

Verse: -

:-

:

أَلَمْ يَكُنْ نُطْفَةً مِّن مَّنِيِّ يَمْنَىٰ ﴿٣٧﴾ ثُمَّ كَانَ عَلَقَةً فَخَلَقَ فَسَوَّىٰ ﴿٣٨﴾ فَجَعَلَ مِنْهُ
الزَّوْجَيْنِ الذَّكَرَ وَالْأُنثَىٰ ﴿٣٩﴾ أَلَيْسَ ذَلِكَ بِقَدْرِ عَلَىٰ أَنْ يُحْيِيَ الْمَوْتَىٰ ﴿٤٠﴾

Dedication



Acknowledgement

All my gratitude and respect to Dr. Tariq Elfatih Elmisbah for his supervision, time, patience, effort, critical comments and discussion concerning my research study.

My thanks extended to the all Heglig hospital members and laboratory Colleques of Jafar Ibnouf Pediatrics hospital, sickle cell anemia teaching unit employees for their assistance in collection of samples from the patients.

My sincere thanks to all individuals whom agreed to participate in this study patients and controls to achieve the objectives.

I also acknowledged the assistance of all authors listed in the reference list for the literature that I have reviewed.

My sincere gratitude is extended to my all friends, colleagues and relatives whom assist me in one way or another, especially Dr. Mardi Ibrahim, Dr. Sami, Dr. Ismail, Dr. Ahmed Elias, and Mr. Ahmed Mohamed, Mr. Mohamed Eljamriy, Magzoup and Yousif Elzubaier.

I would be remiss without mentioning whom I called “The Candles”, Nizar Abdelhafiez, and Elmonzir Mohamed Ali whom extreme generosity will be remembered always.

I am indebted to my family member for their patience, encouragement and moral support help and facilities during this study.

I would like to thank all those offered me their assistance, and help me to success this study in whatever needed that without missing any one, to all of the above, I extend my deepest appreciation.

My prays and thanks should be first and last to God (ALLA) the almighty most gracious and most merciful who enabled me to conduct the study by the grace of him and giving me the confidence and grated me the serenity, means of strength and patience to accomplish this work.

Abstract:

This is an observational analytical case control study conducted to compare haemoglobin F levels in pediatric patients of sickle cell anaemia SCA, either treated with Hydroxyurea (HU) or treated with conventional treatments. Three hundred twelve individuals were selected and informed about the study objectives and their agreement for participation was obtained by using of a study questionnaire, this was performed in the period between May-2009 to June-2010. Sixty one SCA patients had been treated with HU were called group **A₁** and thirty six SCA patients had been treated with conventional managements but not with HU called group **A₂** those were visiting Jafar Ibnouf Pediatric Hospital in Khartoum state, all were compared to 50 healthy individuals selected randomly to be matched in age, sex and tribe as control called group **B**. The study also included SCA patients at Heglig hospital in Heglig area (the petroleum filed) Western Sudan, seventy three SCA patients had been treated with conventional managements were resident inside the petroleum filed called group **C₁** and forty two SCA patients had been treated with conventional managements from outside the petroleum filed called group **C₂**, all are compared to twenty five healthy individuals as control from inside the petroleum filed and twenty five from outside called group (**D₁** and **D₂**) respectively. 2.5 ml of blood was collected from each individual into anticoagulated container. Hb F levels determined by used of Denaturation method, complete blood count CBC done by (Sysmex KX 21N) analyzer. Statistical package for social sciences (SPSS) program version 13 was used for data processing. The mean \pm Standard deviation (Mean \pm SD) for the patient's age was (6.1 \pm 3.1), and the mod was 3.0 years. Fifty two % of patients were males and the rest were females. The highest frequencies of patients were 121 found in Meseria tribe 57.1%. HbF levels in group **A₁**, **C₁** (8.0 \pm 2.6), (4.7 \pm 2.0) percent of the total Hb respectively, this were found to be raised significantly when compared by group **A₂** (0.8 \pm 0.2) % and **C₂** (0.6 \pm 0.1) % with significant probability value (p=0.000) in each groups. The CBC parameters and cell counts showed some differences in groups **A₁** and **C₁** in compared with **A₂** and **C₂** respectively. Total white blood cells (TWBCs) lower in group **A₁** and **C₁**. Red blood cells (RBCs) and haemoglobin (Hb) increased significantly in group **A₁** but no statistical changes in group **C₁**. Packed cell volume (PCV), mean cell volume, (MCV) and mean cell haemoglobin (MCH) showed higher values in group **A₁** and **C₁**. No significant change was seen in mean cell haemoglobin concentration (MCHC) in group **A₁**, but was reduced in **C₁**. Platelets and reticulocytes showed significantly reduced level in group **A₁** and **C₁**. RBCs morphology showed no statistical changes in group **A₁** and **C₁**, When compared to **A₂** and **C₂** of (p=0.325) and (p=0.878) respectively.

So, the study concluded that Hb F levels were raised significantly in SCA patient treated with HU compared to the conventionally treated patients and the drug can be used successfully in Sudan. Also as an important observation, patients resident inside the petroleum filed showed significantly higher percentage of Hb F, though less than the HU treated group, but opened an important new avenue for sickle cell anemia researches. In addition patients inside the petroleum field showed similar behavior to the HU treated group of significantly reduced Hospitalization and blood transfusion requirements, and showed low frequencies of crises onset.

أجريت هذه الدراسة الترقية التحليلية، التحكيمية في ٣١٢ شخص تم اعلامهم عن الدراسة واخذت مولفقتهم علي المشاركة بولسطة إستبيان , خلال الفترة من مايو ٢٠٠٩م وحتى يونيو ٢٠١٠م , بغترق ——— مدير مستوياتهم ——— و قلوبين ف في ——— مرضى فقر الدم المنجلي المعالجين وغير المعالجين و ٣٦ (A₁) بع قار الهيدروكسيوريا, منهم ٦١ مريض يعالجون بواسطة ع قار الهيدروكسيوريا تمت تسميتهم بالمجموعة في مستشفى جعفر ابنعوف لطب (A₂) مريض يعالجون بواسطة العلاجات التقليدية تمت تسميتهم بالمجموعة الاطفال بولاية الخرطوم, قورنت نتائجهم بمجموعة من الاصحاء عددهم ٥٠ تم اختيارهم عشوائيا ليتناسبوا في كما شملت الدراسة مجموعة من مرضى (B) العمر والجنس والقبيلة مع المرضي تمت تسميتهم بالمجموعة فقر الدم المنجلي يعالجون بواسطة العلاجات التقليدية بمستشفى هجليج داخل حقل البترول بمنطقة هجليج في و ٤٢ من خارج الحقل, (C₁) غرب السودان منهم ٧٣ يقطنون داخل حقل البترول تمت تسميتهم بالمجموعة قورنوا بمجموعة من الاصحاء ٢٥ من داخل الحقل و ٢٥ من خارج الحقل, (C₂) تمت تسميتهم بالمجموعة على التوالي, جمعت عينات الدم ٥.٢ مل في وعاء مانع للتجلط, تم (D₂) و (D₁) تمت تسميتهم بالمجموعة تقدير مستويات هيموكلوبين اف بطريقة استخلاص النيتروجين, تم تعداد الدم الكامل باستخدام جهاز و تم استخدام برنامج الحزم الإحصائية للعلوم الاجتماعية نسخة ——— ١٣ للتحليل (KX 21N) (سيسميكس الإحصائي), كان المستوى الوسطي ± الانحراف المعياري لأعمار المرضي المشاركين بالدراسة ١٦ ± ١٣ وكان العمر الشايع ٠.٣ سنوات وجد ان ٥٢% من المرضي كانوا ذكور و ٤٨% كانوا إناث, أعلى تردد لا قبائل في مجموعة المرضي كان ١٢١ من قبيلة المسيرية بنسبة ١٥٧%. وجدت زيادة احصائية في مستوي الهيموكلوبين ف في % ٠.٢ ± ٧.٤ C₁ وفي المجموعة % ٠.٨.٢ ± ٦ A₂ عند مقارنتها بمجموعة % ٠.٨.٢ ± ٦ A₁ المجموعة في كل من المجموعتين على التوالي. كما (p=١٠% بدلالة إحصائية) ٠.٠ ± ٦.٠ C₂ عند مقارنتها بالمجموعة عند مقارنتهم A₁ و C₁ وجدت ان هنالك تأثيرات احصائية في نتائج فحص الدم الكامل في المجموعتين ارتفع عدد كريات الدم, A₁ و C₁ علي التوالي, بحيث قل تعداد الدم الابيض في كل من C₂ و A₂ بالمجموعتين ارتفع حجم كريات, C₁ بينما لم يظهر فرق احصائي في المجموعة A₁ الحمراء وكمية الهيموكلوبين في المجموعة لم, A₁ و C₁ الدم المضغوطة و متوسط حجم الخلية الحمراء ومتوسط هيموكلوبين الخلية في كل من المجموعتين, (C₁) بينما ظهر تأثير احصائي بالنقصان عند المجموعة A₁ يتأثر متوسط تركيز هيموكلوبين الخلية في المجموعة عند C₁ و A₁ قل تعداد الصفائح الدموية و ظهر نقصان إحصائي في الخلايا الشبكية في كل من المجموعتين عند C₁ و A₁ لم تكن هناك فروق إحصائية في الشكل الظاهري لخلايا الدم الحمراء في المجموعة . علي التوالي (p=٠.٨٧) و (p=٠.٢٢) A₂ و C₂ مقارنتهما بالمجموعتين وعليه خلصت الدراسة علي ان مستوي هيموكلوبين مرتفع بصورة معنوية في المرضي المعالجين . بالهيدروكسيوريا والمرضى القاطنين داخل حقل البترول .

المرضى المعالجين بالهيدروكسيوريا والمرضى داخل حقل البترول اظهروا نتائج احصائية بنقصان فترة الرقاد بالمستشفى ونقصان الاحتياج لعمليات نقل الدم, كما قلت مضاعفات وحدة المرض.

List of abbreviations

A	All sickle cell anaemia patients in Khartoum
A ₁	Sickle cell anaemia patients treated with Hydroxyurea in Khartoum
A ₂	Sickle cell anaemia patients untreated with Hydroxyurea in Khartoum
ALL	Axis Loss Light
ANC	Absolute Neutrophils Count
AST	Aspartate transaminase
AVN	A vascular necrosis
B	All Control healthy subjects in Khartoum
C	All sickle cell anaemia patients in Heglig area
C ₁	Sickle cell anaemia patients inside the petroleum field
C ₂	Sickle cell anaemia patients outside the petroleum field
CO ₂	Carbon dioxide gas
CBC	Complete blood count
CT	Computed Tomography
D	All control healthy subjects in Heglig area
D ₁	Control healthy subjects in Heglig area inside the petroleum field
D ₂	Control healthy subjects in Heglig area outside the petroleum field
DPG	Diphosphoglycerate
E/G	Erythropoiesis: Granulopoiesis ratio
DNA	Deoxyribonucleic acid
EDTA	Ethylene-Diamine-Tetra-Acetic acid
G-6-PD	Gglucose-6-phosphate Dehydrogenase
Hb	Haemoglobin
Hb A	Haemoglobin A
Hb C	Haemoglobin C
Hb D	Haemoglobin D
Hb E	Haemoglobin E
Hb F	Haemoglobin F
Hb S	Haemoglobin S
HCT	Haematocrit
HiCN	Haemiglobincyanide
HPFH	Hereditary Persistence of Fetal Haemoglobin
HPLC	High performance Liquid Chromatography
HU	Hydroxyurea
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISCs	Irreversibly Sickled Cells
LDH	Lactate Dehydrogenase Enzyme
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume

Mhb	Methemoglobin
NRBC	Nucleated Red Blood Cell
NO	Nitric Oxid Gass
O ₂	Oxygen
ODCs	Oxygen Dissociation Curves
PCV	Packed Cell Volume
PDQ	Pediatrics Departments Quality control
P 50	Partial pressure of oxygen at which hemoglobin is 50% saturated with oxygen
PO ₂	Partial Oxygen
PLT	Platelet
RBCs	Red Blood Cells.
RDW	Red blood cell Distribution Width
RNA	Ribonucleic acid
SCD	Sickle Cell Disease
SCP	Sickle Cell Patient
SD	Stander Deviations
SPSS	Statistical Package for Social Sciences
TCD	Trans-Cranial Doppler
TEB	Tris/EDTA/Borate (Buffer)
TRCA	Transient Red Cell Aplasia
TRBCsC	Total Red Blood Cells Count.
WBC	White Blood Cell.
2,3-BPG	2,3-Bisphosphoglycerate
2,3-DPG	2,3-Diphosphoglycerate

List of Tables

No	Title	Page
Table (1.1)	Structural defects that produce either a change in hemoglobin-oxygen affinity or a physically unstable molecule.	7
Table (1.2)	Normal CBC values.	10
Table (1.3)	Classification of anemia based on red blood cell indices.	12
Table (1.4)	The classification of anemia's based on morphology (erythrocyte size).	12
Table (1.5)	Pathophysiological classification of anemia	16
Table (1.6)	The common Haemoglobinopathies.	20
Table (1.7)	The common abnormal hemoglobin.	21
Table (1.8)	The prevalence of Haemoglobinopathies and thalassemia among Blacks.	25
Table (1.9)	The Areas of high prevalence of sickle mutation.	31
Table (1.10)	The Sickling syndromes.	32
Table (1.11)	The common abnormal hemoglobin.	40
Table (1.12)	The Symptomatology and Clinical Manifestations of Vaso-occlusive Crises	44
Table (1.13)	The differential diagnosis in sickle cell syndromes.	49
Table (1.14)	The presenting symptoms of acute chest syndrome. Major Pain Syndromes in Patients with Sickle Cell Disease.	51
Table (1.15)	Hydroxyurea Therapy in Sickle Cell Anemia.	59
Table (3.1)	Show the classifications and definitions of study groups.	79
Table (3.2)	Show the (Mean \pm SD) participator ages of study groups.	79
Table (3.3)	Show gender frequencies of study groups.	79
Table (3.4)	Revealed the (Mean \pm SD) of Hb F levels among all study groups.	80
Table (3.5)	Hb F (Mean \pm SD) and p values within A1, A2 and B groups	80
Table (3.6)	Hb F (Mean \pm SD) and p values within C1, C2 and D1 & D2 groups	81
Table (3.7)	CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, Reticulocytes count and p values in A1 & B groups.	81
Table (3.8)	CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, Reticulocytes count and p values in A2 & B groups.	82
Table (3.9)	CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, Reticulocytes count and p values in A1& A2 groups.	82
Table (3.10)	3.10: CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, Reticulocytes count and p values in C1& D1 groups.	83
Table (3.11)	CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, Reticulocytes count and p values in C2& D2 groups.	83
Table (3.12)	CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, Reticulocytes count and p values in C1& C2 groups.	84
Table (3.13)	CBC (Mean \pm SD) (TWBC, Hb, RBC count, PCV, MCV, MCH, MCHC, platelet count, and Reticulocytes count) and p values in D1& D2 groups.	84
Table (3.14)	Crises onset, Hospitalization period (admission days) and blood transfusion requires and the P values among test group A1, A2 and C1, C2.	85

List of Figures

No	Title	Page
Figure (1.1)	The genetic control of human hemoglobin production in embryonic, fetal and adult life (Adapted from John P. Greer, John Forester and John N. Lukens).	6
Figure (1.2)	The globin chain production and development. Fetal red blood cells contain primarily haemoglobin F ($\alpha_2\gamma_2$). Soon after birth, γ -globin chain synthesis is suppressed and β -globin and δ -globin chain production increase, which results in the appearance of hemoglobins A and A2 (Adapted from Robert S. Hillman, Kenneth A. Ault and Henry M. Rinder).	6
Figure (1.3)	Shown the Haemoglobin structure. Each haemoglobin molecule is composed of 2 α -globin (light shading) and 2 β -globin chains (dark shading), each of which contains a single haeme group in a hydrophobic pocket. A central cavity between the 2 β -globin chains houses a molecule of 2,3-DPG. This is important for the respiratory function of haemoglobin (Adapted from Robert S. Hillman, Kenneth A. Ault and Henry M. Rinder).	8
Figure (1.4)	The CBC and reticulocyte index is used to classify anemia morphologically (Adapted from Robert S. Hillman, Kenneth A. Ault and Henry M. Rinder).	14
Figure (1.5)	The Functional classification of anemia. Each of the major categories of anemia (hypoproliferative, maturation disorders, and hemorrhage/hemolysis) can be further sub classified according to the functional defect in the several components of normal erythropoiesis. (Adapted from Robert S. Hillman, Kenneth A. Ault and Henry M. Rinder).	14
Figure (1.6)	How sickle cell genes are inherited (Adapted from James V. Neel.).	23
Figure (1.7)	The geographic distribution of Hemoglobinopathies.	24
Figure (1.8)	Multifocal origin and spread of the β^S gene.	31
Figure (1.9)	The pathophysiology of sickle cell disease (Adapted from Philip Lanzkowsky).	34
Figure (1.10)	The endothelial red cell adhesion and vaso-occlusion in sickle cell disease.	39
Figure (1.11)	The clinical problems of sickle cell disease by age.	42
Figure (1.12)	Mechanisms of Action of Hydroxyurea in Sickle Cell Disease. By selectively killing cells in the bone marrow, Hydroxyurea increase the number of erythroblasts that produce hemoglobin F. It has no known direct effects on gene expression. Bone marrow cellularity may also be diminished. Higher concentrations of hemoglobin F reduce the polymerization of hemoglobin S and the numbers of deformed, dense, and damaged erythrocytes. Cells with high hemoglobin F content survive longer, attenuating hemolysis and leading to a reduction in reticulocytes counts. The numbers of circulating granulocytes, monocytes, and platelets are diminished. The likelihood of vaso-occlusion is reduced by the reduction in the number of dense, poorly deformable erythrocytes that can adhere to and perturb the endothelium.	52
Figure (1.13)	Pathophysiological Characteristics of Sickle Cell Anemia and the Effect of Hydroxyurea	53
Figure (1.14)	Structure of Hydroxyurea. The shaded area highlights the Hydroxamic acid core of Hydroxyurea. Hydroxamic acids avidly bind heavy metals and inactivate a	57

	variety of enzymes. Hydroxyurea inhibits the iron-containing enzyme ribonucleotide reductase, thereby killing cycling cells. Hydroxyurea also produces nitric oxide and stimulates the heme-iron-containing enzyme guanylate cyclase. Both activities have the potential to increase F-cell production.	
Figure (3.1)	Show the tribes frequencies of test group A, (A= SCA patients in Khartoum)	85
Figure (3.2)	Show the tribes frequencies of test group C, (C= SCA patients in Heglig)	86
Figure (3.3)	Hb F determination (Means) within study groups.	86
Figure (3.4)	Show morphology of A1 & A2 groups	87
Figure (3.5)	Show morphology of C1 & C2 groups.	87
Figure (3.6)	Circles percent among A1& A2 group	88
Figure (3.7)	Circles percent among C2 & C2 group	88

List of Content		Page
Preface	Verse آية	I
	Dedication	II
	Acknowledgement	III
	Abstract	IV
	Arabic abstract	V
	List of abbreviations	VI
	List of tables	VIII
	List of Figurers	IX
	List of contents	XI

Chapter One

No	Title	Page
1.	Introduction and literature review	2
1.1	General Introduction	2
1.1.1	Normal hemoglobin	4
1.1.2	Structure and function of haemoglobin	5
1.1.3	Structure of globin	5
1.1.4	Embryonic hemoglobin	7
1.1.5	Fetal hemoglobin	8
1.1.6	Hemoglobin in adult (Post neonatal) RBCs	8
1.1.7	Anemia	9
1.1.7.1	Classification of anemia	11
1.1.7.1.1	Morphological classification	11
1.1.7.1.1.1	Normocytic anaemia	11
1.1.7.1.1.2	Microcytic anaemia	12
1.1.7.1.1.3	Macrocytic anaemia	12
1.1.7.1.2	Etiological classification	13
1.1.7.1.2.1	Hypo proliferative anemia	13
1.1.7.1.2.2	Maturation defects	15
1.1.7.1.2.3	Increased RBCs destruction	15
1.1.7.2	Clinical features of anemia	15
1.1.7.3	Physiological adaptations in anemia	17
1.1.7.3.1	Tissue hypoxia	17
1.1.7.3.2	Increased release of oxygen from red cells	18

1.1.7.3.3	Increased blood flow	18
1.1.7.3.4	Maintenance of blood volume	18
1.1.7.3.5	Redistribution of blood flow	18
1.1.8	Haemoglobinopathies	19
1.1.8.1	Genetics and inheritance of Haemoglobinopathies	19
1.1.8.2	Geographical distribution and prevalence of Haemoglobinopathies	23
1.2	Literature Revue	26
1.2.1	General introductions to sickle cell anemia	27
1.2.1.1	History and backgrounds	27
1.2.1.2	Prevalence and geographical distribution of sickle cell anemia	29
1.2.2	Type of sickle cell anaemia	32
1.2.3	Pathophysiology of sickle cell disease	32
1.2.4	Factors that influence the severity of sickle cell disease	35
1.2.4.1	Oxygen	35
1.2.4.2	Concentration of Hemoglobin S in the Red Cell	35
1.2.4.3	Temperature	36
1.2.4.4	Presence of Other Hemoglobin in the cell	36
1.2.4.5	Deoxygenation	36
1.2.4.6	Vascular Stasis	36
1.2.4.7	Acidosis	37
1.2.4.8	Corpuscular Hemoglobin concentration	37
1.2.4.9	Blood Flow in the Microvasculature	37
1.2.4.10	Infections	38
1.2.4.11	Adhesion	38
1.2.5	Clinical Features of Sickle Cell Disease	39
1.2.6	Sickle Cell Anemia	41
1.2.7	Growth and Development	41
1.2.8	Sickle Cell Crises	42
1.2.8.1	Acute vaso-occlusive (painful) Crises	43
1.2.8.2	Acute a Plastic Crisis	44
1.2.8.3	Sequestration Crisis	45
1.2.8.4	Altered Splenic Function And Splenic Infarcts	45
1.2.8.5	Hemolytic Crisis	45
1.2.9	Complications of Sickle Cell Disease	46
1.2.10.	Hemoglobin S Syndromes	46
1.2.10.1	Sickle Cell Trait	46
1.2.12.2	Hemoglobin SC Disease	47
1.2.10.3	Sickle cell- β -Thalassemia	47

1.2.10.4	Sickle Cell Anemia with Coexistent A-thalassemia	47
1.2.10.5	Hemoglobin S/ HPFH	48
1.2.10.6	Hemoglobin SE Disease	48
1.2.10.7	Hemoglobin SD Disease	48
1.2.10.8	Hemoglobin SO-Arab disease	48
1.2.10.9	Hemoglobin S and Hb Lepore Disease	49
1.2.11	Laboratory Features of Sickle Cell Anemia	50
1.2.12	Treatments	50
1.2.13	Treatment Directed at the Prevention of Complications	52
1.2.13.1.	Hydroxyurea Managements	52
1.2.13.1.1	The Tolerated Dose of Hydroxyurea	57
1.2.13.2	Other Experimental Treatments (Nitric oxide)	58
1.3	Rationale	62
1.4	Objectives	63
1.4.1	General Objectives	63
1.4.2	Specific Objectives	63

Chapter Two

No	Title	Page
2	Materials and Methods	65
2.1	Study Design	65
2.2	Study Duration	65
2.3	Study Area	65
2.4	Study Population	65
2.4.1	Target Population	65
2.4.2	Specific Population	65
2.5	Sampling Procedure	65
2.6	Inclusion criteria	65
2.7	Exclusion Criteria	66
2.8	Sample Size	66
2.9	Data Collection Tools	66
2.10	Data Collection Procedure	66
2.11	Data Entry and Processing Analysis	66
2.12	Ethical Consideration	67
2.13	Methods	67
2.13.1	Sample Collection	67
2.13.1.1	Requirements	67

2.13.1.2	Procedure	67
2.14	Method of Automated Analyzer System (Complete Haemogram):	68
2.14.1	Requirements	68
2.14.2	Detection Principles	68
2.14.3	Procedure	69
2.15	Method of Preparation and Staining of Blood Films	70
2.15.1	Requirements	70
2.15.2	Procedure	70
2.16	Reticulocytes Count	71
2.16.1	Requirements	71
2.16.2	Principle	71
2.16.3	Procedure	71
2.17	Sickling Test	71
2.17.1	Requirements	71
2.17.2	Principle	72
2.17.3	Procedure	72
2.18	Electrophoresis Method	72
2.18.1	Requirements	72
2.18.2	Principle	73
2.18.3	Procedure	73
2.19	Hemoglobin F Estimation	74
2.19.1	Requirements	74
2.19.2	Principle	74
2.19.3	Procedure	75
2.19.3 .1	Preparation of Haemolysate	75
2.19.3 .2	Hb F Estimation Steps	75

Chapter Three

No	Title	Page
3.	The Results	77

Chapter Four

No	Title	Page
4.1	Discussion	90
4.2	Conclusions	93

4.3	Recommendations	94
-----	-----------------	----

Chapter Five

No	Title	Page
5.	References	96

Appendices

No	Title	Page
6.1	Study Questionnaire	107
6.2	Heglig Hospital (information)	108
6.3	Fuel Composition and Nitrogen Oxide (NO _x) Productions	109