in thalassemic RBCs. However,

there is some evidence that heme participates in damaging RBCs in other types of hemoglobinopathies *(Shinar and Rachmitewitz, 1990).*

Free heme is capable of interacting with either the lipid bilayer or cytoskeletal membrane protein, however, the direct contribution of the heme molecule to the creation of oxidative damage in thalassemia still needs to be elucidated *(Wolfe, 1989).*

Massive splenomegaly is a characteristic feature of the natural course of severe thalassemia. Splenectomy is often necessary in the management of a patient with severe β-thalassemia. Splenectomy offers the long lasting benefit of reduction of transfusion requirement together with improving the quality of life of β-thalassemia patient *(Pinna et al., 1988).*

The objective of this study was to evaluate the possible role of heme in the pathophysiology of β-thalassemia through studying the conformational state of heme, heme concentration, conductivity of hemoglobin and methemoglobin concentration in β-thalassemia patients, and comparing these characteristics with those of heterozygous thalassemia individuals as well as with a normal control group. In addition, the effect of splenectomy on the heme pattern was assessed.

# Review of Literature

## STRUCTURE OF HEMOGLOBIN

One vital function of the red blood cell is to mediate the exchange of respiratory gases, oxygen and carbon dioxide, between the lungs and the tissues. Of fundamental importance to this process is the oxygen-transport red-pigmented protein, hemoglobin. It is the major constituent of the red cell cytoplasm, accounting for about 90% of the dry weight of the mature cell *(Telen, 1993)*.

Hemoglobin is a tetramer with a molecular weight of 64,400 daltons. it consists of two pairs of unlike globin polypeptide chains. An iron-containing porphyrin derivative called heme, a ferroprotoporphyrin IX, is linked covalently at a specific site to each chain. When heme iron is in the reduced (ferrous) state, it can bind reversibly with gaseous ligands, such as, oxygen and carbon monoxide. In developing human erythroblasts, eight genes direct the synthesis of six structurally different globin polypeptide chains designated by a Greek letter α, β, γ, δ, ε, and ζ *(Bunn, 1993)*.

### GLOBIN

The globin polypeptide chains in hemoglobin differ from one another in amino acid sequence. The α-chain contains 141 amino acids, whereas β-chain (as well as γ, δ, and ε) have 146. The δ-hain differs from

the β-chain in only 10 of the 146 amino acid residues, whereas the γ- and β-chains differ by 39 amino acids *(Bunn and Forget, 1986).*

Protein structure is routinely described using four different aspects of structure: 1) Primary structure, or the linear sequence of amino acids; 2) Secondary structure, which describes how the amino acids within segments of the protein are spacially organized, e.g., by folding into an alpha helix or beta pleated sheet; 3) Tertiary structure, which refers to the steric relationships of sequence domains that are separate from each other when analysed as part of the linear sequence of the protein; and 4) Quaternary structure, or the way in which several polypeptide chains join to form a single molecule *(Bunn and Forget, 1986).*

The primary structure of the globin chains are different from each other, however, their secondary structures are remarkably similar. Each has eight helical segments designated by the letters A through H. The helices are of nearly identical length in all four normal chains except for the D helix, which contains seven amino acids in the β-, γ-, and δ-chains, but only two amino acids in the β-chain. The helices make up about 75% of the molecule. Interspersed between them are seven nonhelical segments. This arrangement is important structurally, because the helices are relatively rigid and linear, whereas the nonhelical segments allow bending *(Perutz, 1987).*