Sickle Cell Disease

Sickle Cell Disease:

From the Laboratory to Clinical Practice

Edited by

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The book is dedicated to the populations plagued with the menace of Sickle Cell Disease

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PREFACE

The Centre for Disease Control (CDC), USA stated that "Sickle cell disease (Sickle Cell Disease) affects millions of people throughout the world and is particularly common among those whose ancestors came from sub-Saharan Africa; Spanish-speaking regions in the Western Hemisphere (South America, the Caribbean, and Central America); Saudi Arabia; India; and Mediterranean countries such as Turkey, Greece, and Italy" (CDC, August 9, 2017). It was estimated that about 100,000 Americans suffer from Sickle Cell Disease. About 1 out of every 365 Black or African American births have Sickle Cell Disease, while it is 1 out of every 16,300 Hispanic American births. The sickle cell trait occurs at a more alarming rate of 1 in 13 of Black or African American babies.

The commonest haemoglobinopathy in the world is SS gene in terms of distribution and the population affected. Sickle cell disease is majorly a disease of black Africa with 25-30% having sickle cell trait AS and 2-3% being homozygous SS in Nigeria.

Sickle cell disease poses a huge burden on the economic, psychological and social well-being of not only the patients but their families and the nations of the world.

The disease is therefore of great public health importance globally even as people continue to migrate due to security, economic, educational and other sundry reasons.

With advances in knowledge of the pathophysiology of the disease and the advent of drugs to ameliorate its effect, affected individuals tend to achieve near normal life expectancy for age, sex and environment. Gene replacement therapy and stem cell transplantation offer cure but both are not readily available across the world in terms of expertise and cost. Even where they are available, there are other issues to contend with. Stem cell transplantation has its risks (including death) and complexities and many sick patients cannot stand the processes and procedure, moreover some may reject the donor cells. Therefore, cure is still a mirage.

The dissemination of knowledge that this book offers is needed at this time to increase awareness, improve preventive strategies and be abreast of current advances in treatment and cure of the disease.

The book has chapters on all the organs and systems affected in the body, written by people with in-depth knowledge and expertise.

This laudable project, its conception and execution by the authors, is a noble idea.

I recommend this book to everybody interested in sickle cell disease.

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FOREWORD

Sickle cell disease is a generic terminology used for a group of disorders in which there is an inheritance of the sickle β – globin gene (HbS) which results in abnormal haemoglobin production. It comprises homozygous Sickle Cell anaemia (HbSS), Sickle Cell haemoglobin C disease (HbSC), Sickle Cell thalassaemia (HbSThal). Others are compound heterozygous conditions with rarer combinations of SD, SE, SO, and hereditary persistent fetal haemoglobin.

The HBSS is the most troublesome form and is most common among African descent. Though it was first discovered in 1907 and first published in America, history has it that the first symptoms were seen in a Ghanaian family back in the 16th century. Epidemiologically, the prevalence is highest in Sub Saharan Africa especially among Nigerians (about 2% of the population), Congolese, Cameroonians and then the Asians. It is also very common in malaria-endemic zones because of the protective effect of the heterozygous groups on malaria.

It is a disorder that affects every system and organ in the body because it is a form of blood disorder. In as much as it is hereditary, no cure has been found. This makes it a very disturbing disease of public health significance as an estimated 4.4 million people were affected by 114,800 deaths recorded in 2015. In 2018 "the global meta-estimate for the birth prevalence of homozygous sickle cell disease was 112 per 100 000 live births and a birth prevalence in Africa of 1125 per 100 000 compared with 43.12 per 100 000 in Europe." [Wastnedge E et al. J Glob Health. 2018; 8(2): 021103]

This disease can be classified as hereditary and non-communicable. The only way to reduce its prevalence is by having increased knowledge and awareness of its occurrence, which this book has attempted to achieve.

The authors of this book have delved into what is known to be the current concept within the time and period of writing this book. The writers are collaborating professions, teachers and health practitioners from institutions in Nigeria. The book gives in-depth knowledge on all the

systems in the body such as eyes, kidneys, hearts, bones, the pregnancy and delivery state, the mental and psychological states of a person. It also talks about the state, prevention and occurrence in children. The writing of this book went through scientific research processes of review and plagiarism tests and so makes it a scientifically sound and standard academic write up.

The book, therefore, is a very good source of information for health workers and practitioners, students, teachers, policymakers and the African populace in general as a way of caring, preventing and treating sickle cell diseases. It is highly recommended to enlighten the youths, particularly as a counselling guide in the preparation of their marital life.

We, however, look forward to a day we can find a cure to this disease and make SCD placed among the NCD of the global SDG prevention strategies. The mortality of the under-fives is also going to be reduced being one of the global health priorities.

I wholeheartedly and strongly recommend this book to every person of African and Non-African descent.

Professor Michaeline Asuquo ISAWUMI,
Immediate Past Ag. Provost, College of Health Sciences,
Osun State University, Nigeria
November 2019

LIST OF ABBREVIATIONS

ABG:	Arterial Blood Gas
ACS:	Acute chest syndrome
AIDS:	Acquired immune deficiency syndrome
AKI:	Acute kidney injury
ALA:	δ-Amino Levulinate
ALT:	Alanine aminotransferase
AP:	Alkaline Phosphatase
ASS:	Acute splenic sequestration
AST:	Aspartate aminotransferase
ATP:	Adenosine triphosphate
AVN:	Avascular necrosis
BCAM:	Basal Cell Adhesion Molecule
BMI:	Body Mass Index
BNP:	Brain natriuretic peptide
BTL:	Bilateral tubal ligation
CAN:	Cardiovascular autonomic neuropathy
CBC:	Complete Blood Count
CBT:	Cognitive behavioural therapy
CD:	Cluster of Differentiation
CDC:	Centre for Disease Control
CHCM:	Comprehensive Health Care Management
CO:	Carbon monoxide
CO_2:	Carbon dioxide
COPD:	Chronic obstructive pulmonary disease
CPAP:	Continuous positive airway pressure
CRP:	C-reactive protein
CT:	Computerized Tomography
CTEPH:	Chronic thromboembolic pulmonary hypertension
CVA:	Cerebrovascular accident
CVS:	Chorionic Villus Sampling
DMPA:	Depo-medroxyprogesterone acetate
DNA:	Deoxyribonucleic acid
EBT:	Exchanged blood transfusion
ECG:	Electrocardiogram
ED:	Erectile dysfunction

ELISA: Enzyme Linked Immunosorbent Assay
EmeA: European Medicines Agency
eNOS: Endothelial NO synthase
ESRD: End stage renal disease
ET-1: Endothelin-1
FIO_2: Fraction of Inspired Oxygen
FLACC: Face, Legs, Activity, Cry and Consolability
FSH: Follicular Stimulating Hormone
FVC: Forced vital capacity
G-6-PD: Glucose-6-Phosphate Dehydrogenase
GFR: Glomerular filtration rate
GIT: Gastrointestinal tract
GM-CSF: Granulocyte macrophage colony stimulating factor
GVHD: Graft versus host disease
Hb: Haemoglobin
HbA_{1C}: Glycated haemoglobin
Hb A: Haemoglobin A
Hb AS: Haemoglobin AS
Hb F: Foetal haemoglobin
Hb S: Haemoglobin S
Hb Sthal: Haemoglobin Sickle cell thalassaemia
HCG: Human Chorionic Gonadotropin
HDL-C: High density lipoprotein cholesterol
Hib: Haemophilus influenzae type b
HIV: Human Immunodeficiency Virus
HLA: Humal Leucocyte Antigen
HPLC: High performance liquid chromatography
HRSA: Health Resources and Services Administration
HRV: Heart rate variability
HSC: Haematopoietic stem cells
HSCT: Haematopoietic stem cell transplantation
HU: Hydroxyurea
ICAM: Intercellular adhesion molecules
IEF: Iso-electric focussing
IFN-γ: Interferon-γ
Ig: Immunoglobulins
IL: Interleukins
INR: International Normalized Ratio
IST: International Stroke Trial
IUGR: Intrauterine growth restriction
LAE: Left Atrial Enlargement

LDL-C: Low density lipoprotein cholesterol
LFT: Liver function test
LH: Luteinizing Hormone
LV: Left Ventricle
LVH: Left Ventricular Hypertrophy
LVNC: Left ventricular non-compaction
MAC: Myeloablative conditioning
MACSS: multicenter acute chest syndrome study
Mb: deoxygenated myoglobin
MbO_2: Oxygenated myoglobin
MCH: Mean corpuscular haemoglobin
MCHC: Mean corpuscular haemoglobin concentration
MCV: Mean corpuscular volume
MI: Myocardial infarction
MMF: Mycophenolate mofetil
MOD: Multi-organ dysfunction
MPAP: Mean pulmonary artery pressure
MPI: Myocardial Performance Index
MRA: Magnetic Resonance Angiography
MRI: Magnetic Resonance Imaging
MSH: Multi-center Study of Hydroxyurea
MTD: Maximum tolerable dose
NBS: Newborn screening
NCD: Non communicable diseases
NEMLIST: National Essential Medicine List
NGCMSCD: National guidelines for the control and management of SCD
NGO: Non-Governmental Organization
NHLBI: National Heart Lung & Blood Institute
NHR: National Haemoglobinopathy Registry
NIH: National Institute of Health
NK: Natural Killer
NMAC: Non-Myeloablative conditioning
NO: Nitric oxide
N-PRS: Numerical Pain Rating Scale
NSAIDS: Non-steroidal anti-inflammatory drugs
NSCDN: Nigerian Sickle Cell Disease Network
NYHA: New York Heart Association
O_2: Oxygen
OCD: Obsessive-compulsive disorders
OSAS: Obstructive sleep apnoea syndrome

PAH: Pulmonary arterial hypertension
PCI: Percutaneous coronary intervention
PCR: Polymerase chain reaction
PCV 13: 13-valent pneumococcal-conjugated vaccine
PGF: Placenta growth factor
PHC: Primary Health Care
PHT: Pulmonary hypertension
PMN: Polymorphonuclear
PNH: Paroxysmal nocturnal haemoglobinuria
PO_2 Partial Pressure of Oxygen
PT: Prothrombin time
PTTk: Partial thrombopastin time
PVT: Pulmonary venous hypertension
RAE: Right Atrial Enlargement
RBC: Red blood cell
RDW: Red cell distribution width
RES: Reticuloendothelial system
RHC: Right heart catheterization
RTA: Renal tubular acidosis
RV: Right Ventricle
RVH: Right Ventricular Hypertrophy
SaO_2: Arterial Oxygen Saturation
SCA: Sickle cell anaemia
SCD: Sickle cell disease
SCCLD: Sickle cell chronic lung disease
SCI: Silent cerebral infarction
SCN: Sickle cell nephropathy
SCT: Sickle cell trait
SCTA: Sickle Cell Treatment Act
S_PO_2: Peripheral Oxygen Saturation
SS-RBC: Sickled red blood cells
STRs: Short tandem repeat allele mutations
TCD: Transcranial Doppler
TIA: Transient Ischaemic Attack
TLC: Total lung capacity
TMA: Thrombotic microangiopathy
TNF: Tumor necrotic factor
TRV: Tricuspid Regurgitant Velocity
TSP: Thrombospondin
UK: United Kingdom
USA: United States of America

US-FDA:	United States Food and Drug Administration
UTI:	Urinary tract infection
VCAM-1:	Vascular cell adhesion molecule-1
VEGF:	Vascular endothelial growth factor
VLA-4:	Very late antigen-4
VOC:	Vaso-occlusive crisis
VTE:	Venous thromboembolism
WBC:	White Blood Cell
WB-PRS:	Wong-Baker Faces Pain Rating Scale
WHO:	World Health Organization

HAEMOGLOBIN: STRUCTURE, SYNTHESIS AND OXYGEN TRANSPORT

OLADOKUN OO AND ATERE TG

Introduction

Haemoglobin is a word that was coined from two words "haemo" which means blood and "globin" meaning protein. Globin is a protein substance of four different polypeptide chains that have amino acids ranging between 141 to 146. Haemoglobin is a conjugated globular protein having a molecular weight of about 64500 (1). There are two important oxygen-binding proteins in vertebrates namely haemoglobin (Hb or Hgb) and myoglobin. (1) Haemoglobin supplies oxygen (O_2) to tissues.

Haemoglobin's function is to transport oxygen (O_2) in the blood from the lungs to other tissues of the body and provide cells with the oxygen they need for foodstuff oxidative phosphorylation. Haemoglobin is found in the blood within erythrocytes (red blood cells (RBC)) and is the most common family of carriers of O_2. (1) Haemoglobin is the main component of red blood cells which number about 250 million per cell and its combination with iron (Fe) and oxygen forms the bright red colour of RBC. Haemoglobin comprises more than 95% of the erythrocyte, it also carries nitric oxide, which controls vascular tone and blood pressure. (3) Haemoglobin is equally involved in the transport of respiratory carbon dioxide (about 20–25% of the haemoglobin as carbamino-haemoglobin) in which carbon dioxide is bound to the globin protein. (4) Erythrocytes further contain carbonic anhydrase, an enzyme that rapidly interconverts carbon dioxide and bicarbonate allowing the efficient transport of carbon dioxide, produced by respiration in the peripheral tissues, to the lungs, where it is exhaled. The haemoglobin combination with O_2 and CO_2 is reversible and this forms the basis for the gas-transport capability of hemoglobin. However, the combination of Hg with carbon monoxide (CO) is irreversible. This reduces the cell capacity to transport O_2 during carbon monoxide poisoning. (5) Myoglobin, the other

O_2-binding protein, stores oxygen in body tissues until cells need it. The highest levels of myoglobin are found in cardiac muscles and skeletal muscle, which require large amounts of oxygen during contraction. (6)

Catabolism of haemoglobin splits off the globin portion into an amino acid pool while the haem portion is converted into biliverdin. In humans, biliverdin is converted to bilirubin and secreted in the bile. Iron from haem is however reused for haemoglobin synthesis. (3, 6)

Haemoglobin is known to have an O_2-binding capacity of 1.34 cm^3 of dioxygen per gram which increases the total oxygen capacity in the blood by 70 times compared to dissolved oxygen in the blood. (6) For normal level tissue oxygenation, an optimum haemoglobin level must be maintained. The normal Hb level for males is 14 to 18 g/dl, and for females it is 12 to 16 g/dl. (7) A low level of haemoglobin results in anaemia, while a level above the normal is called erythrocytosis. A complete blood cell (CBC) test which expresses the level of haemoglobin is clinically used to diagnose anaemia, dehydration, and malnutrition. (7) Myoglobin and haemoglobin describe both protein structure-function relationships and the molecular basis of genetic diseases such as hereditary persistence of foetal haemoglobin, thalassaemias and sickle cell anaemia. (8, 9)

Structure

Haemoglobin has a quaternary structure, it is a tetrameric protein with two α chains and two β chains (α2β2), each with a haem unit as a prosthetic group, each polypeptide chain having a very strongly three-dimensional structure similar to the unique polypeptide chain in myoglobin. However, their amino acid sequences differ by 83%.

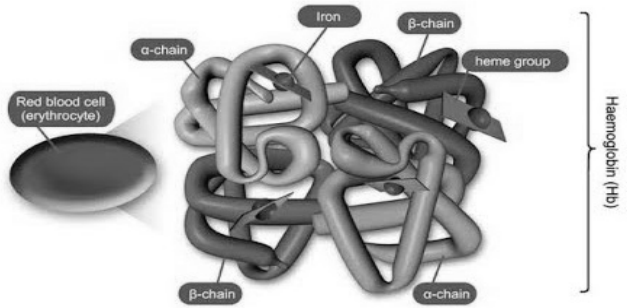

Figure 1: Structure of haemoglobin (10)

The globular protein units of haemoglobin comprise two identical pairs of polypeptide chains, i.e. two identical alpha (α) chains containing 141 amino acids and two identical non-α chains (beta (β), gamma (γ), delta (δ) or epsilon (ε) chains).

The main type of haemoglobin present in adults (HbA) consists of the α chain, which contains 141 amino acid residues, and the β chain, which consists of 146 amino acid residues ($\alpha_2\beta_2$, Figure 1). Each chain, comprises eight α helices and each group contains a prosthetic haemic group (Figure 1). As a result, haemoglobin can bind to four O_2 molecules. The four polypeptide chains, two α chains and two β chains are tightly grouped into a tetrahedral set to form a globally spherical molecule held together by several non-covalent interactions. (6) The two dimers are held tightly primarily by hydrophobic interactions. Ionic and hydrogen bonds also occur between the members of the dimers. Two dimers are able to move with respect to each other being held together primarily by polar bonds. The weaker the interaction between these mobile dimers results in the two dimers occupying different relative positions in deoxyhaemoglobin compared to oxyhaemoglobin. (11)

The tetrameric structure of common haemoglobin follows that found in an adult. However, there are other variations. For instance, the combination of two alpha chains and two gamma chains ($\alpha 2\gamma_2$) forms foetal haemoglobin, called haemoglobin F; sickle cell anaemia HbS (α_2S_2); and HbA_2 (α2δ2) (a minor haemoglobin A variant found in about 2.5% of adults). In a normal adult, with HbA, the α chain has 141 amino acid residues, while β has 146 amino acid residues, some minor haemoglobin types are shown in Table 1.

Table 1: Some minor haemoglobin types

Haemoglobin type	Chain composition	Fraction of total haemoglobin
HbA	$\alpha_2\ \beta_2$	90%
HbA_2	$\alpha_2\delta_2$	2-5%
HbA_{1c}	$\alpha_2\ \beta_{2\text{-glucose}}$	3-9%
HbF	$\alpha_2\delta_2$	<2%

Myoglobin, a haemoprotein present in skeletal and heart muscle, functions as both a reservoir and carrier of oxygen. Unlike haemoglobin that is tetrameric in structure, myoglobin is of a single polypeptide chain.

Foetal haemoglobin

In the foetus, there is another type of haemoglobin, haemoglobin F (HbF), which, unlike adult haemoglobin (HbA, $\alpha_2\beta_2$), consists of two α chains and two γ ($\alpha_2\gamma_2$) chains. HbF has a greater affinity for O_2 than HbA under physiological conditions, optimizing the transmission of oxygen from the maternal circulation to the foetal circulation through the placenta. The molecular basis of this difference in affinity for O_2 is that HbF 2,3-bisphosphoglycerate binds less strongly than HbA. Near birth, the synthesis of the γ chain is deactivated and the β chain (HbA) is activated (Figure 2). (6)

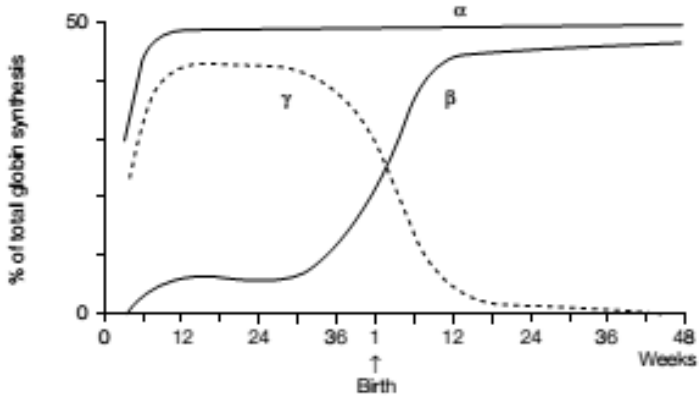

Figure 2: The transformation of human globulin synthesis at birth (6)

The different types of chains in haemoglobin are encoded by different genes. The genes encoding alpha globin chains are located on chromosome 16. The genes encoding non-alpha globin chains are on chromosome 11 in humans. Many human haemoglobinopathies result from inadequate expression of globin genes, and attempts to modulate globin gene expression are a fundamental approach to seek novel avenues to therapy. (12, 13)

Haem

Haem is a complex protoporphyrin IX and Fe^{2+}. Porphyrins are cyclic compounds formed by linking four pyrrole rings via methenyl bridges. A characteristic of porphyrins is the formation of complexes with nitrogen ions bound to metal ions (archive.org) of pyrrole rings. (14)

The iron is held in the centre of the haem molecule by bonds to the four nitrogens of the porphyrin ring. The Fe^{2+} of the haem can form two more bonds. In myoglobin and haemoglobin, one of these positions is coordinated to the side chain of the histidine residue of the globin molecule, while the other position is available for the binding of oxygen. (2)

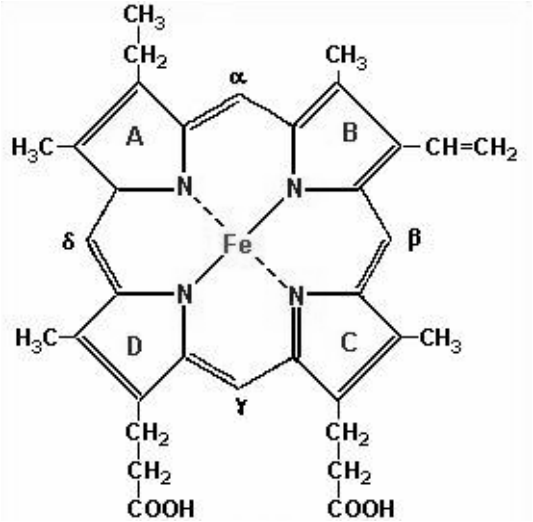

Figure 3: Structure of haem (https://www.namrata.co/structure-of-hemoglobin-an-overview/)

Synthesis of Haemoglobin

Synthesis of Haem

Acetic acid in the Krebs cycle changes into α-keto glutaric acid which combines with glycine to form pyrrole compound, and pyridoxal phosphate is necessary to activate glycine.The product of the condensation reaction between succinyl CoA and glycine is α-amino-β-ketoadipic acid, which is rapidly decarboxylated to form δ-aminolevulinate (ALA). The reaction is catalyzed by ALA synthase, which is the rate limiting enzyme in porphyrin biosynthesis. Two molecules of ALA condensed to form one porphobilogen (PBG) and two molecules of water. This reaction is catalyzed by ALA dehydratase. The formation of tetrapyrrole/porphyrin occurs by condensation of four PBG molecules. This reaction is catalyzed by PBG deaminase. Porphyrin biosynthesis occurs in the mammalian liver. (15, 16)

Four PBG molecules condenses to form linear tetrapyrrole, hydroxymethylbilane. The hydroxymethylbilane cycle spontaneously forms uroporphyrinogen I or it is converted to uroporphyrinogen III by the combined action of uroporphyrinsynthase and uroporphyrinogen III co synthase. (15, 16) In the presence of uroporphyrinogen decarboxylase, uroporphyrinogen III is decarboxylated of all the acetate substituents to methyl substituents to form coproporphyrinogen III. Coproporphyrinogen III is then transported into mitochondria from where it is transformed to protoporphyrinogen III, then to proporphyrin III in the presence of mitochondrial enzyme coproporphyrinogen oxidase while protoporphyrinogen oxidase is needed in the conversion of proporphyrinogen III to protoporphyrin. (15, 16)

The incorporation of Fe^{2+} into the protoporphyrin by the action of haem synthase or ferrochelatase is the last step in the biosynthesis of haem. The iron is held in the haem centre molecule with bonding to the four nitrogens of the porphyrin ring. In myoglobin and haemoglobin, the side chain of histidine residue coordinates the position of Fe^{2+} available for oxygen bonding. (15, 16)

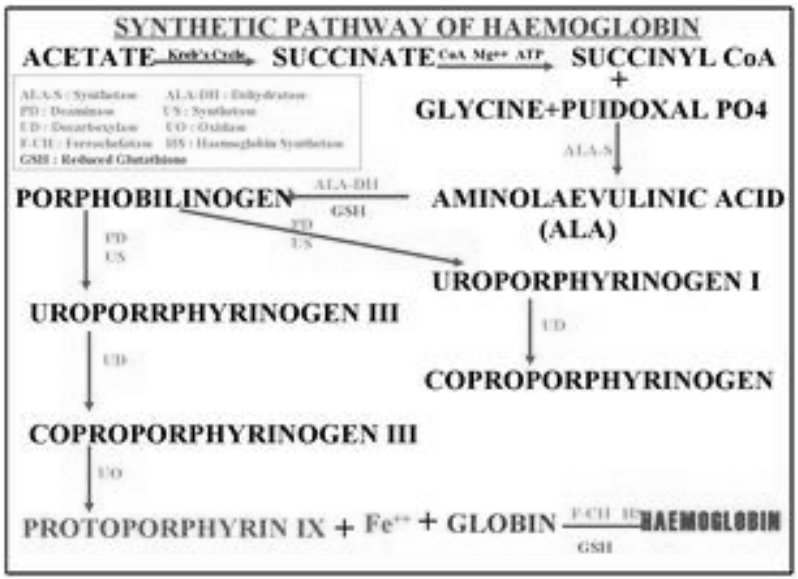

Figure 4: Synthetic pathway of haemoglobin (21)

The synthesis of globin chains α, β, δ, ε, γ and ζ, is controlled by structural genes of chromosomes 16 (α; ζ) (archive.org) and 11 (ζ). The rate of synthesis and the location vary through embryo to foetus to neonatal and to adult (17). The expression of alpha and non-alpha genes is precisely balanced by an unknown mechanism. Normal function of red blood cells requires balanced gene expression. A disturbance of balance leads to thalassaemia. (17, 18)

Binding of oxygen to haemoglobin

The deoxy form of haemoglobin is known as the "T" or taut (tense) form. In this form, the two αβ dimers interact through a network of ionic and hydrogen bonds that constrains the movement of polypeptide chains. This form is the low oxygen affinity form of haemoglobin. The binding of oxygen to haemoglobin causes the rupture of some of the ionic bonds and hydrogen bonds between the dimers. This leads to the "R" structure, or relaxed form, in which the polypeptide chains have more freedom of movement. The R form is the high oxygen affinity form of haemoglobin. (16)

Haemoglobin binds one oxygen molecule (O_2) at each of its four haem groups. The percentage of saturation of oxygen binding sites on haemoglobin molecules can be represented graphically on the oxygen dissociation curve for haemoglobin (Figure 5).

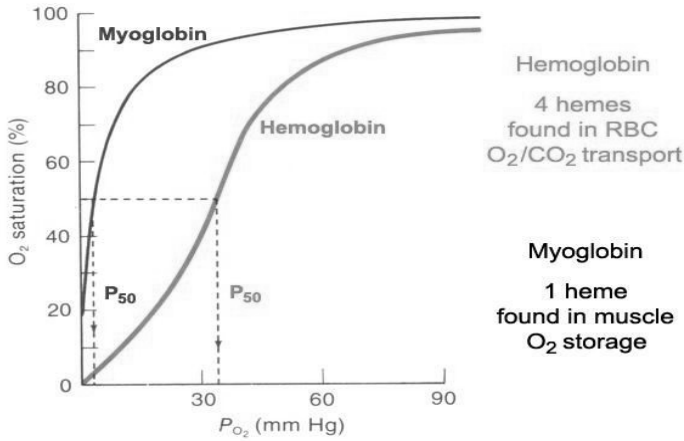

Figure 5: The oxygen binding curve (22)

The partial pressure of oxygen needed to achieve half saturation of the binding sites (P_{50}) is approximately 1 mmHg for myoglobin and 26 mmHg for haemoglobin. This shows that myoglobin has a higher affinity for oxygen. PO_2 in the lung capillary bed is 100 mmHg, myoglobin could effectively be loaded up with oxygen in the lungs. PO_2 of active muscle is about 20 mmHg. Myoglobin cannot deliver a large fraction of its bound oxygen even at 20 mmHg. It cannot serve as an effective vehicle for the delivery of oxygen from the lungs to peripheral tissue but is a better store of oxygen. The oxygen dissociation curve for myoglobin has a hyperbolic shape. This reflects the fact that myoglobin reversibly binds a single molecule of oxygen. The oxygenated myoglobin (MbO_2) and deoxygenated myoglobin (Mb) exist in simple equilibrium. (11, 12)

$Mb + O_2 \quad MbO_2$

Myoglobin therefore releases its oxygen during the deprivation that accompanies severe physical exercise that lowers the PO_2 of muscle tissue to as about 5 mmHg.

Haemoglobin binds $4O_2$ molecules per tetramer (one per subunit of haem) and the oxygen saturation curve is sigmoidal. The facility with which haemoglobin binds O_2 depends on whether other molecules are present at the same tetramer. If O_2 is present, binding of subsequent O_2 is achieved more readily because the binding of oxygen molecules at one haem group increases the oxygen affinity of the remaining haem group in the same haemoglobin molecule i.e. haemoglobin exhibits cooperative binding kinetics – a property that permits it to bind a maximal quantity of O_2 at the respiratory organ (PO_2) 100 mmHg and to deliver a maximal quantity of O_2 at the PO_2 of peripheral tissue (20 mmHg).

$Hb_4 + O_2 \quad Hb_4O_2$

$Hb_4O_2 + O_2 \quad Hb_4O_4$

$Hb_4O_4 + O_2 \quad Hb_4O_6$

$Hb_4O_6 + O_2 \quad Hb_4O_8$ (fully saturated haemoglobin)

These changes profoundly alter haemoglobin's secondary, tertiary and quarterly structure. One pair of α/β subunits rotates with respect to the other α/β pair, compacting the tetramer and increasing the affinity of the haem for O_2. Oxygenation of haemoglobin makes iron atoms of

deoxyhaemoglobin (which lie about 0.06 nm beyond the plane of the haem ring) move into the plane of the haem ring. This motion is transmitted to the proximal (F_8) histidine, which also moves towards the plane and to residues attached to $HisF_8$. (11, 12, 19)

Haemoglobin binds CO_2

Haemoglobin binds CO_2 directly when oxygen is released and about 15% of the CO_2 carried in blood is carried directly on haemoglobin molecules. CO_2 reacts with the amino terminal α-amino group of haemoglobin, forming a carbonate and releasing protons that contribute to the Bohr effect

$$CO_2 + Hb\text{-}NH_3^+ \qquad 2H^+ + Hb - \underset{\displaystyle H}{N} - COO^-$$

Conversion of the amino terminal from a positive to a negative charge favours salt bridge formation between α and β chains so that the taut T state is stabilized resulting in a decrease in its affinity for oxygen. (19)

Increase in the partial pressure of CO_2 and/or increase in H^+ concentration or a decrease in pH enhances the release of oxygen from haemoglobin that is a decrease in Hb affinity for O_2 and the oxygen dissociation curve shifts to the right.

Sources of protons that lower pH

The concentration of both CO_2 and H^+ in the capillaries of metabolically active tissue is higher than that observed in alveolar capillaries of the lungs. As CO_2 is absorbed in blood, the carbonic anhydrase in erythrocytes catalyzes the formation of carbonic acid. Carbonic acid rapidly dissociates into bicarbonate and a proton.

$$CO_2 + H_2O \qquad H_2CO_3 \qquad HCO_3^- + H^+$$

Haemoglobin binds protons for every four oxygen molecules released and thus contributes significantly to the buffering capacity of blood.

In the lungs, the process is reversed. Oxygen binds to deoxyhaemoglobin and protons are released. The protons combine with carbonate forming carbonic acid. Carbonic anhydrase helps to form CO_2 which is exhaled. This reversible reaction is called the *BOHR EFFECT*. (11, 12)

The protons responsible for the Bohr effect are generated by the rupture of salt bridges during the binding of oxygen to the T structure. The protons released from the nitrogen atoms of β-chain residues drive carbonate toward carbonic acid which is released as CO_2 in alveolar capillaries. The Bohr effect can be represented as:

HbO_2^+ HbH^+ + O_2

oxyhaemoglobin deoxyhaemoglobin delivered

2,3 diphosphoglycerate (2,3 DPG) also affects the affinity of haemoglobin for oxygen

2,3 DPG is very plentiful in red cells. It is formed as an intermediate of the glycolytic pathway. It is a highly charged anion that binds to the β-chain of deoxyhaemoglobin. One molecule of deoxyhaemoglobin binds one of 2,3 DPG, in effect,

HbO2 + 2,3-DPG Hb-2,3DPG + O_2

The binding of 2,3-DPG to deoxyhaemoglobin stabilizes the taut conformation of deoxyhaemoglobin and favours the liberation of O_2 from haemoglobin. Haemoglobin from which 2,3-DPG has been removed has a high affinity for oxygen. The presence of 2,3-DPG therefore reduces the affinity of haemoglobin for oxygen thereby shifting the oxygen dissociation curve to the right to release O_2 at the peripheral tissue. (6, 11)

The preferential binding of 2,3-DPG to the β-globin chain is important and in foetal haemoglobin where the β-chain is replaced by the ϑ-chain that binds 2,3-DPG weakly, the affinity of haemoglobin to oxygen is high. This high affinity facilitates the movement of O_2 from mother to foetus despite the hypoxic (anaerobic) environment of the foetus. (20)

2,3-DPG concentration in red blood cells increases in response to chronic hypoxia and anaemia. In conditions like obstructive pulmonary emphysema or high altitude and chronic anaemia, intracellular levels of 2,3-DPG increase. However low pH reduces 2,3 DPG because acidosis inhibits red cell glycolysis. Thyroid hormone, growth hormone and androgens increase 2,3-DPG and the P_{50}. (6,11,12)

Conclusion

Hundreds of haemoglobins have been previously characterized to establish haemoglobinopathies. The best characterized haemoglobinopathy is probably sickle cell anaemia (sickle cell haemoglobin, HbS). The molecular basis of this disease is the substitution of a glutamic acid residue, a polar residue to a valine, a hydrophobic one at the 6-position of the β-chain, resulting in the substitution of a polar residue for a hydrophobic residue. (6) Sickle cell disease is a genetically transmitted haemolytic disease. The crescent-shaped RBCs are more fragile than the normal erythrocytes, they lysate easily and have a shorter half-life, leading to severe anaemia. Sickle cell anaemia is prevalent among people of African descent.

References

1. Khan RH, Siddiqi MK, Salahuddin P. Protein structure and function. Basic Biochemistry. 2017:1-39.
2. Gupta CP. Role of iron (Fe) in body. IOSR Journal of Applied Chemistry (IOSR-JAC). 2014;7(11):38-46.
3. Ponka P, Koury MJ, Sheftel AD. Erythropoiesis, hemoglobin synthesis, and erythroid mitochondrial iron homeostasis. Handbook of Porphyrin Science: erythropoiesis, Heme, and Applications to Biomedicine. 2013;27:41-84.
4. Kim HW, Greenburg AG, editors. Hemoglobin-based oxygen carriers as red cell substitutes and oxygen therapeutics. Springer Science & Business Media; 2013 Dec 18.
5. Kampa M, Castanas E. Human health effects of air pollution. Environmental pollution. 2008 Jan 1;151(2):362-7.
6. Hames D, Hooper N. Bios instant notes in biochemistry. Taylor & Francis; 2006 Sep 27.
7. Dogan S, Mermer E. Comparison of the Hemoglobin Amount between Old and Young Persons in Bosnia and Herzegovina. J Biom Biostat. 2017;8(337):2.
8. Saiki RK, Chang CA, Levenson CH, Warren TC, Boehm CD, Kazazian Jr HH, Erlich HA. Diagnosis of sickle cell anemia and β-thalassemia with enzymatically amplified DNA and nonradioactive allele-specific oligonucleotide probes. New England Journal of Medicine. 1988 Sep 1;319(9):537-41.
9. He S, Wei Y, Lin L, Chen Q, Yi S, Zuo Y, Wei H, Zheng C, Chen B, Qiu X. The prevalence and molecular characterization of (δβ) 0-thalassemia and hereditary persistence of fetal hemoglobin in the

Chinese Zhuang population. Journal of clinical laboratory analysis. 018 Mar;32(3):e22304.
https://thealevelbiologist.co.uk/legacy-topics/haemoglobin/)
10. Champe PC, Harvey RA, Ferrier DR. Biochemistry. Lippincott Williams & Wilkins; 2005.
11. Hardison RC. Evolution of hemoglobin and its genes. Cold Spring Harbor perspectives in medicine. 2012 Dec 1;2(12):a011627.
http://sickle.bwh.harvard.edu/hbsynthesis.html
12. Saito S, Osuka A. Expanded porphyrins: intriguing structures, electronic properties, and reactivities. Angewandte Chemie International Edition. 2011 May 2;50(19):4342-73.
13. Patil N N. Haemoglobin. 2011.
http://dspace.vpmthane.org:8080/jspui/bitstream/123456789/2001/1/Haemoglobin.pdf
14. Murray RK. Porphyrins & Bile Pigments 32. a LANGE medical book. 2003:270.17.
https://www.meded.virginia.edu/courses/path/innes/nh/globin.cfm
15. http://sickle.bwh.harvard.edu/hbsynthesis.html
16. Perutz MF. Mechanisms regulating the reactions of human hemoglobin with oxygen and carbon monoxide. Annual Review of Physiology. 1990. Mar;52(1):1-26.
17. Rudolph AM. Oxygenation in the fetus and neonate—a perspective. In Seminars in perinatology 1984 Jul 1 (Vol. 8, No. 3, pp. 158-167). Elsevier.
https://issuu.com/dr.hilali/docs/hb_synthesis
https://www.pinterest.com/pin/359373245244635091/