

بسم الله الرحمن الرحيم

: قال تعالى

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا {
أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا}

(الإسراء : ٨٥)

Dedication

To those who are bright as the moon, their hearts shine
With light of million heavily stars and who are always
encouraging in many pursuit

My parents

*

*

To my lovely brothers & sisters

To my friends in study and working life

To supervisor who guidance made such progression in my
life

For All patients in the hope of treating the sickle cell
haemoglobinopathies

Acknowledgment

Praise to Allah the almighty who gave us the health and strength.

With great pleasure and thanks to my supervisor **Dr.Mohi Eldein Abass** for his continuous supervision, guidance and useful advises.

With especially grateful to all members of Haematology department in Sudan University of Science and Technology who dedicated their precious time to give me valuable assistance and guidance throughout the whole work and thanks to the staff of Central lab. of Military Hospital for any help provided, also my thanks to Dr.Sana Eltahir whose help me to throughout put this layout.

Lastly gratitude goes to my family

Abstract

This descriptive case control study was conducted to determine the types, patterns and level by percent (%) of the haemoglobins among Sudanese patients with sickle cell haemoglobinopatheis through the period of March 2012 up to May 2012, using Capillary Electrophoresis technology. The total number of study group was one hundred, seventy patients group and thirty as control group. 51.4% of patients group were female while 48.6% were male. The study reveals the distribution rate of sicklling cell haemoglobinopatheis among all age groups.

The study results shows that the frequency of AS patterns were 52.9%, AS/C patterns were 1.4%, S/BThalassemia patterns were 11.4%, SC patterns were 1.4%, SD patterns were 4.3% and SS patterns were 28.6%. However, all patients with sickle cell haemoglobinopatheis might have the abnormal form of haemoglobin (HbS) even in small amount. Hence, the amount of HbS (Mean) show 39.23% of AS pattern, 42.8% of ASC pattern, 47.5% of SC pattern, 11.64% of S/ β Thalassemia, 56.3% of SD pattern and 84.1% of SS pattern.

The study also reveals that the mean of haemoglobin A pattern in patients group were statistically significant lower than means of control group (P value < 0.05).

The study also explain the rate effect of HbF on the type of treatment, if the mean was 13.62 % and it is statistically significant higher than means of control group (P. value > 0.05).

In sickle cell haemoglobinopatheis (with exception S/ β Thalassemia) non significant differences of haemoglobin A2 in comparison with control group (P. value > 0.05), while Hb F and Hb S show significant elevation respectively in comparison with control group (P. value < 0.05).

ملخص البحث

أجريت هذه الدراسة التحليلية الوصفية لتحديد أنواع وأنماط وكمية خضاب الدم بالنسبة المئوية عند المرضى السودانيين المصابين بخضاب الدم المنجلي المعتل في الفترة ما بين مارس 2012 حتى مايو 2012 باستخدام تقنية الرحلان الشعيري الكهربائي. مجموع عدد الحالات في الدراسة مائة ، سبعةون مجموعة المصابين و ثلاثون يمثلون المجموعة الضابطة. مثلت الاناث نسبة 51,4% من المجموعة المصابة بينما مثل الذكور 48.6%. كشفت الدراسة ان معدل الاصابة بخضاب الدم المنجلي المعتل يشمل جميع الفئات العمرية

أظهرت نتائج الدراسة ان تكرار نمط الخضاب ذو الرحلان الشعيري S/ β -hT النمط ، (1.4%) C\SA والنمط ، (52.9%) SA الكهربائي SS والنمط (4.3%) DS النمط ، (1.4%) CS والنمط ، (11.4%) كما أظهرت ان جميع المرضى المصابين بخضاب الدم . (28.6%) و ان كان بكمية S المنجلي المعتل لديهم الخضاب ذو الرحلان الكهربائي SA في المجموعة ذات النمط S بسيطة، وبالتالي كان متوسط النمط في مجموعة 47.5% ، C\SA في مجموعة النمط 42.8% ، 39.23%

في 56.3% ، β -hT في مجموعة النمط 11.64% ، CS النمط SS. و 84.1% في مجموعة النمط DS مجموعة النمط كذلك أوضحت الدراسة انخفاض معدل متوسط الخضاب ذو الرحلان في المجموعة المصابة مقارنة بالمتوسط المتحصل عليه من A الكهربائي المجموعة الضابطة ، ووجد أنه أقل بفرق احصائي ذو دلالة معنوية (القيمة المعنوية < 0.05).

في F كذلك أوضحت الدراسة أثر زيادة الخضاب ذو الرحلان الكهربائي المجموعة المصابة والذي اذا كان متوسطه 13,6% بالمقارنة مع الرحلان الكهربائي عند المجموعة الضابطة والفرق ذو دلالة احصائية معنوية ((القيمة المعنوية < 0.05).

في مرضى فقر الدم المنجلي المتجانس (باستثناء فقر الدم الثلاسيميا) و A ووجد أنه لا اختلاف بين متوسط الرحلان الكهربائي لخضاب الدم نوع 2 متوسطه في المجموعة الضابطة ، بينما يوجد فرق احصائي بين متوسط حيث كانا مرتفعان في S و النوع F الرحلان الكهربائي لخضاب الدم نو (مرض فقر الدم المنجلي المعتل (القيمة المعنوية < 0.05).

Contents

	Page No.
الآية	I
Dedication	II
Acknowledgment	III
Abstract	IV
ملخص البحث	V
<i>Contents</i>	VI
<i>List of Tables</i>	XI
<i>List of Figures</i>	XIII
<i>List of Abbreviation</i>	XV

Chapter One Introduction and literature review	
1.1 Introduction	1
1.2 Literature review	3
1.2.1 Haemoglobin	3
1.2.2 Components of Haemoglobin	3
1.2.2.1 Globin chain	3
1.2.2.2 Protoporphyrin IX and Iron	4
1.2.2.3 2,3Dibphosphoglycerate	4
1.2.3 Heme synthesis and haemoglobin structure	5
1.2.3.1 Heme structure	7
1.2.3.2 Formation of protoporphyrin IX	8
1.2.3.3 Three dimensional structure	9
1.2.3.4 Structural relations of globin, 2,3-DPG and heme	10
1.2.3.5 Nonoxygenated haemoglobin structure : Tense (T) form	10
1.2.3.6 Oxygenated haemoglobin structure: Relaxed "R" Form	10
1.2.4 Genetic coding for globin chain	11
1.2.4.1 Globin chain production	11
1.2.4.2 Globin chain structure	11
1.2.5 Assembly of the haemoglobin molecule	12
1.2.6 Locations of Haemoglobin during function and degradation	12
1.2.7 Physiologic characteristic of haemoglobin: haemoglobin function	13
1.2.8 Factors affecting haemoglobin affinity for oxygen	14
1.2.9 Breakdown of haemoglobin	15
1.2.10 Types of haemoglobin in humans	15
1.2.10.1 Normal variants of haemoglobin	15
1.2.10.2 Variant forms that cause disease, Haemoglobinopathies	18
1.2.10.2.1 Haemoglobin H (β_4)	19
1.2.10.2.2 Haemoglobin-Barts (γ_4):	19
1.2.10.2.3 Haemoglobin-C ($\alpha_2\beta C_2$)	19
1.2.10.2.4 Haemoglobin-E ($\alpha_2\beta E_2$)	20
1.2.10.2.5 Haemoglobin-D	21
1.2.10.2.6 Sick cell Haemoglobinopatheis	21

<i>1.2.10.2.6.1 Nomenclature of sickle cell Haemoglobinopatheis</i>	22
<i>1.2.10.2.6.2 Sickle cell disease</i>	23
<i>1.2.10.2.6.3 Prevalence and Geographic distribution of sickle mutation</i>	23
<i>1.2.10.2.6.4 Molecular basis of sickling</i>	25
<i>1.2.10.2.6.5 The effect of mutated HbS on erythrocytes</i>	27
<i>1.2.10.2.6.6 Variants of sickle cell syndromes</i>	29
<i>1.2.10.2.6.6.1 Sickle cell disease</i>	30
<i>1.2.10.2.6.6.2 Sickle cell trait</i>	30
<i>1.2.10.2.6.6.3 HbSC disease</i>	30
<i>1.2.10.2.6.6.4 Sickle cell-β-thalassaemia</i>	31
<i>1.2.10.2.6.6.5 Sickle cell anaemia with coexistent α-thalassaemia</i>	31
<i>1.2.10.2.6.6.6 Sickle cell-HPFH</i>	32
<i>1.2.10.2.6.6.7 Sickle cell-Hb Lepore disease</i>	32
<i>1.2.10.2.6.6.8 Sickle cell-HbD disease</i>	32
<i>1.2.10.2.6.6.9 Sickle cell-HbO Arab disease</i>	32
<i>1.2.10.2.6.6.10 Sickle cell-HbE disease</i>	33
<i>1.2.10.2.6.7 The inheritance of sickle cell anemia's</i>	33
<i>1.2.10.2.6.8 Pathophysiology of sickle cell disease</i>	34
<i>1.2.10.2.6.9 Clinical manifestations of sickle cell haemoglobinopatheis</i>	35
<i>1.2.10.2.6.10 Laboratory Diagnosis of sickle cell hemogolobinoptheis</i>	36
<i>1.2.10.2.6.10.1 Peripheral blood findings</i>	36
<i>1.2.10.2.6.10.2 Other laboratory tests</i>	36
<i>1.2.10.2.6.10.3 Haemoglobin electrophoresis</i>	37
<i>1.2.10.2.6.10.3.1 Gel electrophoresis</i>	37
<i>1.2.10.2.6.10.3.2 Capillary electrophoresis</i>	38
<i>1.2.10.2.6.10.4 Other tests to detect sickle haemoglobin</i>	40
<i>1.2.10.2.6.11.2 Sickle cell anemia treatment & management</i>	41
<i>1.2.10.2.6.11.3 Transfusion therapy</i>	41
<i>1.2.10.2.6.11.4 Hydroxyurea</i>	42
<i>1.2.10.2.6.11.5 Bone marrow transplantation</i>	43
<i>1.2.10.2.6.11.6 Gene therapy</i>	43

<i>1.2.10.2.6.12 Previous studies</i>	44
<i>1.2.10.2.6.12.1 Clinical capillary electrophoresis</i>	44
<i>1.2.10.2.6.12.2 Capillary Zone Electrophoresis for the Diagnosis of Congenital Haemoglobinopathies</i>	44
<i>1.2.10.2.6.12.3 Multicenter validation of fully automated capillary electrophoresis method for diagnosis of Thalassemia and Haemoglobinopathies in Thailand</i>	45
<i>1.2.10.2.6.12.4 The place of capillary electrophoresis techniques in screening for haemoglobinopathies</i>	46
<i>1.3 Rationale</i>	48
<i>1.4 Objectives</i>	49
<i>1.4.1 General Objective</i>	49
<i>1.4.2 Specific Objectives</i>	49
Chapter Two Materials and Methods	
<i>2.1 Study design</i>	50
<i>2.2 Study area</i>	50
<i>2.3 Study population</i>	50
<i>2.4 Sampling</i>	50
<i>2.5 Sample size</i>	50
<i>2.6 Tools of data collection</i>	50
<i>2.7 Inclusion criteria</i>	50
<i>2.8 Exclusion criteria</i>	51
<i>2.9 Ethical consideration</i>	51
<i>2.10 Safety assurance</i>	51
<i>2.11 Sample collection</i>	51
<i>2.12 Method of sample collection</i>	51
<i>2.13 Sick cell slide test</i>	51
<i>2.14 Capillary electrophoresis</i>	53
<i>2.15 Statistical analysis</i>	56
Chapter Three Results	
<i>3. Results</i>	57
<i>3.1 General overview</i>	57
<i>3.2 Results presentation</i>	57
<i>3.2.1 Demographic data and clinical history of study participants</i>	57
<i>3.2.2 The results (Mean, Range and SD) of</i>	59

<i>haemoglobin pattern in case group comparing with control group</i>	
<i>3.2.3 Frequency of the different types of Hb among patients group</i>	65
<i>3.2.4 Comparison of the HbF level with Hydroxyurea, Blood transfusion therapy among patients group</i>	66
Chapter Four	
Discussion, Conclusion and Recommendations	
<i>4.1 Discussion</i>	68
<i>4.2 Conclusion</i>	71
<i>4.3 Recommendations</i>	72
<i>References</i>	73
<i>Appendix</i>	79

List of Tables

No.	Title	Page
Table (1-1)	<i>Globin chains in haemoglobin</i>	4
Table (1-2)	<i>Normal haemoglobin in adult blood</i>	10
Table (1-3)	<i>Effects of various factors on Oxyhaemoglobin Dissociation curve</i>	14
Table (1-4)	<i>Normal Human Haemoglobin variants</i>	17
Table (1-5)	<i>The common abnormal haemoglobin</i>	21
Table (1-6)	<i>Areas of high prevalence of sickle mutation</i>	25
Table (1-7)	<i>Hb electrophoresis for the sickling syndromes</i>	29
Table (3-1)	<i>The demographic characteristics of patient group</i>	57

Table (3-2)	<i>The demographic characteristics of control group</i>	57
Table (3-3)	<i>Haemoglobin A, Hb A2, Hb F and Hb S level of AS patients group compared with normal control group</i>	59
Table (3-4)	<i>Haemoglobin A, Hb A2, Hb F, Hb S and Hb C level of AS/C patients group compared with normal control group</i>	60
Table (3-5)	<i>Haemoglobin A, Hb A2, Hb F, Hb S and Hb C level of SC patients group compared with normal control group</i>	61
Table (3-6)	<i>Haemoglobin A, Hb A2, Hb F and Hb S level of S/βThalassemia patients group compared with normal control group</i>	62
Table (3-7)	<i>Haemoglobin A, Hb A2, Hb F, Hb S and Hb D level of SD patients group compared with normal control group</i>	63
Table (3-8)	<i>Haemoglobin A, Hb A2, Hb F and Hb S level of SS patients group compared with normal control group</i>	64

List of Figures

No.	Title	Page
Figure (1.1)	<i>Heme synthesis pathway</i>	5
Figure (1.2)	<i>Heamoglobin synthesis in the developing red cell</i>	6
Figure (1.3)	<i>Structure of Heme and Haemoglobin</i>	8
Figure (1.4)	<i>Structure of Hb molecule illustrate the nonoxygenated state “Tense form” form or (T) and “Relax form” or (R).</i>	9
Figure (1.5)	<i>Human globin gene arrangement on chromosomes 11 and 16</i>	11
Figure (1.6)	<i>Effect of temperature and pH on the oxygen-haemoglobin dissociation curve.</i>	14
Figure (1.7)	<i>Gene expression of haemoglobin before and after birth. Also identifies the types of cells and organs in which the gene expression.</i>	18
Figure (1.8)	<i>Normal and mutated HbS sequences</i>	26
Figure (1.9)	<i>Induction of normal red cell to be sickling</i>	26

Figure (1.10)	<i>How sickle cell genes are inherited.</i>	34
Figure (1.11)	<i>Separation of haemoglobins by electrophoresis at alkaline pH.</i>	38
Figure (1.12)	<i>Diagram of capillary electrophoresis system</i>	39
Figure (1.13)	<i>Depiction of the interior of a fused-silica gel capillary in the presence of a buffer solution.</i>	40
Figure (3.1)	<i>Age distribution in study group</i>	58
Figure (3.2)	<i>The frequency of variants sickling haemoglobinopathies among patient group</i>	65
Figure (3.3)	<i>Subscribed therapy among patient group</i>	66
Figure (3.4)	HbF% in comparison with subscribed therapy Blood transfusion and Hydroxyurea among patients group	67

List of Abbreviations

δ-ALA	δ-aminolevulinic acid
BCSH	British Committee for Standards in Haematology
BMT	Bone Marrow Transplantation
CE	Capillary Electrophoresis
CZE	Capillary Zone Electrophoresis
CO ₂	Carbon dioxide
CA	Carbonic Anhydrase
CoA	Coenzyme A
Cl ⁻	Chloride ions
CT	Computerized Tomography
DNA	Deoxyneuclic Acid
2,3-DPG	2,3Dibhosphoglycerate
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic acid
EMS	Emergency Medical Services
EOF	ElectroosmoticFlow
FEP	Free Erythrocyte Protoporphyrin
FDA	Food and Drug Administration
Hb	Haemoglobin
HPLC	High-Performance Liquid Chromatography
HPCEC	High-Performance Cation-Exchange Chromatography
HLA	Human Leukocyte Antigen

H ⁺	Hydrogen
HU	Hydroxyurea
HPFH	Hereditary Persistence of Fetal Haemoglobin
IgG	Immunoglobulin G
IRDS	Infant Respiratory Distress Syndrome
ISCs	Irreversibly Sickled Cells
IEF	Iso-electro Focusing
Kg	Kilogram
MCH	Mean Concentration of Haemoglobin
MCHC	Mean Cell Haemoglobin Content
MCV	Mean Cell Volume
MECC	Micellar Electrokinetic Capillary Chromatography
mg	milligram
MRI	Magnetic Resonance Imaging
mRNA	Massenger Riponuclic acid
NO	Nitric Oxide
NRBCs	Nucleated Red Cells
O ₂	Oxygen
pH	Power of Hydrogen
RES	Reticuloendothelial System
SC*	Sickle cell Hb C disease with Hb A
SC	Sickle cell Hb C disease
SD	Sickle cell Hb D disease
SCD	Sickle Cell Disease
US	United State
VCAM-1	Vascular Cell Adhesion Molecule-1