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Human Fetal Hemoglobin Expression and Erythrocytic Indices in Different Groups Among U.A.E Population

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HUMAN FETAL HEMOGLOBIN EXPRESSION AND ERYTHROCYTIC INDICES IN DIFFERENT GROUPS AMONG U.A.E POPULATION

By

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ABSTRACT

Molecular analysis has revealed that a variety of hemoglobin molecules are produced in humans. All are tetramers consisting of numerous combinations of seven distinct polypeptide chains, each encoded by a separate gene.

Fetal hemoglobin is one of these tetramers that by eight weeks of gestation replace the embryonic hemoglobin forms. Fetal hemoglobin is the major hemoglobin in fetal life. It's concentration in blood decreases after birth to less than 2 per cent of the total hemoglobin by 6 months of age. However, in some cases fetal hemoglobin persist to synthesize in adult life in abnormal concentrations.

Our study concerned the fetal hemoglobin expression in four different groups (normal group, anemic group, thalassemic patients group).

The results show that fetal hemoglobin in the normal group was in the normal ranges observed in other populations, there is no big differences between males and females, cells level and fetal hemoglobin production.

In the anemic group there was more fetal hemoglobin present than in the normal group and there was no differences between males and females. even when due to genetic or environmental factors.

The fetal hemoglobin production in pregnant women is heterogenous and is still within the normal female range that was obtained in our study, this indicates that there are no sex linked genetic factors modulating the fetal hemoglobin expression.

The study shows also, that fetal hemoglobin production is heterogenous in beta thalassemic patients and there were large variations

between the heterozygous and the homozygous patients. The large differences in expression within a homogenous genetic population and, sometimes within the same family, imposes the problem of fetal hemoglobin regulation and leads us to assume that fetal hemoglobin production is under at least 2 determinants: genetic, nongenetic.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Fetal hemoglobin (Hb F) is the major hemoglobin in fetal life. It's concentration in the blood decreases after birth to less than 2 per cent of the total hemoglobin by 6 months of age. However, in some cases fetal hemoglobin continues to be synthesized in adults life.

The production of fetal hemoglobin is under the control of many factors, the majority classified as genetic and the nongenetic factors. The nongenetic factors are physiological factors (i.e. pregnancy, and rapid erythroid regeneration) and the acquired disorders. The most known as genetic factors are the hemoglobinopathies which can be divided into the structural variants (i.e. sickle cell hemoglobin) and disorders of synthesis (i.e. β thalassemias).

Since the expression of fetal hemoglobin appears to be closely related to the population and its environment, many reports showed that fetal hemoglobin is different from one population to another and in the different groups from the same population. We choose to study the expression of fetal hemoglobin in different groups of the UAE population as well as some erythrocytic indices.

Four groups were studied, the normal population (nonanemic) which was considered to be the reference group and three other special groups of population in whom it is expected to have a differences in fetal hemoglobin expression (anemic group, pregnant women group, and a group of β^+ thalassemic patients of UAE population bearing the same mutation).

PART I: REVIEW OF LITERATURE

The Hemoglobin Molecule

Hemoglobin, the oxygen transporter in vertebrate erythrocytes is a tetrameric protein, composed of two pairs of subunits (**Figure 1**). Each subunit consists of a protoheme to which oxygen binds and a globin chain made up of amino acids arranged in a definite sequence. The four chains are held together by noncovalent attraction. A tetrameric hemoglobin molecule has a molecular weight of

spherical (**Asakura and Schwartz,1990**). Although the structure of the heme group is common to all hemoglobin subunits (**Figure 2**),

chains differ in the number and sequence of

designated by Greek letters, including α (alpha),

(delta),

into two groups, those for the α-like globins which have 141 amino acids and those for the β-like globins which have 146 amino acids. The sequence of amino acids is different in each chain (**Zeringer and Harmening,1992**). In a hemoglobin molecule each α-like chain is in contact with both β-like chains. In contrast, there are few interactions between the two α-like chains or between the two β-like chains leading to a quaternary structure of hemoglobin. The structure of these chains are summarized in **figure 3** (**Stryer,1988**).

Ontogeny of Hemoglobin

The hemoglobin composition of the erythrocyte varies depending on when in gestation or postnatal

of

the α and non-α globin gene cluster (**Figure 4**) (**Steinberg and Benz,1991**). In adult life two major hemoglobins are present : Hb A ($\alpha_2 \beta_2$)

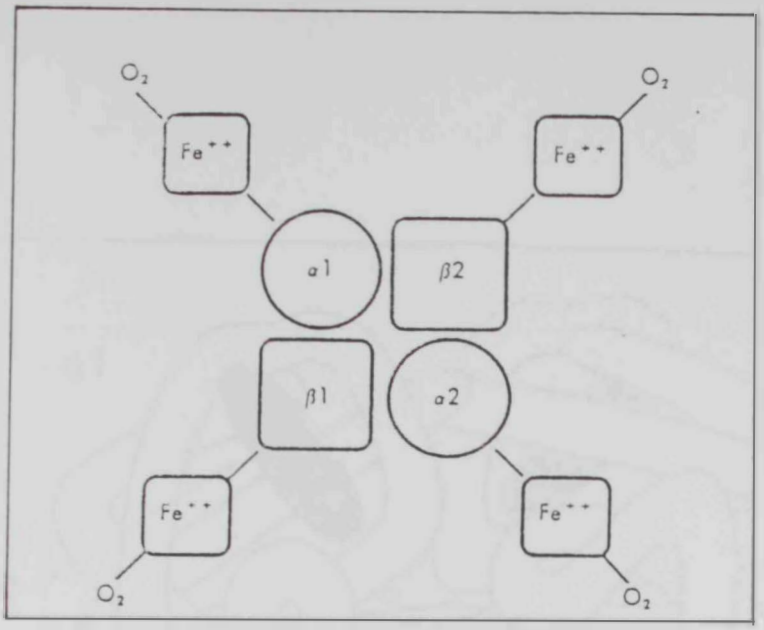

Figure 1: Schematic drawing of human hemoglobin. Hemoglobin is composed of two α - and two β-chains, with each of the subunits having a heme prosthetic group. Oxygen combines with the ferrous iron (from: Asakura and Schwartz, 1990, p: 359).

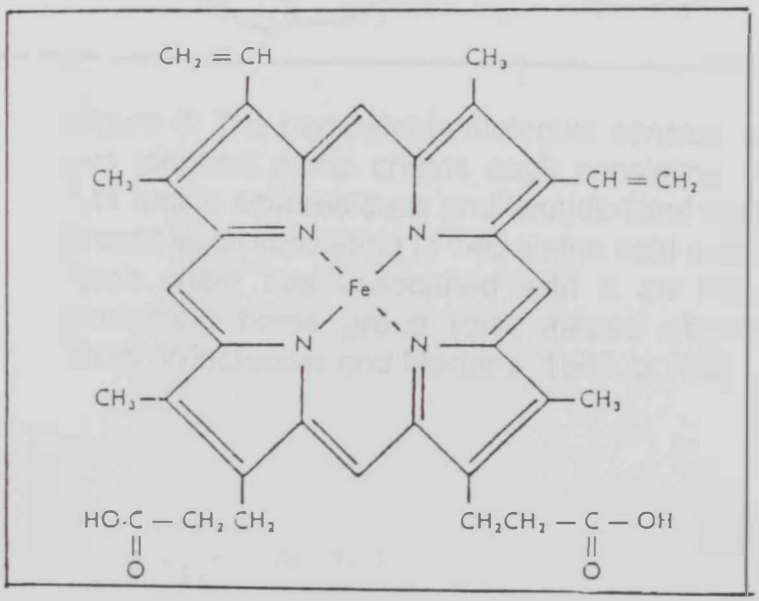

Figure 2: Structure of protoheme (from: Asakura and Schwartz, 1990, p: 359).

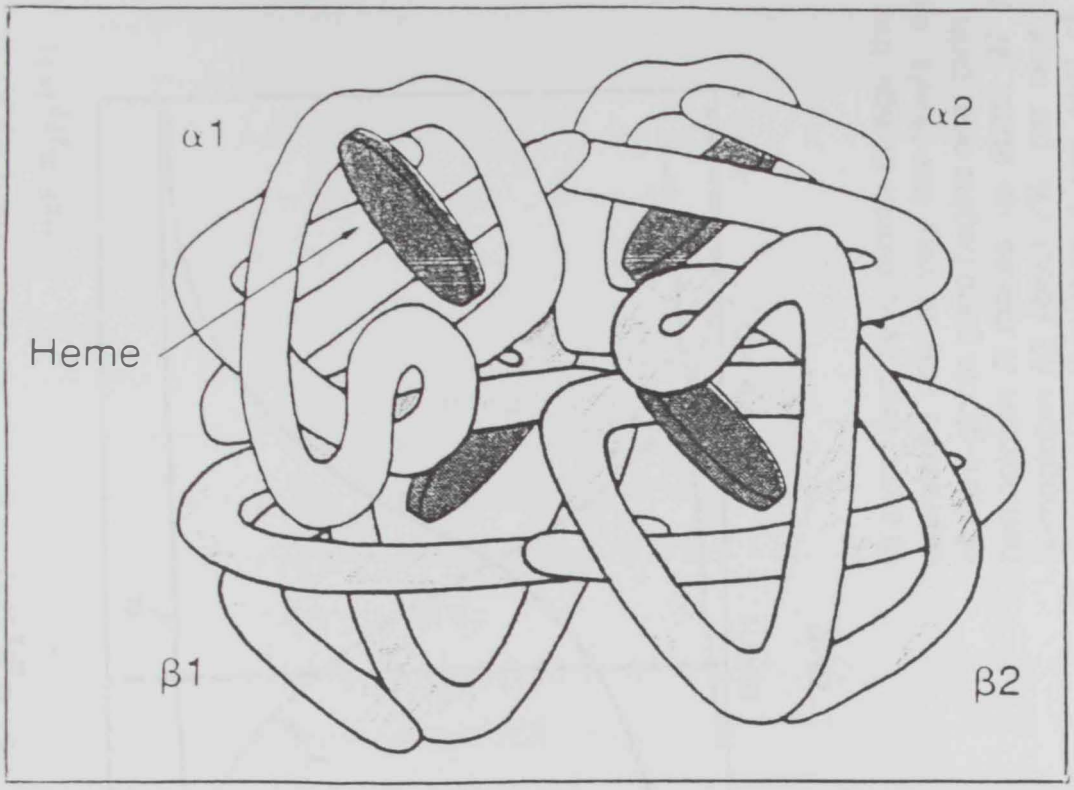

Figure 3: The hemoglobin molecule consists of two identical alpha chains each consisting of 141 amino acid residues and two identical beta chains each consisting of 146 amino acid units. Each chain has associated with it an iron-containing heme group (disc-shaped object) (from: Winchester and Mertens, 1983, p:161).

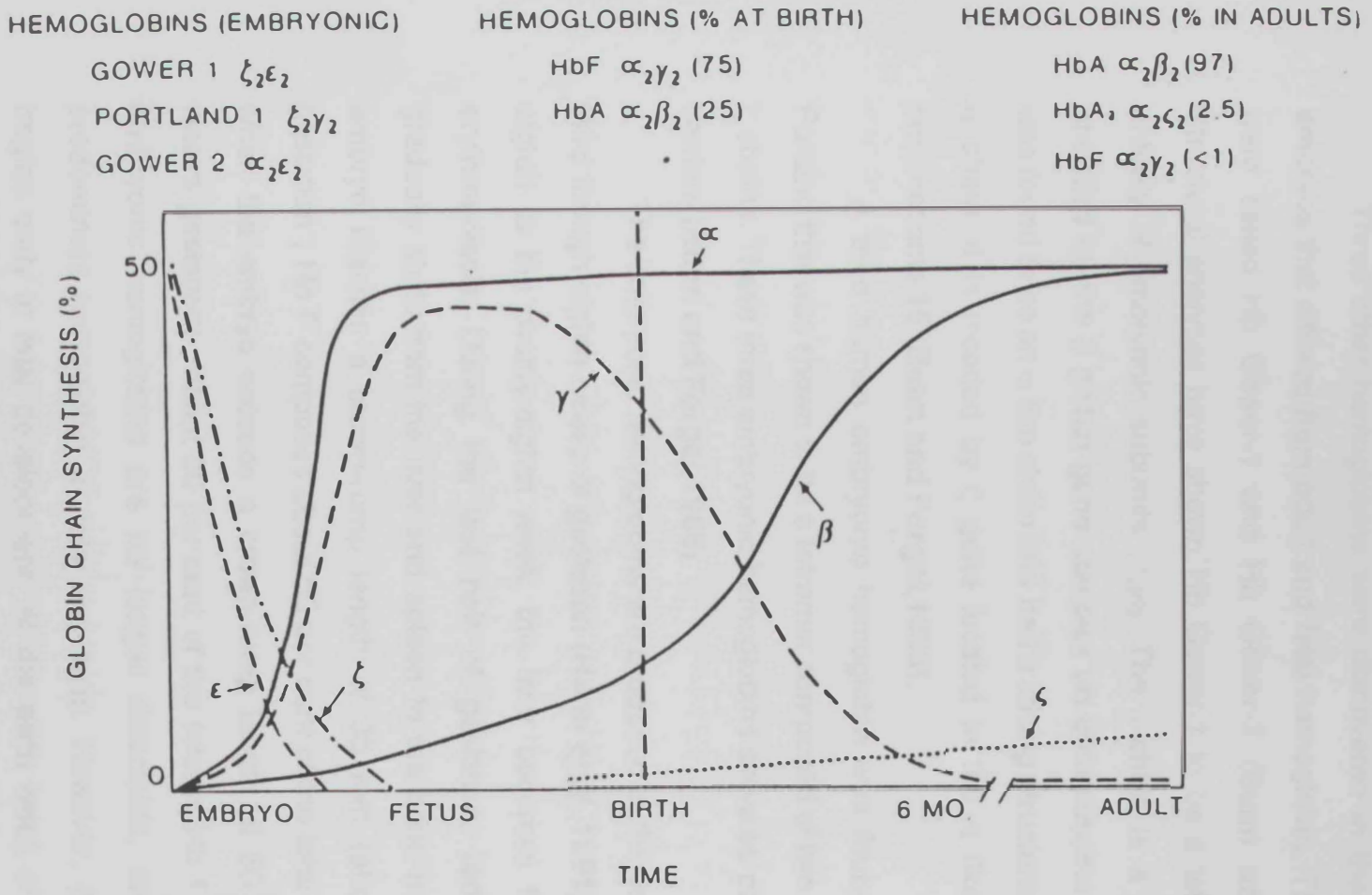

Figure 4: Hemoglobin switching during embryonic, fetal, and adult development. The ζ and ε genes are transcribed during embryonic development and soon replaced by the fetal γ- and adult α-globin gene. At birth, fetal hemoglobin forms about 75 per cent and hemoglobin A 25 per cent of the total. Transcription of the γ gene begins to fall prior to birth, and by 6 month of age this gene is expressed only at very low levels. expression of the δ-globin gene begins near birth. In adults hemoglobin A makes up about 97 per cent, hemoglobin A2 about 2½ per cent, and fetal hemoglobin less than 1 per cent of the total (from: Steinberg, and Benz, 1991, p: 298).

and Hb A_2 (α_2 δ_2). In childhood special hemoglobin has been discovered: fetal hemoglobin Hb F (α_2 γ_2).

Three other hemoglobins were discovered in the blood of human embryos that differed from adult and fetal hemoglobin. These components were called Hb Gower-1 and Hb Gower-2 (**Bunn and Forget,1986**). Structural analyses have shown Hb Gower-1 to be a tetramer composed entirely of embryonic subunits : $\zeta_2\varepsilon_2$. The ε chain is a β like chain and encoded by the β globin gene complex on chromosome 11. The ζ chain was found to be an α like chain and had a strong structural homology to the α chain, it is incoded by ζ gene located in the α like gene family on chromosome 16 (**Bunn and Forget,1986**).

A third human embryonic hemoglobin was found and called Hb Portand that was shown to be a tetramer composed of two γ chains and two ζ chains. These three embryonic hemoglobins serve as physiologic oxygen carriers (**Bunn and Forget,1986**).

The embryonic hemoglobins are produced in the yolk sac during the third though eighth weeks of gestation (**Hann et al.,1991**). From about the eighth to the twenty-eighth week, the liver becomes the major site of erythropoiesis. During the last half of gestation, red cell production gradually shifts from the liver and spleen to the bone marrow. When the embryo reaches a crown-rump length of 35 mm (about seven weeks gestation) Hb F composes about 50 per cent of the total hemoglobin, and when the embryo exceeds a crown-rump length of 50 mm (about nine weeks gestation) , about 90 per cent of the total is Hb F. There after, the embryonic hemoglobins are no longer detectable, and Hb F is the predominant hemoglobin in fetal red cells. However, β-chain synthesis begins early in fetal development. At the sixth week of gestation, Hb A composes about 7 per cent of the total hemoglobin and increases slowly until about the thirtieth week, when there is a much more concerted "switch" from γ-chain to β-chain production (**Bunn and Forget,1986**).

On the other hand,
appears in adult red cells later around six months of age, when fetal hemoglobin tend to disappear (**Bunn and Forget,1986**).

Hemoglobin Genes

The α like and β like globin subunits are incoded by two clusters of genes each of which is expressed sequentially during development. The α-genes are clustered on the short arm of chromosome 16 in a 25 kb (25,000 base pair) region. In the α-cluster there are two expressed α-globin structural genes (α_1 and α_2) located less than 3 kb apart. Three other genes belong to this cluster ; the embryonic ζ genes and the pseudogenes $\psi\alpha_1$ and $\psi\zeta$. Pseudogenes are genes that have sequence homology with the active genes but contain mutations that prevent their expression. The location of α-like globin genes are shown in **figure 5**. The β-globin genes cluster is found on the short arm of chromosome 11 in a 50 kb region. The arrangement of the embryonic ε-globin genes,
genes, the δ and β (adult) globin genes, as well as the pseudogene $\psi\beta_1$ is shown in **figure 5**.

Every expressed globin gene has three exons (sequences most of which code for the globin chains)
intervening sequences (IVS) are transcribed in erythroid cells so that the initial RNA transcript is a mosaic of coding and intervening sequences. Within the nucleus RNA processing enzymes excise the intervening sequences and ligate the coding blocks together to assemble the final mRNA molecule. The precise biochemical steps DNA sequences of many normal genes has indicated that there are preferred sequences (consensus sequences) at the boundaries of the coding blocks and the IVS.
always begin with the dinucleotide GT and end with an AG (the so-called Chambon rule).
as well as all the other genes of these clusters have been determined using current rapid nucleotide sequencing methods (**Antonarakis et al., 1985**).

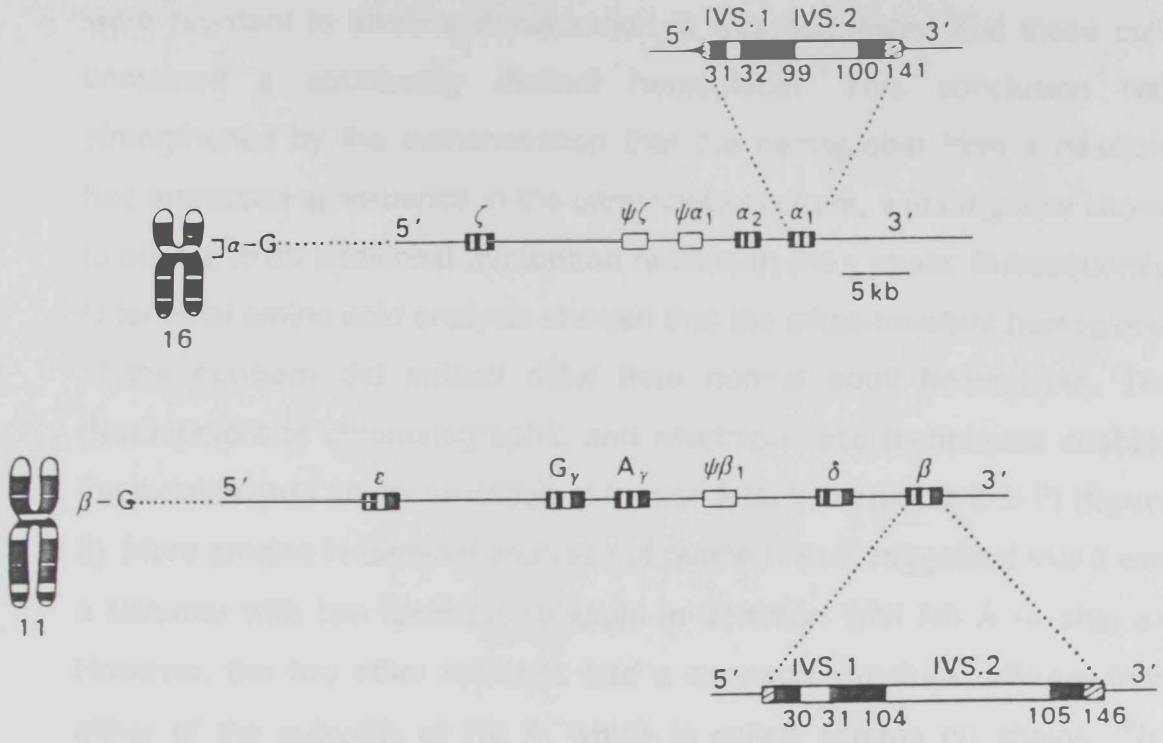

Figure 5: The chromosomal localization and genomic organization of the human genes. For every gene, black boxes represent coding regions, white boxes represent intervening sequences, and hatched boxes are the 5'and 3'untranslated regions. α-G: alpha-globin genes, β-G: beta-globin genes. IVS-1 and IVS-2: first and second intervening sequence. The numbers below the area of coding sequences represent the number of the amino acid residue coded by this particular sequence (from: Serjeant, 1985, p:4).

FETAL HEMOGLOBIN

It has been discovered that the hemolysates of newborn red cells were resistant to alkaline denaturation. It was suggested that these cells contained a structurally distinct hemoglobin. This conclusion was strengthened by the demonstration that the hemoglobin from a newborn had increased absorbance in the ultraviolet spectrum, a finding now known to be due to an additional tryptophan residue in the γ chain. Subsequently, N-terminal amino acid analysis showed that the alkali-resistant hemoglobin of the newborn did indeed differ from normal adult hemoglobin. The development of chromatographic and electrophoretic techniques enabled the isolation and characterization of human fetal hemoglobin (Hb F) (**figure 6**). More precise N-terminal analyses of purified Hb F suggested that it was a tetramer with two identical subunits in common with Hb A (α chains). However, the two other subunits had a common structure different from either of the subunits of Hb A, which is called gamma (γ) chains. The gamma chains lie near the gene for beta chains on human chromosomes number 11; the gene for alpha chains resides on chromosome 16 (**Bunn and Forget,1986**).

Hemoglobin F is the major hemoglobin in fetal life. It constitute 90 to 95 per cent of the total hemoglobin in the fetus until about 34 to 36 weeks of gestation. The fetal hemoglobin concentration in blood decreases after birth by approximately 3 per cent per week and is generally less than 2 to 3 per cent of the total hemoglobin by 6 months of age. This rate of decrease in Hb F production is closely related to the gestational age of the infant and does not appear to be affected by the changes in environment and oxygen tension that occur at the time of birth (**Segel and Oski,1990**).

Near the time of birth, synthesis of gamma globin is almost completely switched off, while synthesis of beta globin is switched on. How such switching occurs remains one of the major unsolved problems of human molecular biology (**Edelstein,1986**).

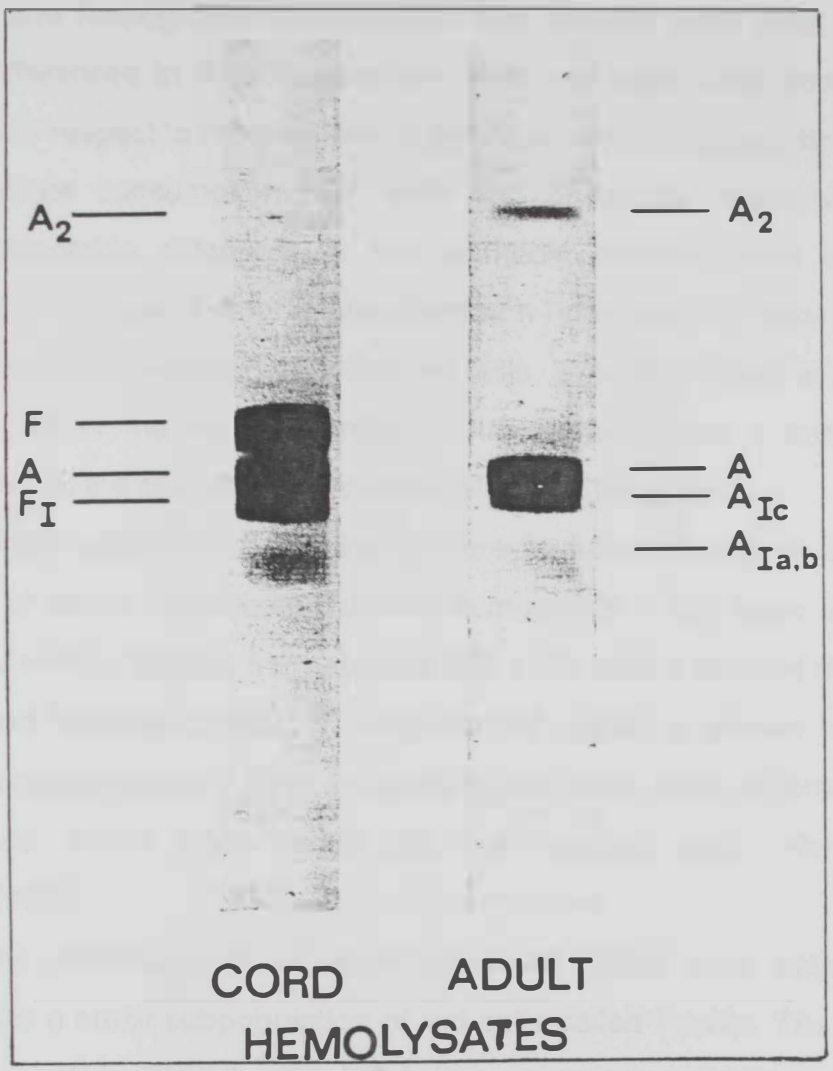

Figure 6: Analysis of human umbilical cord and adult blood hemolysates by gel electrofocusing. The gels have been overload in order to demonstrate Hb A_2 (from: Bunn, and Forget, 1986, p: 62).

F CELLS and Hb F EXPRESSION

1-F Cell Definition

Red cells of newborn infants have a higher mean corpuscular volume and hemoglobin concentration than normal adult cells and show many differences in their metabolism. Fetal red cells differ from those of adults with respect to the activities of glycolytic and non-glycolytic enzymes, carbohydrate consumption and ATP and phosphate metabolism. One easily detectable difference is the markedly reduced level of red-cell carbonic anhydrase, the most abundant non-hemoglobin protein of the red cell, in fetal as compared with adult red cells. In addition there are a variety of changes in the red-cell antigens; in particular the i antigens are expressed on the cell surface (**Weatherall and Clegg,1981**).

Small amount of hemoglobin F are found in all normal adult. The majority of adults have less than 1% hemoglobin F but there is a broad scatter of values ranging from about 0.3 to 1.2% with a skewed distribution (**Benz and Schwartz,1990**). The amount of scatter is greater in females who as a group probably have a slightly higher mean level of hemoglobin F. Note here, these adult levels are not reached until after puberty (**Wood,1989**).

The small amount of Hb F produced during post natal life are confined to a small subpopulation of red cells called F cells. The numbers of F cells produced and the (Hb F/F cell) content of these cells appear to be genetic polymorphisms (**Dover et al.,1981**). F cells seem to be adult cells carrying Hb F, and are distinct from true fetal red cells (**Benz and Schwartz,1990**).

It has been established with immunological techniques that normal adults contain 0.2-7% F cells. The proportion of F cells is linearly correlated with the percentage of hemoglobin F in the peripheral blood when the latter is either within the normal range or under conditions in which it is slightly elevated up to about 5%. This correlation suggests that the mean level of hemoglobin F per F cell remains constant in these conditions and that the

elevation of hemoglobin F results from an increase in the number of F cells rather increased production of hemoglobin F per red cell. Calculation of the mean hemoglobin F per red cell from the percentage of F cells, mean cell hemoglobin, and the percentage of hemoglobin F in the blood gives a value of 4-8pg (**Weatherall and Clegg,1981**).

Serial studies on individuals over periods of up to two years show that the F cell level remains constant and reproducible, and furthermore once the adult level of hemoglobin F and F cells is reached it appears to be relatively constant.

Separation of peripheral blood cells according to age by centrifugation produces no difference in the distribution of F cells, arguing that they have a similar life span or turnover as non-F cells.

Two possible models for the origin of adult F cells were set out. The first suggested that there might be a distinct stem-cell population which gives rise to F cells and another to adult hemoglobin containing cells. The second suggested that both F cells and hemoglobin A containing cells are derived from the same stem cell population. There is now reasonably good evidence drived from both in-vitro culture techniques and from studies of the distribution of F cell number in various clonal myeloproliferative disorders that both F cells and adult-hemoglobin containing cells are derived from the same stem-cell population and that F cells represent a subpopulation of cells that are derived from terminal maturation of less differentiated red cell progenitors (**Weatherall and Clegg,1981**).

2-Hb F expression

Red cells of newborns contain 80 ± 10 per cent Hb F. The amount of Hb F drops continuously during the first six months of life, owing to a progressive decrease in the synthesis of Hb F. The rate of decline of Hb F is slower in babies born prematurely as well as in infants of diabetic mothers and in those with D trisomy. In contrast, infants with Down's syndrome and with C/D translocation have precocious synthesis of Hb A

and therefore a more rapid decline in the amount of Hb F (**Bunn and Forget,1986**).

During early childhood, there is a further decrease in Hb F. About 80 per cent of children six months to two years of age have greater than 1 per cent Hb F, whereas only 4 per cent of normal adults have greater than 1 per cent (**Bunn and Forget,1986**).

The small amount of hemoglobin F present in adult red cells is restricted to between 0.1 per cent and 7 per cent of the total red cells. It also appears that in most normal adults the proportion of F cells remains relatively constant throughout life. However, there are wide range of conditions in which hemoglobin F production may be increased over that seen in normal individuals (**Bunn and Forget,1986**).

An elevated level of hemoglobin F in adult life may result from a genetic disorder of hemoglobin production or may be acquired in a variety of different hematological conditions. There is increasing evidence that the underlying mechanisms for increased hemoglobin F production in these various disorders are quite different in each case (**Weatherall and Clegg,1981**).

3-Factors Affecting Hb F Production

Hb F production is under the control of many factors, genetic and nongenetic.

a-Nongenetic Factors

i-Physiological Factors

Many physiological factors are involved in changes in Hb F production, of which pregnancy is the best known.

-Fetal Hemoglobin and Pregnancy

The most important condition in which there appears to be a physiological variation in the number of F cells is pregnancy

(**Anyaegbunam et al.,1989**). Here there is consistant elevation of the number of F cells at about mid-term, the level of which returns to normal towards the end of pregnancy (**figure 7**) (**Weatherall and Clegg,1981**). This elevation is caused primarily by increased production of maternal F cells rather than by fetal red cells that have entered the maternal circulation through small breaks in the placental barrier (**Dunn et al.,1989**). Thus, F cell production is partially responsive to chorionic gonadotrophin and perhaps other hormones related to pregnancy (**Bunn and Forget,1986**).

-Rapid erythroid regeneration

There is increasing evidence that during periods of rapid regeneration after bone - marrow hypoplasia there may be a transient increase in the number of F cells and levels of hemoglobin F in the peripheral blood. This case has been well documented in patients recovering from bone - marrow depression following the transient erythroblastopenias of childhood, or infection, and less marked increase in F cell production has been observed within a week following treatment of iron deficiency anemia (**Weatherall and Clegg,1981**).

ii-Acquired Disorders

Some other physiological factors led to increase in Hb F production they are shown in **table 1**.

b-Genetic Factors

The best known genetic factors affecting expression and elevation of fetal hemoglobin in adult life are the presence of gene mutations leading to hemoglobinopathies , in which populations are divided into at least two major groups: populations with high level of fetal hemoglobin and populations with low level of fetal hemoglobin.

-Populations with high level of Hb F

Many patients with sickle cell anemia (SCA) are known to synthesize increased amounts of fetal hemoglobin (**Miller et al.,1968**). In some

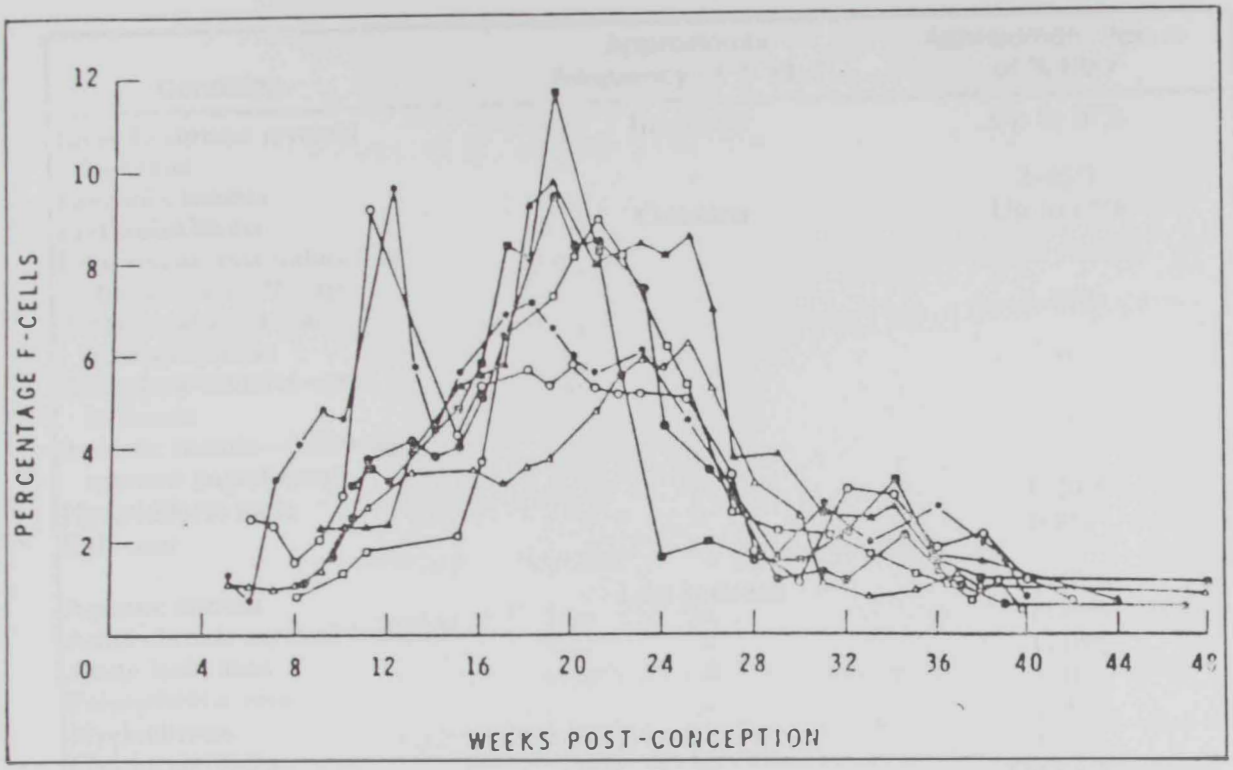

Figure 7: Studies of the sequential changes in F cell numbers during pregnancy (from: Weatherall, and Clegg, 1981, p:77).

Table 1: Acquired disorders associated with elevated Hb F (from: Bunn, and Forget, 1986, p: 74).

Condition	Approximate Frequency of ↑ Hb F	Approximate Range of % Hb F
Juvenile chronic myeloid leukemia	Invariable	Up to 70%
Fanconi's anemia	"	2–85%
Erythroleukemia	Common	Up to 60%
Erythroleukemia induced by chronic chemotherapy	"	"
Paroxysmal nocturnal hemoglobinuria	"	2–20%
Refractory anemias—pre-leukemia	"	"
Aplastic anemia—following marrow engraftment	"	"
Hydatidiform mole	"	1–10%
Kala-azar	"	1–8%
Aplastic anemia	Less common	1–10%
Adult chronic myeloid leukemia	"	1–12%
Acute leukemias	"	1–10%
Polycythemia vera	"	1–10%
Myelofibrosis	"	1–8%
Choriocarcinoma	"	1–5%
Osteopetrosis	"	Up to 20%
Testicular malignancies	"	2–15%
Bronchogenic carcinoma	"	Up to 38%
Hepatoma	"	Up to 22%
Thyrotoxicosis	"	2–20%

situations, the levels attained are so high that the course of the disease is ameliorated since Hb F does not participate in the polymerization process characteristic of the sickling phenomenon (**El-Hazmi et al.,1990**). It has been reported that the simultaneous inheritance of an α thalassemia gene reduces the severity of SCA (**Charache,1990**). From the examination of levels of Hb F in Saudi Arabians, there are at least two levels of Hb F (high and low) (**Al-Awamy et al.,1986a**). The eastern group of Saudi Arabia is similar to Iranian and some Indians (**Kulozik et al.,1987**) which have higher Hb F levels of 15-40% and mild clinical conditions (**Al-Awamy et al.,1986b**; **El-Mouzan et al.,1989**; **Pembery et al.,1978**; **Wood et al.,1980**).

-Populations with low level of Hb F

The *southwestern population of Saudi Arabia has lower* total hemoglobin and lower Hb F than the Eastern population has (**Padmos et al.,1991**). The southwestern levels of Hb F are similar to that of American and Jamaican populations. In Nigerians, the observations reported show *that the levels of fetal hemoglobin are raised in SCA patients in this* population. The Hb F levels obtained (5.9%) are similar to those in Jamaicans, black Americans and Brazilians with 5.6% (**Falusi and Kulozik,1990**), but are, however, markedly lower than those observed in Saudi Arabia and parts of India. The levels of Hb F observed in this population are not very high and the fetal hemoglobin levels observed may not affect the clinical severity of their illness. The patients which are doubly heterozygous for the sickle gene and the Negro type of HPFH produce very mild symptoms, and have fetal hemoglobin concentration in the range 15-40%. The lower levels of Hb F found in males compared with females (**Adedeji et al.,1988**) is still unexplained since there is no evidence that there was selective loss through hemolysis of cells with low amounts of this hemoglobin in females resulting a higher Hb F levels in them (**Falusi and Esan,1989**).

In the Tunisian population the mean levels of Hb F were not high compared to those observed in Senegalese and Saudi Arabian population. However, the heterogeneity of Hb F expression in each sickle cell patient (ranging from 2-16%) could not be solely explained by age differences and is presumably under the control of other factors yet to be discovered (**Abbes et al.,1991**).

In the Sudanese population, Hb F levels were significantly higher in young sickle cell patients (below 10 years) than in older patients. However, in general it is slightly different from black Americans and Jamaicans but distinctly different from Saudis. The ameliorating effect of Hb F seen among Saudi populations was not observed (**Bayoumi et al.,1988**).

-Fetal Hemoglobin in UAE Population

The present study attempts to examine the ability of the UAE population to synthesize Hb F and to compare it with other populations. It attempts to demonstrate the mechanism controlling this production of Hb F and the factors that contribute most to the difference in the Hb F levels among the UAE population. This study is known to be the first studying the UAE population.

PART II: GOALS OF THE STUDY

*- To establish the normal range of fetal hemoglobin expression in UAE population.

*-To compare other special groups of population in whom there is a rise in fetal hemoglobin expression (anemic, pregnant women and thalassemic families) with normals.

*-To examine whether there are variations in fetal hemoglobin expression within the various groups of UAE population.

*-To demonstrate the importance of genetic determinant as opposed to environmental determinant in the expression of fetal hemoglobin.

CHAPTER II.

SUBJECTS AND METHODS

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SUBJECTS AND METHODS

-Subjects

A total 832 blood samples have been studied. Samples were obtained by venepuncture (2 ml) into EDTA as anticoagulant. Each sample has been studied at the hemological level and Hb F study. Our study covered :

- 261 pregnant women from the Obstetrics and Gynaecology clinic.
- 506 patients attending clinics for different reasons.
- 65 β^+ thalassemic patients studied already at molecular levels.

The strategy was to perform:

1-Red Blood Cell Counts (R. B. C.)

2-F Cells Counts

3-Fetal Hemoglobin Quantitation

a-Alkaline Denaturation

b- Immunologic Method

4-Hemoglobin Separation

a-Cellulose Acetate Electrophoresis at Alkaline pH

b- Quantitation of Hb A_2

-Methods

1-Red Blood Cell Counting (RBC)

A complete blood count (C.B.C.), including red blood count (RBC), white blood count (WBC), hemoglobin level (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) , provides valuable information with respect to the diagnosis of both alpha and beta thalassemia and for the normal, anemic, and pregnant women samples.

The C.B.C. was done by Coulter Counter (type S plus) at the hematology laboratory in Al-Ain hospital. It gives the following parameters: RBC, WBC, Hb, Hct, MCV, MCH, MCHC, RDW.

2-F Cells Counting

F cells are small subpopulation of red cells that contain fetal hemoglobin. The acid elution cytochemical method adopted is a sensitive procedure which identifies individual cells containing Hb F even when few are present (**Dacie and Lewis,1991**). F cells are conted in fresh blood under the microscope. A blood cell smear is prepared for each sample and **Chiewslip et al. (1991)** protocol has been followed.

Principle

The identification of cells containing Hb F depends upon the fact that they resist acid elution to a greater extent than do normal cells; thus, in the technique described below, Hb A and its variants are readily eluted from red cells on blood smears when incubated in acid/alcohol/amido black solution at pH 2, while Hb F remains on the slide and appears as isolated darkly-stained cells amongst a background of paly staining ghost-cells (**Figure 8**).

Technique

1) The air dried fresh blood smear (not over 24 hours) was fixed in 80% ethanol for 3 min.

2) Then immersed in acid/ alcohol/ amido black solution for 2 min at room temperature (100 mg amido black, in 100 ml of 80% ethanol, pH adjusted to 2 with HCl).

3) The smear was washed with tap water for 15 seconds and air dried.

4) The stained smear was examined under an oil immersion lens.

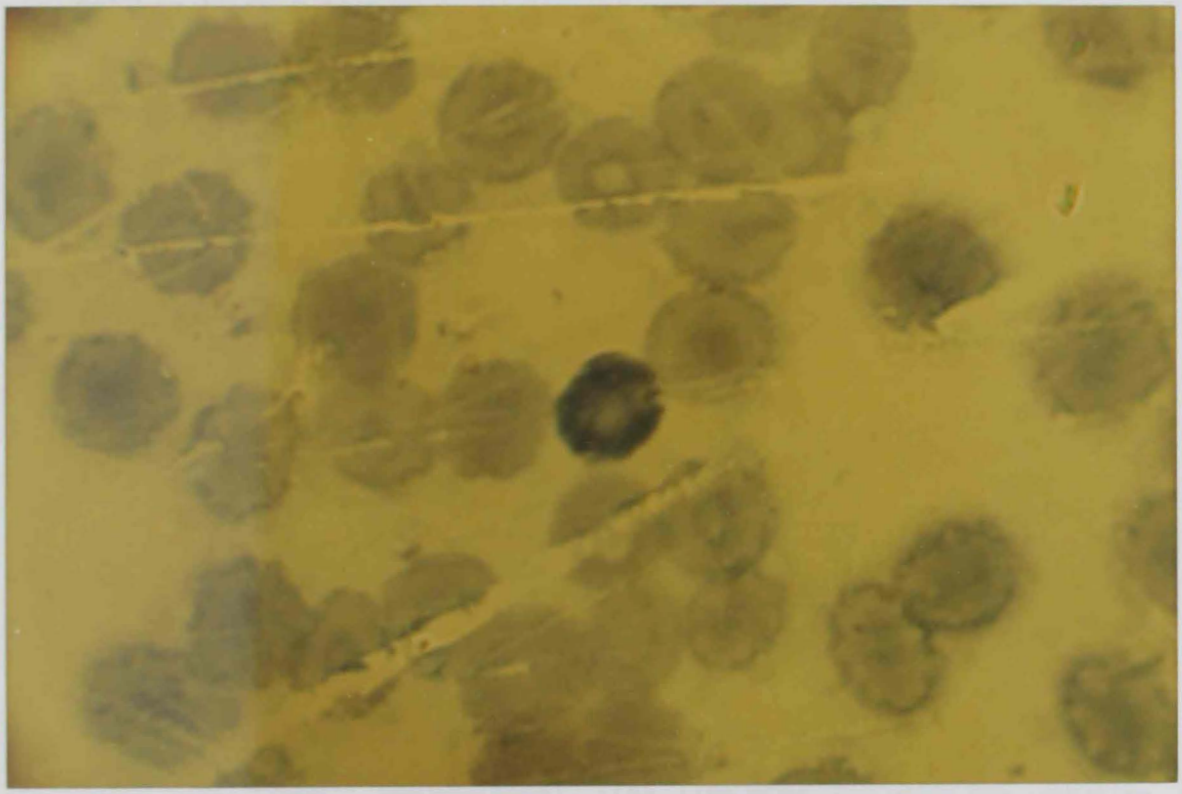

Figure 8: Acid elution preparation from a peripheral blood smear showing F cells.

5) Total of 100 cells were counted in the field and the percentage of F cells was taken according to the number of F cells counted in the field.

3-Fetal Hemoglobin Quantitation

In order to quantitate the total amount of fetal hemoglobin, two different techniques have been used.

a-Alkaline denaturation

Blood Washing

The anticoagulated blood was washed with isotonic solution (saline solution NaCl 0.9%) and red blood cells were used for fetal hemoglobin determination.

Blood washing procedure

1) Whole blood was added with an equal volume of saline solution (NaCl 0.9%).

2) Diluted blood was centrifuged for 10 min at 2000 rpm.

3) The supernatant was removed by a vaccum pump.

4) This operation was repeated 3 times until the supernatant becomes very clear.

Principle

Among all hemoglobins (Hb A, Hb A_2, Hb S etc...) the Hb F is resistant to alkaline denaturation. This characteristic can be used to determine the amount of Hb F in a solution (hemolysate) containing Hb F.

To measure the percentage of Hb F in a mixture of hemoglobins, denaturation is made by addition of sodium hydroxide (NaOH) for a given time, and stopped by adding saturated ammonium sulphate. The ammonium sulphate lowers the pH and precipitates the denatured hemoglobin. The above mixture is centrifugated, and two layers will appear: a precipitate of denatured hemoglobin and supernatant containing fetal

hemoglobin. The proportion of alkali-resistant (i.e. fetal) hemoglobin is then calculated as a percentage of the total amount of hemoglobin present (**Dacie and Lewis,1991**).

Technique

The amount of fetal hemoglobin is obtained by measuring the absorbance density of the supernatant against a control.

-prepare a hemolysate as follows:-

a- 300 µl of washed red blood cell.

b- 4 ml Drabkin (3% potassium cyanide, 3% potassium ferricyanide).

Reactions

-Prepare the following reaction tubes:

	Control	Dosage (Test)
Hemolysate	1400µl	1400µl
NaOH	-	200µl
Water	200µl	-

Allow to react for exactly 2 minutes and then add the following:

$(NH_4)_2 SO_4$	1000µl	1000µl

Allow to stand for at least 5 minutes then centrifuge at 2000 rpm for 10 minutes.

-Dilute the control to 1/20 (200 μl hemolysate + 3800 μl H_2O).

- Allow to stand for 5 min. at least and centrifuge for 10 minutes at 2000 rpm ; two phases will apear:

i- the supernatant (containing the Hb F).

ii- the precipitate containing the other types of Hb.

-The upper solution is removed carefully and the absorbance is read at 540 nm against the control.

The amount of Hb F is done by the equation:-

$$\frac{\text{Absorbance of Dosage (Test)}}{\text{Absorbance of Control}} * 100 = \%\ \text{HbF}$$ **(Fucharoen et al.,1989)**

b- Immunologic method

Fetal hemoglobin can be measured immunologically by using radial immunodiffusion (RID) with an antibody specific for Hb F (**Adams and Steinberg,1991**). **Helena laboratories (b)** protocol has been followed. Antiserum specific for Hb F is incorporated in the agarose gel of the QUIPlate. Hb F Standard and patient hemoglobin dilutions (antigens) are applied to wells in the agarose. The antigens are allowed to diffuse into the agarose matrix where Hb F in the sample react with the Hb F antiserum, producing an opaque precipitin ring around the wells. The precipitin ring diameter for each standard and sample is measured after 24 hours of incubation. The diameter squared of the precipitin ring is directly proportional to Hb F concentration at the end of incubation. A reference curve is prepared from the standards and used to determine the Hb F concentration in the samples.

Technique

i. Preparation of sample

1) Determine the total hemoglobin concentration using a standard laboratory method (**Dacie and Lewis, 1991**).

2) Dilute 0.1 ml of the sample with purified water for a final hemoglobin concentration of 1.0 g/dl. Use the following table to determine the amount of purified water required. The following table lists the volume of purified water to use in the sample dilution.

3) Round off the total Hb concentration to the nearest 0.5 g/dl.

4) Find the volume of water in the table to be added to the 0.1 ml of whole blood sample.

Hb	H_2O	Hb	H_2O	Hb	H_2O
2	0	8	0.6	14	1.2
2.5	0.05	8.5	0.65	14.5	1.25
3	0.1	9	0.7	15	1.3
3.5	0.15	9.5	0.75	15.5	1.35
4	0.2	10	0.8	16	1.4
4.5	0.25	10.5	0.85	16.5	1.45
5	0.3	11	0.9	17	1.5
5.5	0.35	11.5	0.95	17.5	1.55
6	0.4	12	1	18	1.6
6.5	0.45	12.5	1.05	18.5	1.65
7	0.5	13	1.1	19	1.7
7.5	0.55	13.5	1.15	19.5	1.75

5) After dilution, agitate samples to ensure complete hemolysis which is essential to the accuracy of the test.

6) This preparation will be used for application on the Hb F QUIPlate except samples containing greater than 10% Hb F.

7) For samples containing greater than 10% Hb F, add 0.1 ml of the previous hemolysate to 0.9 ml of purified water to make a 1:10 dilution to be used for application on the Hb F QUIPlate after mixing it well.

ii. QUIPlate preparation

Allow the plate to come to room temperature. The plate can be opened easily by placing a thumb and forefinger on each of the plate flanges and then twisting the sections apart. Remove excess moisture from the wells if present.

iii. Application of Standards and Samples

1) Apply 5 µl of each Hb F Standard (0.5%, 5% and 10%) to three separate wells on the plate using a microdispenser.

CAUTION: Damage to a well during application will result in asymmetric precipitin rings and erroneous results.

2) Apply 5 µl of each diluted patient sample to separate wells in the plate.

iv. Incubation

1) Place the cover on the plate and put it in the Humidity Chamber. Ensure that the top and bottom flanges do not align with each other.

2) Incubate the covered plate a minimum of 24 hours at room temperature (15° to 30° C).

Note: The plate incubation time (24 hours) must not be shortened. Incubation for less than 24 hours will cause erroneous results.

3) Remove the plate from the chamber at the end of incubation. The plate can be read at this time or after storage.

v. Precipitin ring measurement

1) Position a precipitin ring under the QUIP Comparator (ocular).

2) Hold your eye as close as possible to the ocular for accurate, reproducible readings.

3) Read the ring diameter to the nearest 0.1 mm and record.

vi. QUIPlate reuse

If some wells on the QUIPlate are not used, the covered plate may be stored at 2° to 6° c in a humidity chamber. Under these storage conditions, a plate may be reused for up to 28 days without establishing a new reference curve. The plate can be used after 28 days if a new reference curve is established.

vii. Calculation of results

1) Prepare a reference curve using the graph paper provided. Plot the known sample concentration (x-axis) against the ring diameter squared (y-axis) for each Hb F Standard. Draw a straight line-of-best-fit through the points.

2) Using the square of the diameter of each sample precipitin ring value, read the %Hb F from the reference curve.

3) Results for samples with Hb F values between 0.5% and 10% are read directly from the reference curve.

4) Results for samples containing greater than 10% Hb F must be multiplied by a dilution factor of 10 to obtain the final value.

4-Hemoglobin Separation

a-Cellulose Acetate Electrophoresis at Alkaline pH

Principle

Electrophoresis on cellulose acetate membrane is one of the best methods for separating abnormal hemoglobins. This method is simple,

rapid and sensitive. It is generally satisfactory for distingushing the common hemoglobin variants.

At alkaline pH (8.4 - 8.6) hemoglobin is a negatively charged protein and in an electric field will migrate toward the anode (+). Most structural variant of hemoglobin will separate due to surface charge differences, thus allowing identification of abnormal forms.

Technique

Dacie and Lewis (1991) protocol has been followed.

1) Fill the compartments of the electrophoresis tank with TEB buffer (Tris/EDTA/Borate (TEB), pH 8.5). Tris - (hydroxymethyl) - amino - methane 10.2 g; ethylenediaminetetra - acetic acid (EDTA) 0.6 g; boric acid 3.2 g; water to 1 liter. Soak and position the wicks of filter paper, the outer edges should be in the buffer.

2) In a separate dish soak the cellulose acetate membranes in TEB buffer for at least 5 min. It is important to immerse the membranes slowly so as to avoid trapping air bubbles.

3) Blot the membranes between two pieces of absorbent paper, but do not allow to dry out before applying the hemolysates.

4) Lyse the washed blood (packed cells) by adding to it 2 volumes of distilled water. After shaking, centrifuge the mixture at 2000 rpm for 10 min.

5) Place a small volume (10 μl) of the top layer of each hemolysate sample in a sample well into which the applicator is dipped in order to transfer the sample to the cellulose acetate membrane.

6) Apply the hemolysate samples to the cellulose acetate approximately 2 cm from one end of the strip. Allow the applicator tips to remain in contact with the strip for approximately 3 seconds.

7) Place the strips upside down in the tank so that the wicks are in contact with the buffer and the cellulose acetate so that the application line is toward the cathode.

8) Apply the power and run at 250 - 350 V for 20 min or until adequate separation is obtained.

9) Switch off the power, remove the strips from the tank and stain for 3 - 5 min with Ponceau S (Protein stain: Ponceau S (4 g/100 ml) in trichloracetic acid 5 %.

10) Remove the strips, drain and elute the excess stain with three consecutive 15 min washes with destaining solution (5 % (v/v) acetic acid, 50 ml/l) then air dry.

11) Make certain the strips are adequately labelled and place in a protective container, e.g. a plastic envelope.

b- Quantitation of Hb A_2

Principle

The accurate quantitation of hemoglobin A_2 (Hb A_2) in the clinical laboratory is essential for the differential diagnosis of several anemias and the thalassemias. Elevated Hb A_2 is widely regarded as sufficient evidence for the diagnosis of β-thalassemia trait. The Helena Beta - Thal Hb A_2 Quick Column is an anion exchange chromatography method. The anion exchange resin is a preparation of cellulose covalently coupled to small positively charged molecules. The positively charged cellulose attracts negatively charged molecules. Proteins, such as the hemoglobins, contain many positive and negative charges due to the ionizing properties of the component amino acids. In the anion exchange chromatography of Hb A_2, buffer and pH levels are controlled to cause different hemoglobins to possess different net negative charges. These negatively charged proteins are attracted to the positively charged cellulose and bind accordingly. Following binding, the proteins are removed selectively from the resin by altering the pH or ionic strength of the elution buffer. Due to the pH of the resin and the ionic strength of the Hb A_2 Developer, Hb A_2 does not bind to the positively charged cellulose and is eluted as the developer moves

through the column. The other normal and most abnormal hemoglobins are retained by the resin. The Hb A_2 fraction is compared to a total hemoglobin fraction by determining the absorbance of each using a spectrophotometer and then calculating the percentage of Hb A_2.

Technique

Helena laboratories (a) protocol has been followed.

1) For each sample quantitation to be performed obtain:

1 Quik Column from Helena laboratories

1 Hb A_2 Collection tube (small)

1 Total Fraction tube (large)

2) Allow the appropriate number of columns and the reagents to come to room temperature before performing the test.

3) Prepare the samples as follows:

Lyse the washed blood (packed cells) by adding a drop of it to 400 µl of distilled water. Vigorously shake the tube to achieve complete hemolysis of the sample. Complete lysis of the sample is essential for accurate results.

4) Prepare the columns for use as follows:

a. Upend each column twice to remove any resin from the top cap closure. Remove the top cap closure and completely resuspend the entire contents of the column using a Pasteur pipette with a small rubber bulb. Be sure all the resin is resuspended above the filter.

b. Immediately after resuspension of each column, hold the column over a sink or absorbant paper and remove the bottom tip closure allowing the buffer to drain. If the column is allowed to stand with the bottom tip closure in place, resuspension must be repeated.

c. As the resin repacks, you will see an interface (with a slurry above) slowly move up the tube. As soon as the slurry settles to form an interface of resin and remaining supernatant

above, aspirate the undrained supernatant (making sure not to disturb the resin) and discard.

d. Place the column in the Quik Column Rack aligned over a small collection tube.

5) Slowly and carefully apply 100µl of the hemolysate to the column. During application, do not allow the sample to form bubbles or run down the side of the tube. Excessive force used during application will disturb the resin and may cause erroneous results.

6) Immediately after sample application to the column, add 100µl of the same sample preparation to a Total Fraction (TF) collection tube using the same Quickpette. Add purified water to the tube to the scribed line. The total volume will be 15 mL.

7) Allow the sample to completely absorb into the resin. The hemolysate will have a glossy appearance when viewed from above until the sample is completely absorbed by the resin. Upon complete absorption, the top of the resin will have a dull, mat-like appearance.

8) Following the absorption of the sample into the resin bed, slowly apply 2.5 mL of Hb A_2 Developer to the column. Excessive force when applying the developer may disturb the resin and cause erroneous results.

9) Allow all of the Developer to pass through the column into the Hb A_2 (small) collection tube. The eluate contains the Hb A_2.

10) add 0.5 mL of purified water to the small collection tube. The total volume will be 3 mL.

11) Invert both collection tubes several times to mix contents thoroughly.

12) Determine the Hb A_2 percent in each sample using a standard spectrophotometer.

a. Adjust the wavelength to 415 nm.

b. Zero the instrument with purified water.

c. Read and record the absorbance of both the Hb A_2 and TF collection tubes.

d. Determine the Hb A_2% by this formula:

$$\frac{\text{Abs of Hb } A_2 \text{ Fraction}}{5*\text{Abs of TF Solution}} *100 = \text{Hb } A_2\%$$

-In the formula:

*Hb A_2% = percentage of Hb A_2 in the sample.

*Abs of Hb A_2 Tube = absorbance of the contents of the small collection tube at 415 nm (Hb A_2 fraction).

*Abs of the TF Tube = absorbance of the contents of the large collection tube at 415 nm (other hemoglobin fractions).

*5 = dilution factor (15 mL of TF Tube /3mL of Hb A_2 tube =5)

*100 = percentage conversion factor.

CHAPTER III.

RESULTS AND DATA PRESENTATIONS

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A- Normal Population

1- General Characteristics

A preliminary study has been conducted on a normal sample consisting of 335 healthy subjects (127 females and 208 males) aging from less than one year to 85 years. In this group no clinical symptoms related to anemia were observed and the hemoglobin level was always equal to or more than 12 g/dl (**Appendix I**).

2-Hematological Data

Hematological data were obtained using Coulter Counter, type S. This device gives the total red blood corpuscular count (RBC), hemoglobin level (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), but we are interested only in Hb, MCV, and MCH (**Appendix I**).

a-Hemoglobin Level (Hb)

In all our normal sample hemoglobin is always in the range of 12-19.4 g/dl, however, it is noticeable as expected that the amount of hemoglobin is higher in males (12-19.4 g/dl) with a mean level of 14.83 g/dl than that observed in females (12-15.9 g/dl) with a mean level of 13.02 g/dl (**Table 2**).

Table 2: The hematological data range and mean values among normal population.

PARAMETERS		GENERAL POPULATION TOTAL NO.=335	MALES TOTAL NO.=208	FEMALES TOTAL NO.=127
Hb (g/dl)	RANGE	12 - 19.4	12 - 19.4	12 - 15.9
	MEAN VALUE	14.18 (± 1.74)*	14.83 (± 1.58)[a]	13.02 (± 1.38)
MCV (fl)	RANGE	61.2 - 102.8	61.2 - 99.8	68.1 - 102.8
	MEAN VALUE	83.80 (± 6.28)	83.36 (± 6.40)[b]	83.02 (± 6.11)
MCH (pg)	RANGE	18.9 - 38.9	18.9 - 38.9	20.5 - 36.3
	MEAN VALUE	27.36 (± 3.43)	27.41 (± 3.87)[c]	27.52 (± 2.59)

*Mean (± S.D.)

At $P < 0.05$ the two means between males and females were not significantly different, using a t-test for the difference between two means (Sokal and Rohlf,1981; Ronald and Yates,1963).

a t-test for Hb= $4.507*10^{-42}$ b t-test for MCV= 0.905

c t-test for MCH= 0.399

b-Mean Corpuscular Volume (MCV)

The normal MCV obtain in our study ranges from 61.2 fl to 102.8 fl with a mean level of 83.80 fl. This parameter is slightly higher in males with mean level 83.36 fl than in females with mean level 83.02 fl (**Table 2**).

c-Mean Corpuscular Hemoglobin (MCH)

The mean value of this parameter is higher in females than in the males with respective mean values of 27.52 pg for females and 27.41 pg for males, but it ranges from 20.5 pg to 36.3 pg in females and 18.9 pg to 38.9 pg in males (**Table 2**).

3-Fetal Hemoglobin Expression

a-Fetal Cells (F cells)

Fetal cells (F cells) are a hemoglobin F containing red blood cells. The presence of fetal hemoglobin in the erythrocytes make them resistant to acid elution. The amount of F cells has been obtained by counting 100 cells in a random microscopic field. The F cell appears darkly stained while other erythrocytes appear light (**Figure 8**).

According to this technique, the F cells appear to be variable and differ from sample to another. The count ranges from 0%-4% in males and from 0%-5% in females, with a mean level 1.16% in males and 1.29% in females. However, we have seen separate cases with high level of F cells namely 41% and 44% in males (samples no. 6 and 10 respectively) and 41% in one female (sample no. 289). As mentioned in the hematological data, no noticeable differences have been observed between males and females (**Table 3**) (**Figure 9, 10, 11**).

b-Fetal Hemoglobin (Hb F)

This parameter is higher in females than the males with respective mean level of 0.485% for females and 0.430% for males, but it ranges from

Table 3: The Hb F % and F cells % range and mean values among normal population.

PARAMETERS		Hb F (%)	F cell (%)
TOTAL POPULATION	RANGE	0.104 - 0.895	0 - 5
	MEAN VALUE	0.449(± 0.2)*	1.23(± 1)
MALES	RANGE	0.108 - 0.89	0 - 4
	MEAN VALUE	0.430(± 0.2)[a]	1.16(±1)[b]
FEMALES	RANGE	0.104 - 0.895	0 - 5
	MEAN VALUE	0.485(± 0.2)	1.29(± 1)

*Mean (± S.D.)

At P< 0.05 the two means between males and females were not significantly different, using a t-test for the difference between two means (Sokal and Rohlf,1981; Ronald and Yates,1963).

a t-test for Hb F= 0.028 b t-test for F Cell= 0.892

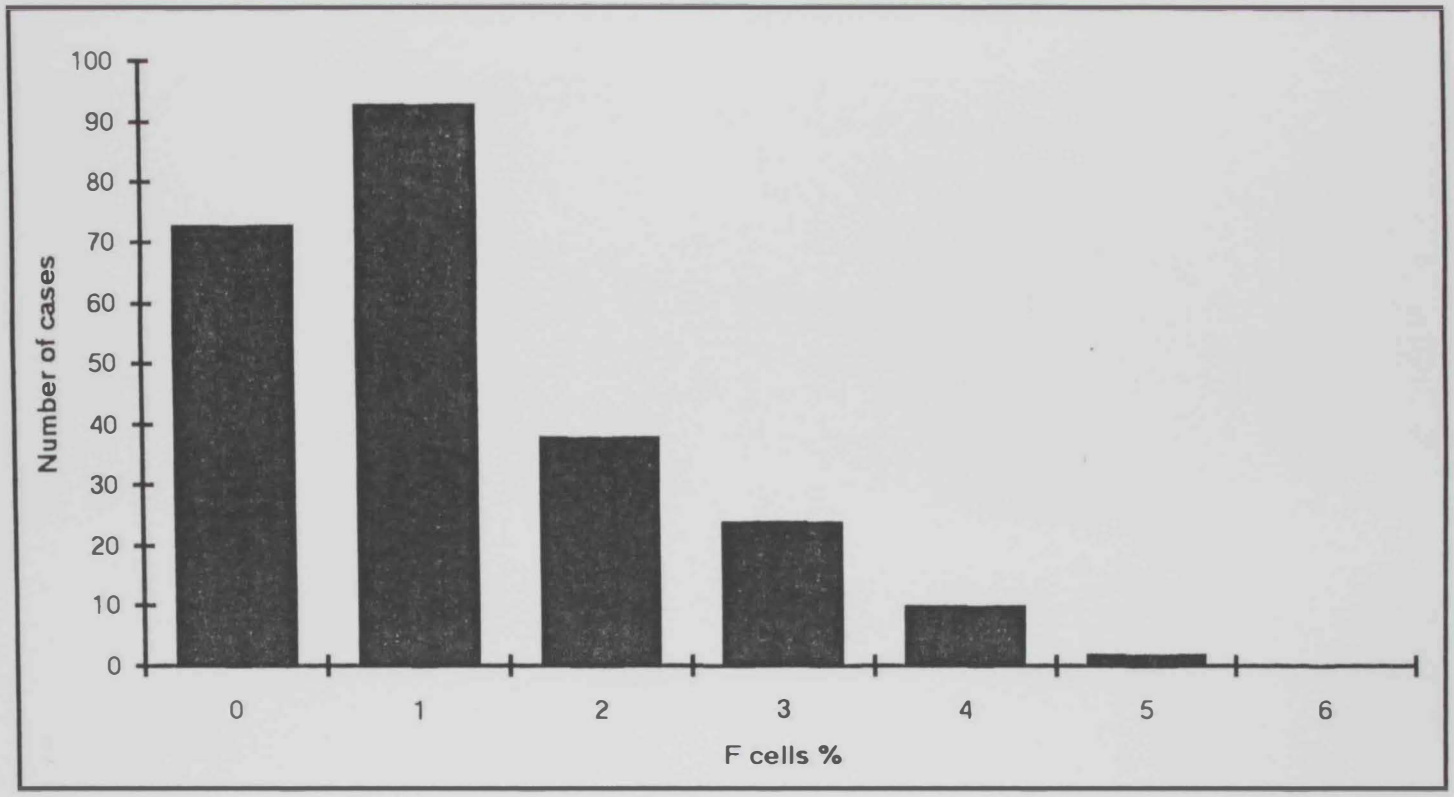

Figure 9: Distribution of F cells % among normal population.

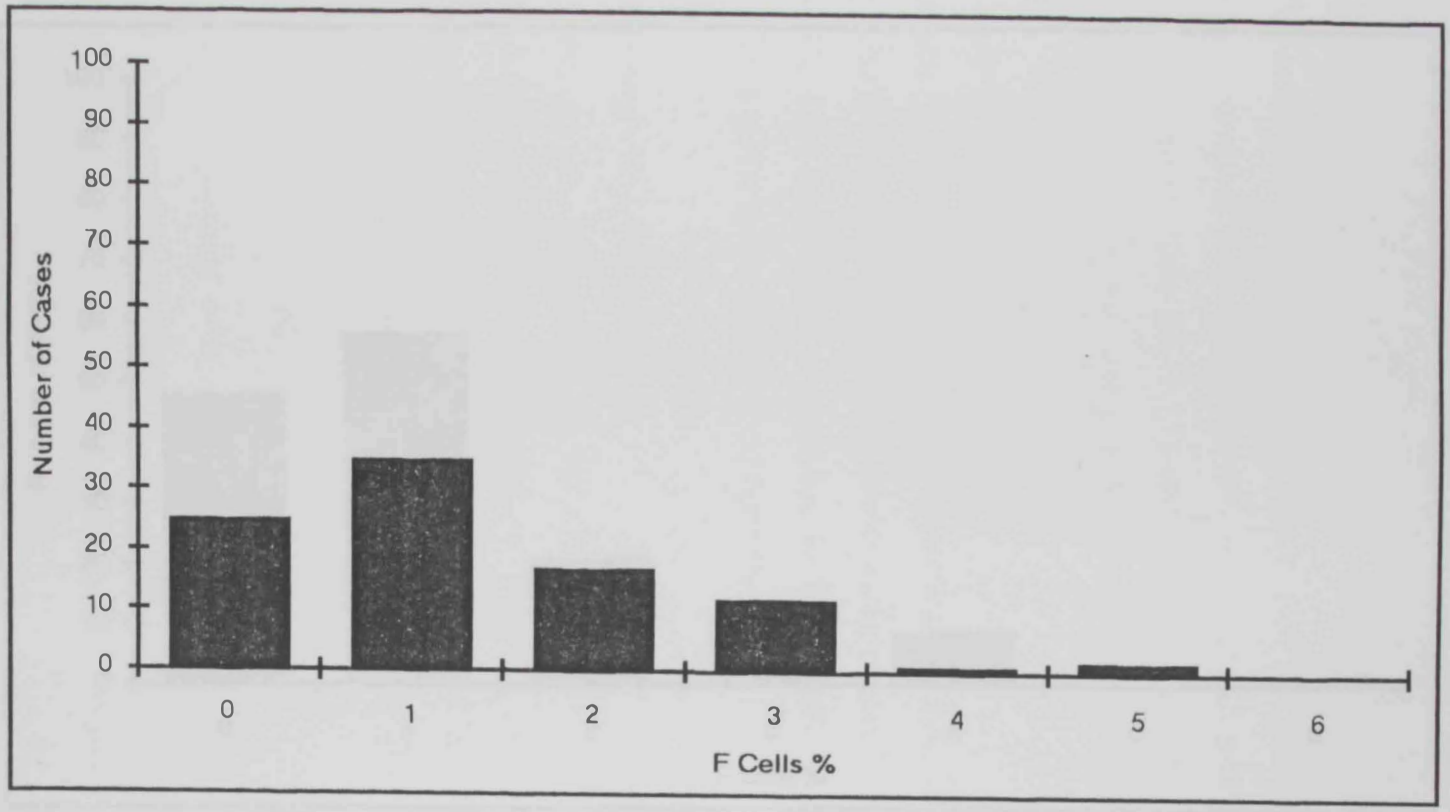

Figure 10: Distribution of F cells % among normal female population.

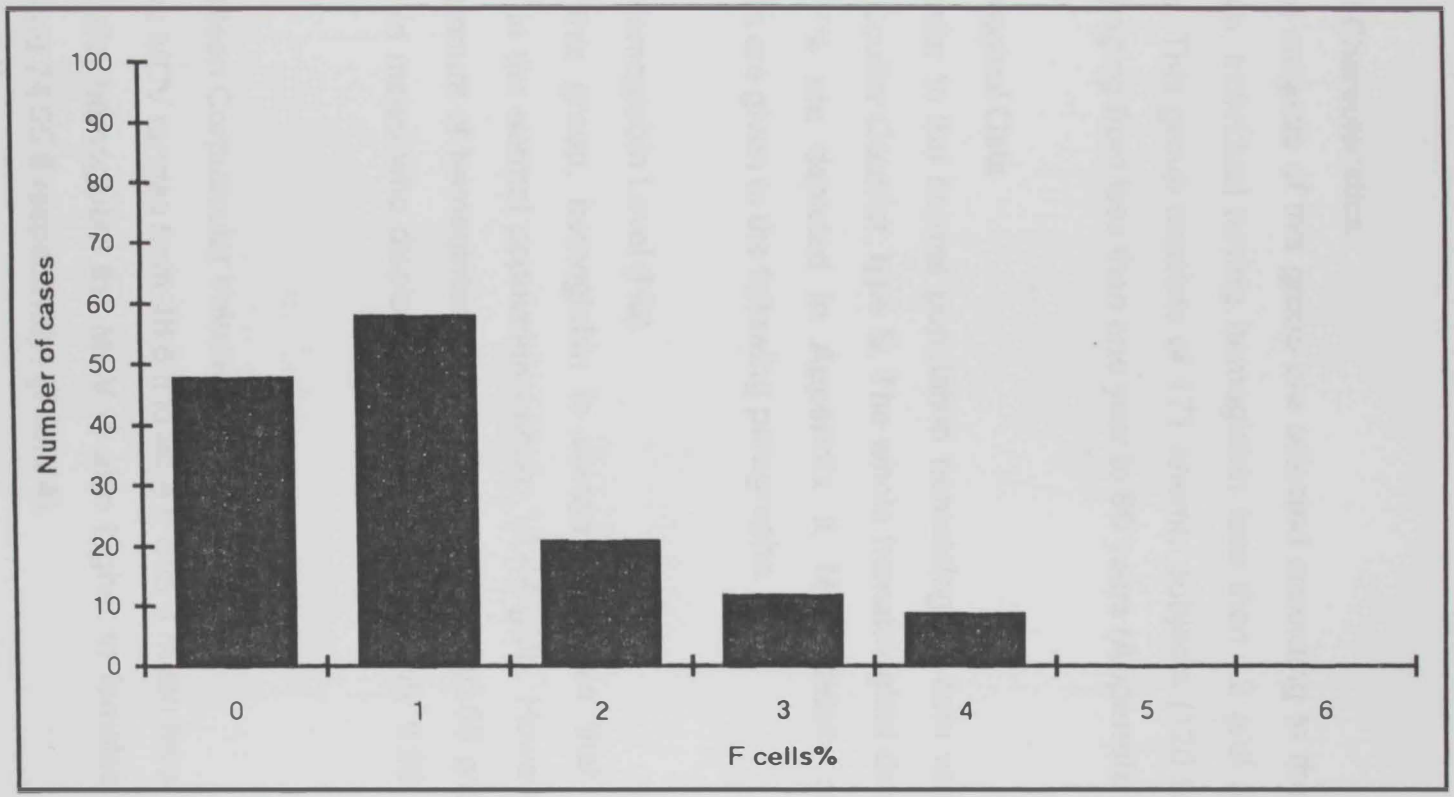

Figure 11: Distribution of F cells % among normal male population.

0.104% to 0.895% in females and from 0.108% to 0.89% in males (**Table 3**) (**Figure 12,13, 14**).

B- Anemic Population

1-General Characteristics

The subjects of this group are selected according to the amount of hemoglobin. Individual having hemoglobin less than 12 g/dl are taken in this group. This group consists of 171 anemic subjects (120 females and 51 males) aging from less than one year to 80 years (**Appendix II**).

2-Hematological Data

Similar to the normal population hematological data were obtained by using Coulter Counter, type S. The whole hematological data, F cells% and Hb F% are depicted in **Appendix II**. More details about some parameters are given in the following paragraphs.

a-Hemoglobin Level (Hb)

In this group, hemoglobin is always less than that which was observed in the normal population (mean: 10.44 g/dl). However. we note that the amount of hemoglobin is higher in females (10.55 g/dl) than that observed in males who displayed a mean value of only 9.98 g/dl (**Table 4**).

b-Mean Corpuscular Volume (MCV)

The MCV ranges from 48.6 fl to 98.4 fl with a mean level of 75.71 fl . However, like hemoglobin the MCV is also higher in females than males (76.03 fl and 74.95 fl respectively) (**Table 4**).

c-Mean Corpuscular Hemoglobin (MCH)

Concerning this parameter, we observed that there are many individual variations with a great decrease in MCH reaching very low values

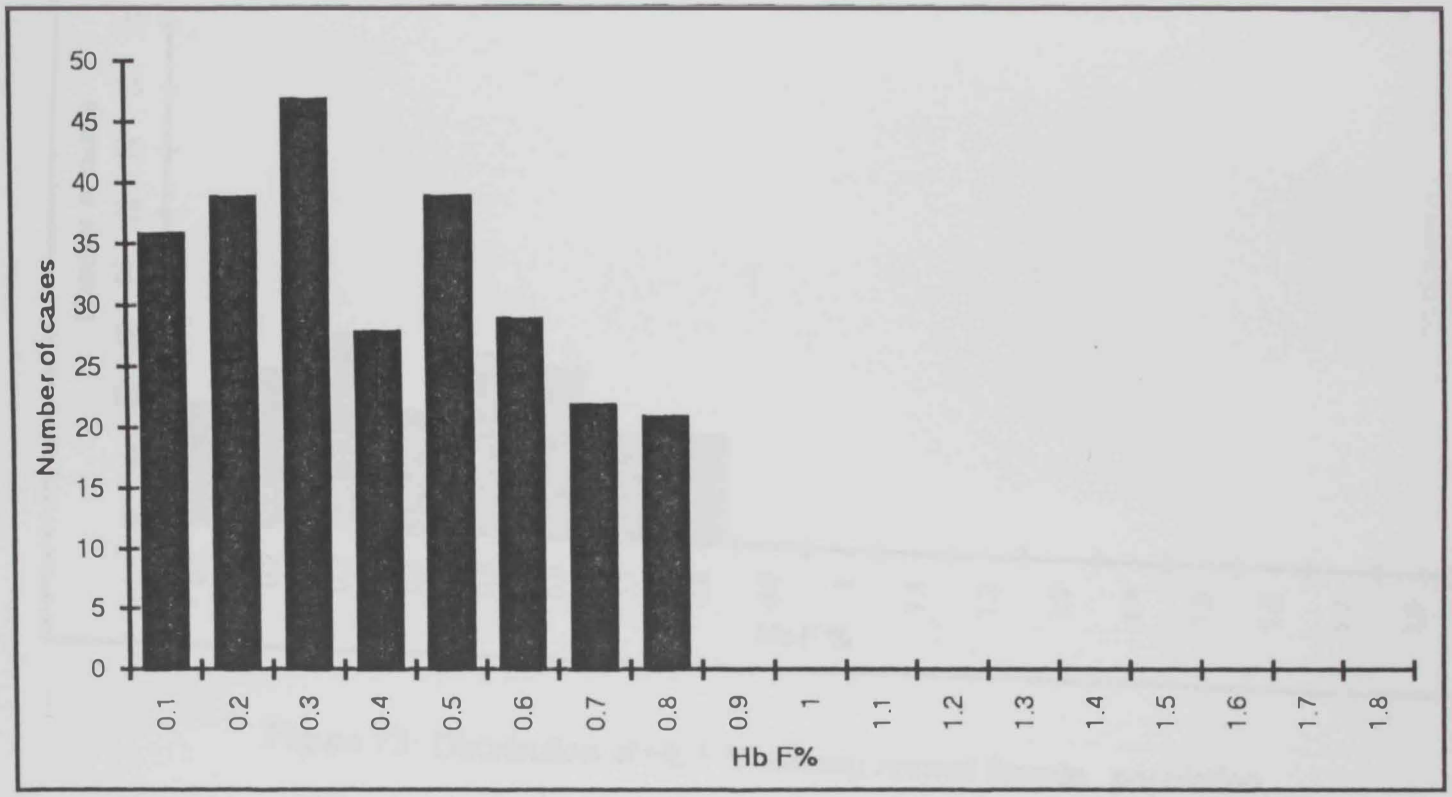

Figure 12: Distribution of Hb F % among normal population.

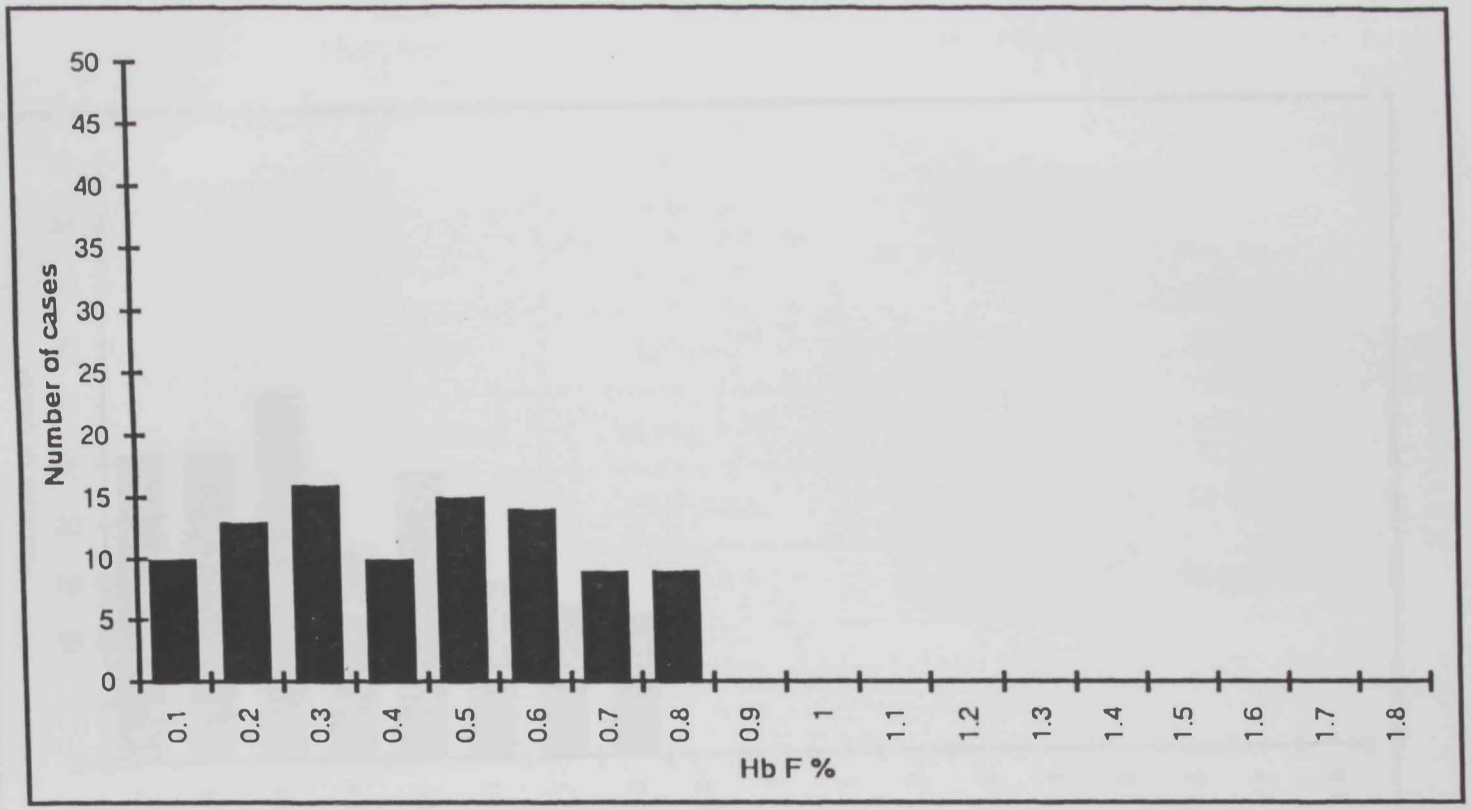

Figure 13: Distribution of Hb F % among normal female population.

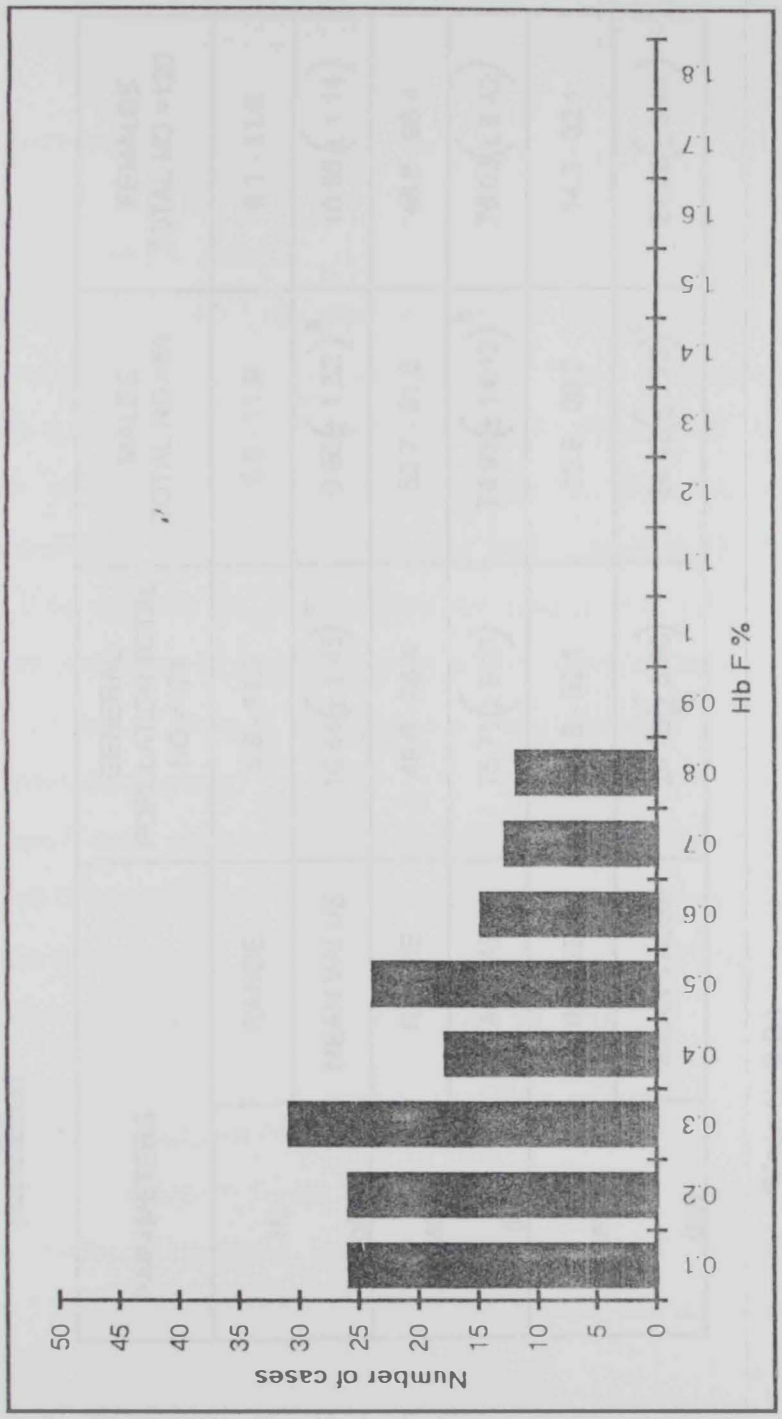

Figure 14: Distribution of Hb F % among normal male population.

Table 4: The hematological data range and mean values among anemic population.

PARAMETERS		GENERAL POPULATION TOTAL NO.=171	MALES TOTAL NO.=51	FEMALES TOTAL NO.=120
Hb (g/dl)	RANGE	5.8 - 11.9	5.8 - 11.9	6.1 - 11.9
	MEAN VALUE	10.44 (± 1.45)*	9.98 (± 1.32)[a]	10.55 (± 1.14)
MCV (fl)	RANGE	48.6 - 98.4	52.7 - 91.6	48.6 - 98.4
	MEAN VALUE	75.71 (± 9.21)	74.95 (± 14.12)[b]	76.03 (± 9.42)
MCH (pg)	RANGE	14.3 - 32.1	15.9 - 30.2	14.3 - 32.1
	MEAN VALUE	24.06 (± 3.90)	24.02 (± 3.25)[c]	24.08 (± 3.44)

*Mean (± S.D.)

At $P < 0.05$ the two means between males and females were not significantly different, using a t-test for the difference between two means (Sokal and Rohlf,1981; Ronald and Yates,1963).

a t-test for Hb= 0.024 b t-test for MCV= 0.403

c t-test for MCH= 0.554

such as 14.3 pg, 15.9 pg and 17.7 pg. This observation is expected since we are working in anemic subjects, having different causes of anemia. However, no noticeable differences were observed between males and females. The mean level is around 24 pg for the whole anemic population (**Table 4**).

3-Fetal Hemoglobin Expression

a-Fetal Cells (F cells)

The presence of F cells was observed in blood smears from anemic patients, but with a broad individual variation. The F cells ranged from 0% to 6% with a mean value of 1.81% (**Table 5**). **Figure 15** illustrates the distribution of this parameter within the anemic population. Unlike the normal population, most anemic patients had F cells in their blood. This indicates that anemia is a condition in which the organism tend to compensate and there are many young cells containing fetal hemoglobin in the blood stream. These F cells have a long life span and thus remain visible in the blood smear.

When F cells were compared in the same population between males and females it appears that there is no significant difference was observed between the two sexes in spite of a slight increase of the F cells mean value in males than the females (**Table 5**) (**Figure 16, 17**).

b-Fetal Hemoglobin (Hb F)

The distribution of Fetal Hemoglobin (Hb F) in anemic patients is also heterogenous. However, most patients displayed normal values, no more than 1%. Some patients had Hb F more than 1% and reached in few patients 1.8% (**Figure 18**).

We know in this group that there was a possible cases of β thalassemia and this could be a reason for the increase in Hb F production. Indeed, patients with Hb F around 1.5% to 1.8% have hematological

Table 5: The Hb F % and F cells % range and mean values among anemic population.

PARAMETERS		Hb F (%)	F cell (%)
TOTAL POPULATION	RANGE	0.121 - 1.864	0 - 6
	MEAN VALUE	0.654 (± 0.4) *	1.810 (± 1)
MALES	RANGE	0.121 - 1.793	0 - 6
	MEAN VALUE	0.643 (± 0.4) [a]	1.93 (± 1) [b]
FEMALES	RANGE	0.13 - 1.864	0 - 6
	MEAN VALUE	0.659 (± 0.4)	1.746 (± 1)

* Mean (± S.D.)

At $P < 0.05$ the two means between males and females were not significantly different, using a t-test for the difference between two means (Sokal and Rohlf,1981; Ronald and Yates,1963).

a t-test for Hb F= 0.966 b t-test for F Cell= 0.338

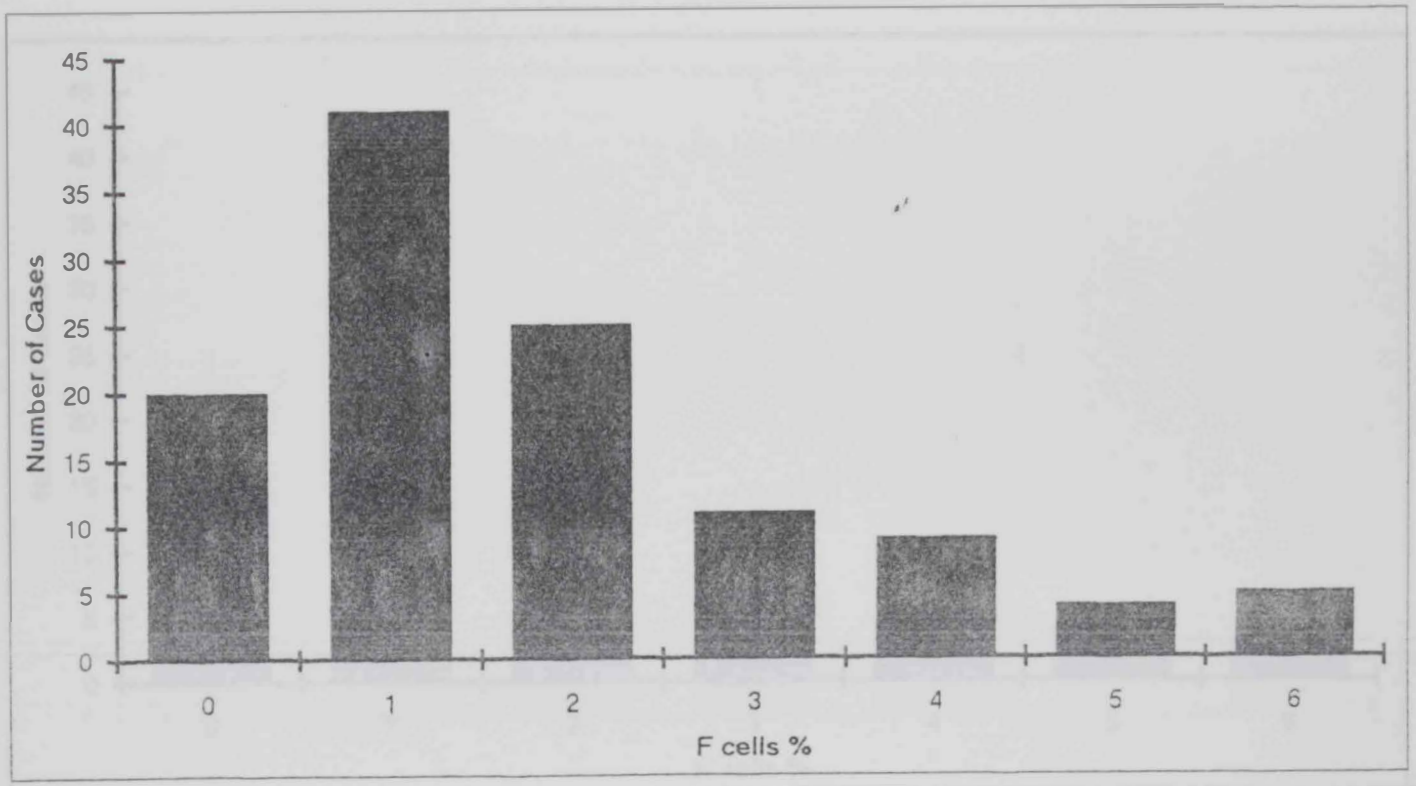

Figure 15: Distribution of F cells % among anemic population.

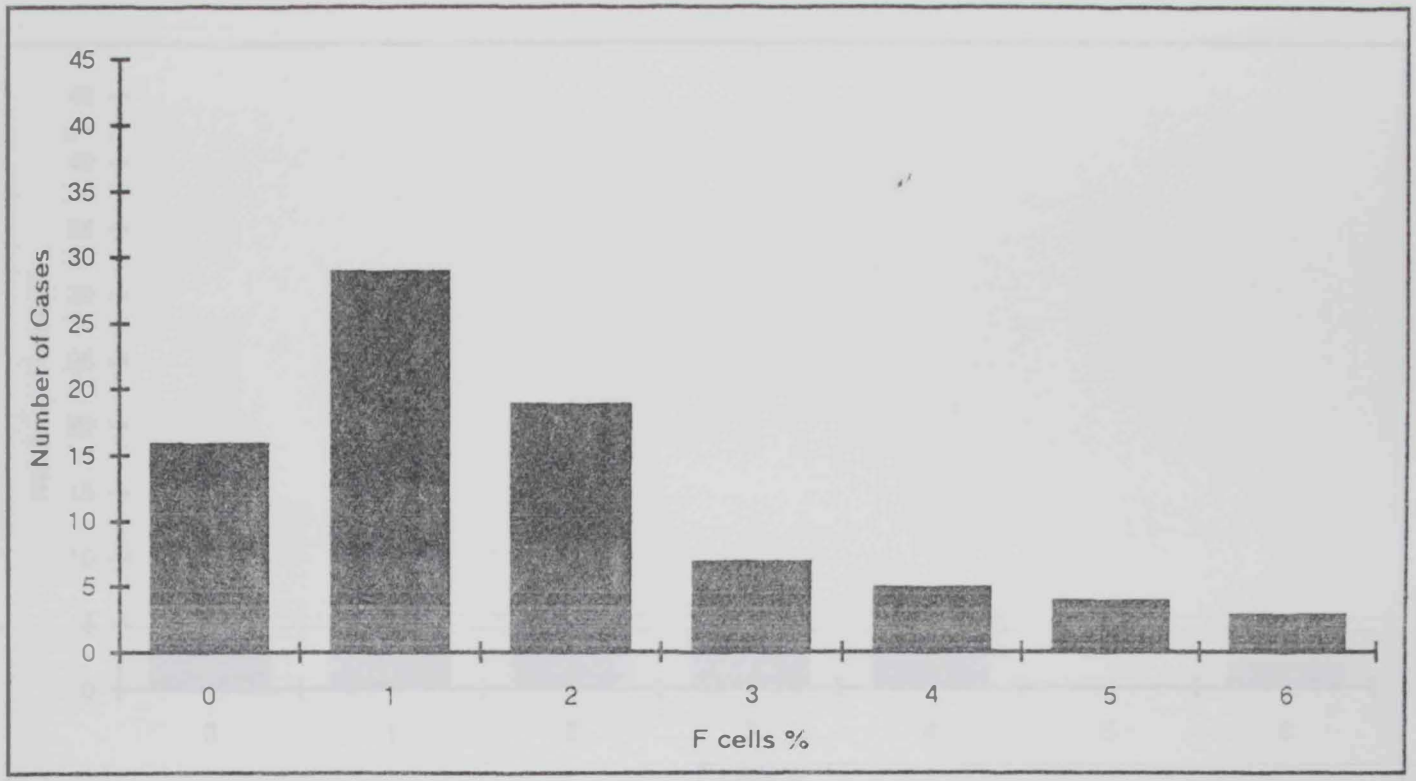

Figure 16: Distribution of F cells % among anemic female population.

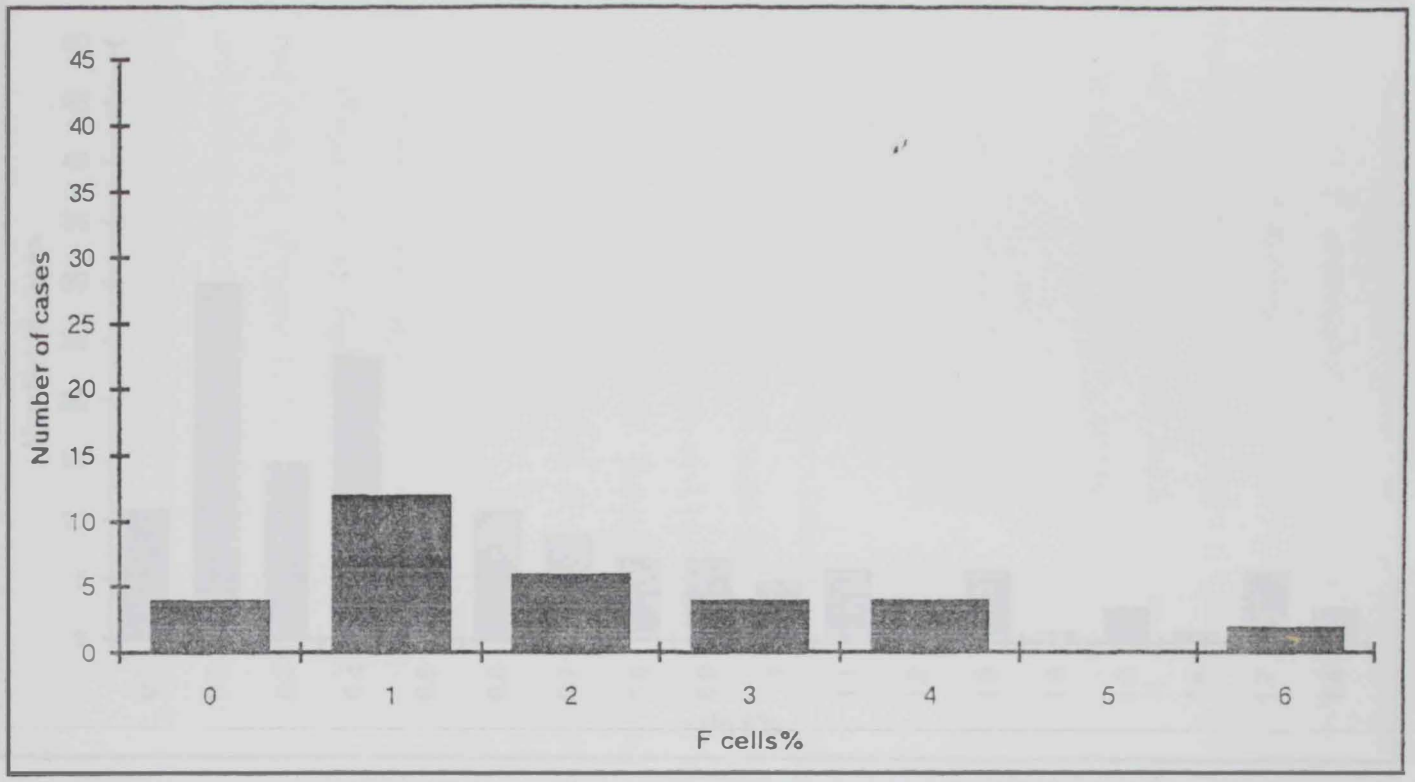

Figure 17: Distribution of F cells % among anemic male population.

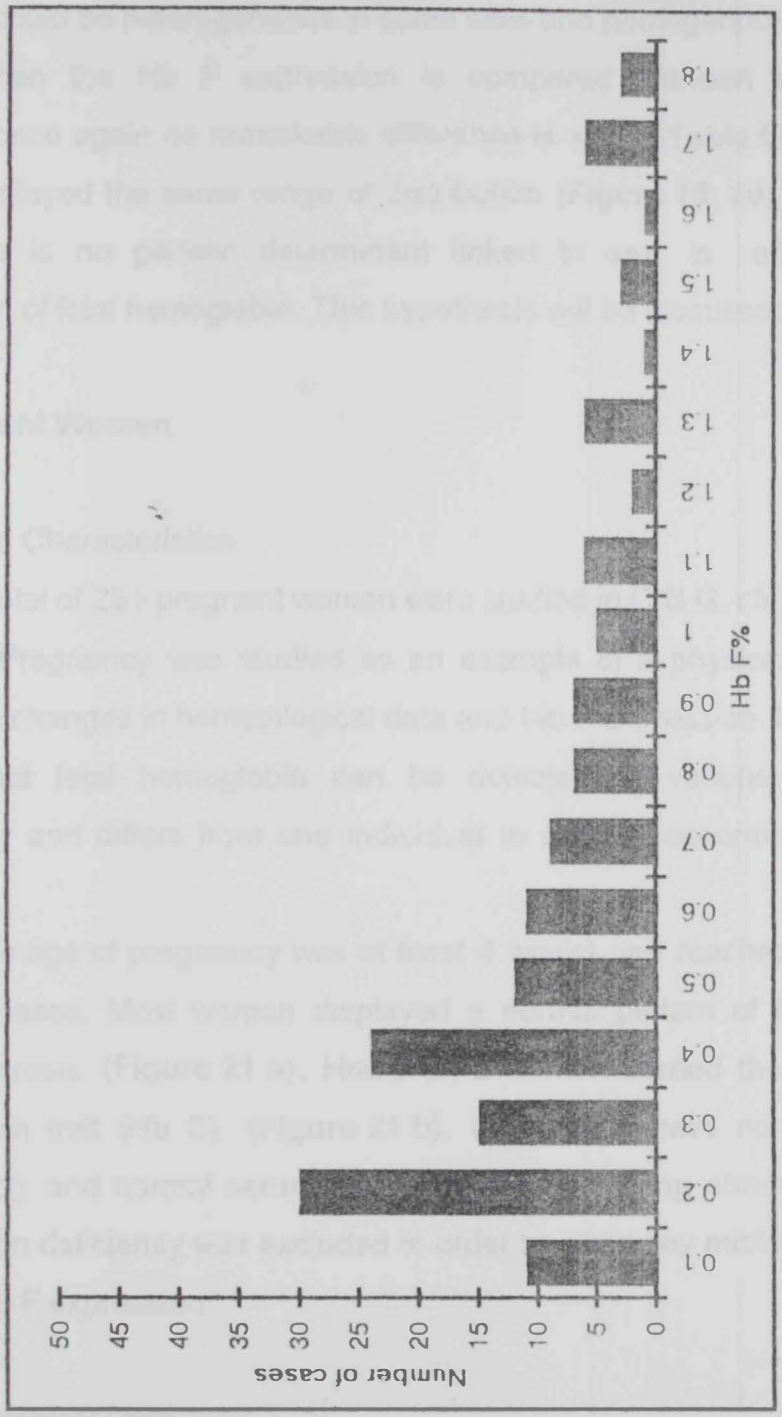

Figure 18: Distribution of Hb F % among anemic population.

parameters with microcytic hypochromic anemia characterizing typical β thalassemia.

The high level of Hb F in this population does not coincide with the high level of F cells, indicating that the distribution of fetal hemoglobin in red cells could be heterogeneous in some cells and homogenous in others

When the Hb F expression is compared between males and females, once again no remarkable difference is seen (**Table 5**) and both sexes displayed the same range of distribution (**Figure 19, 20**), indicating that there is no genetic determinant linked to sex is affecting the production of fetal hemoglobin. This hypothesis will be discussed later.

C- Pregnant Women

1- General Characteristics

A total of 261 pregnant women were studied in O.B.G. clinic of Al-Ain hospital. Pregnancy was studied as an example of a physiological state leading to changes in hematological data and Hb F expression. Indeed, it is known that fetal hemoglobin can be detected in various stages of pregnancy and differs from one individual to another according to many factors.

The age of pregnancy was at least 4 weeks and reached 40 weeks in some cases. Most women displayed a normal pattern of hemoglobin electrophoresis (**Figure 21 a**). However, 5 women carried the sickle cell hemoglobin trait (Hb S) (**Figure 21 b**), all of them have normal Hb A_2 (**Figure 22**) and normal serum iron. Any woman having abnormal Hb A_2 level or iron deficiency was excluded in order to avoid any misinterpretation of high Hb F expression.

2-Hematological Data

The general hematological data are depicted in **Appendix III** and summarized in **table 6**.

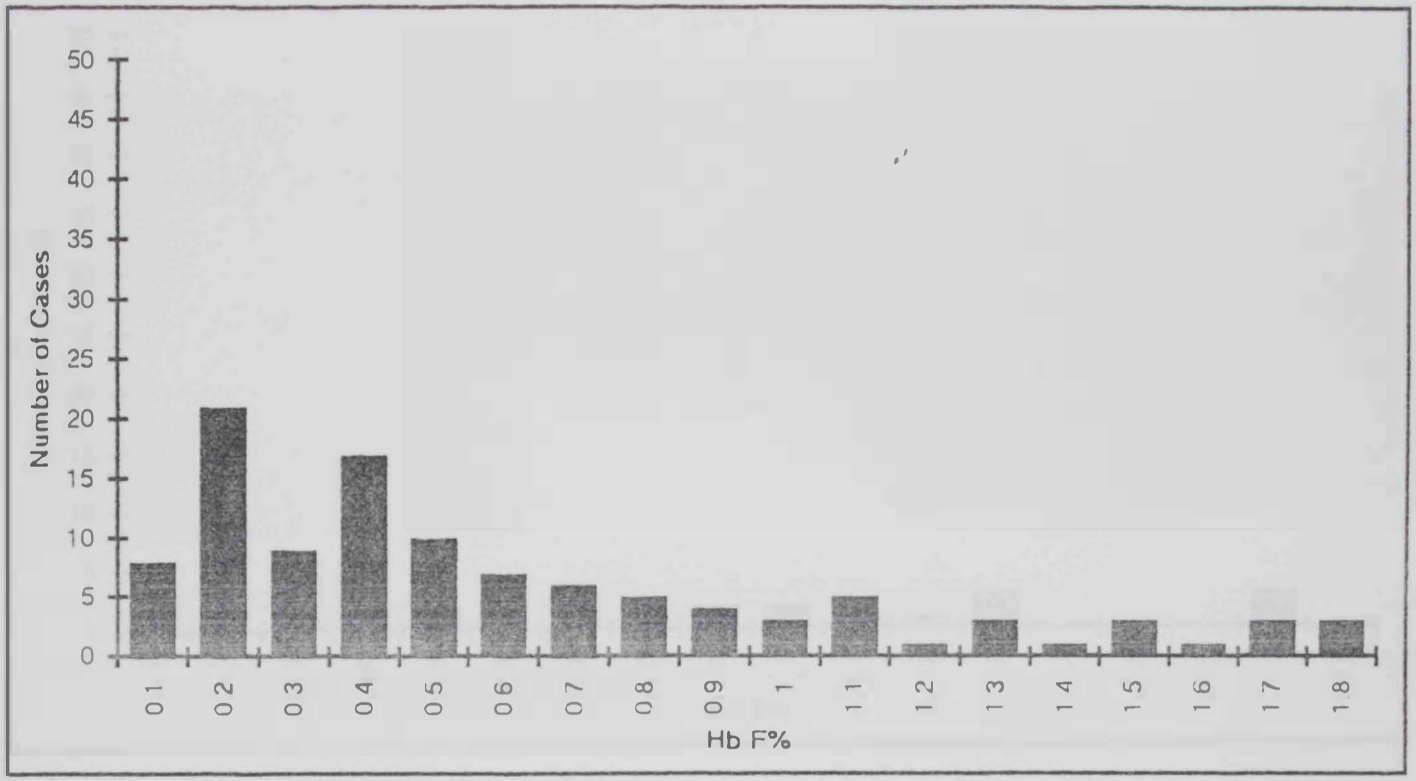

Figure 19: Distribution of Hb F % among anemic female population.

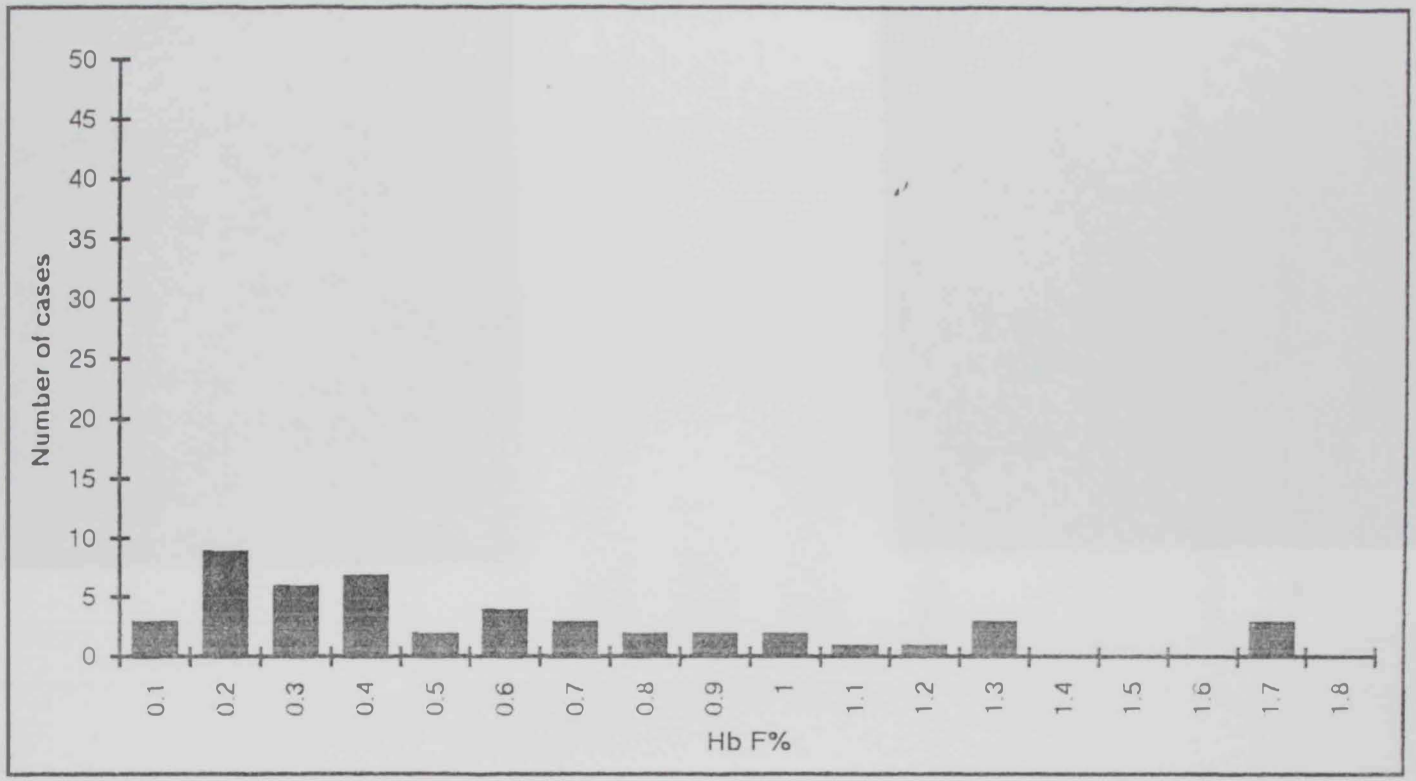

Figure 20: Distribution of Hb F % among anemic male population.

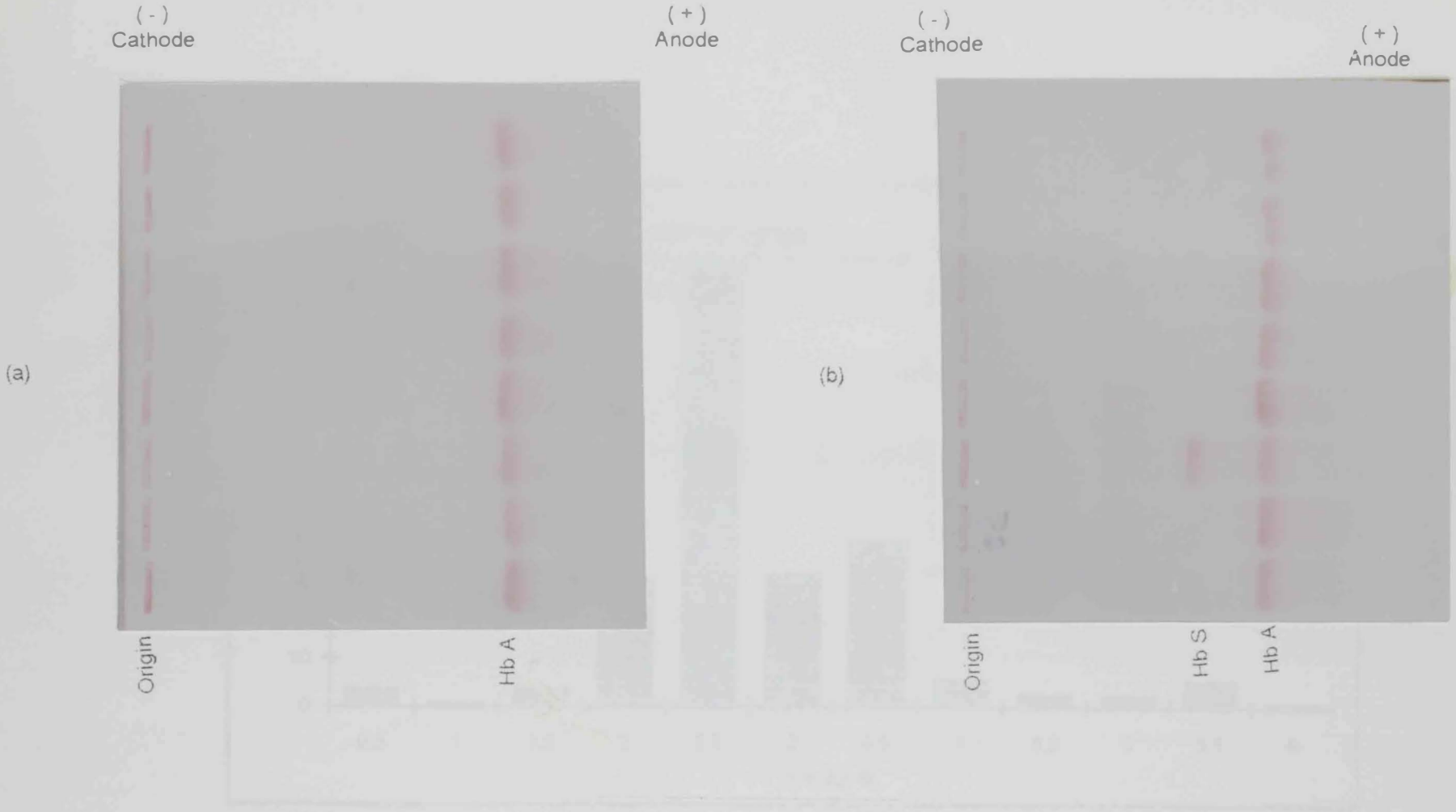

Figure 21: (a) Electrophoresis on cellulose acetate membrane at alkaline pH showing separation of normal hemoglobin. (b) Electrophoresis on cellulose acetate membrane at alkaline pH showing separation of S trait.

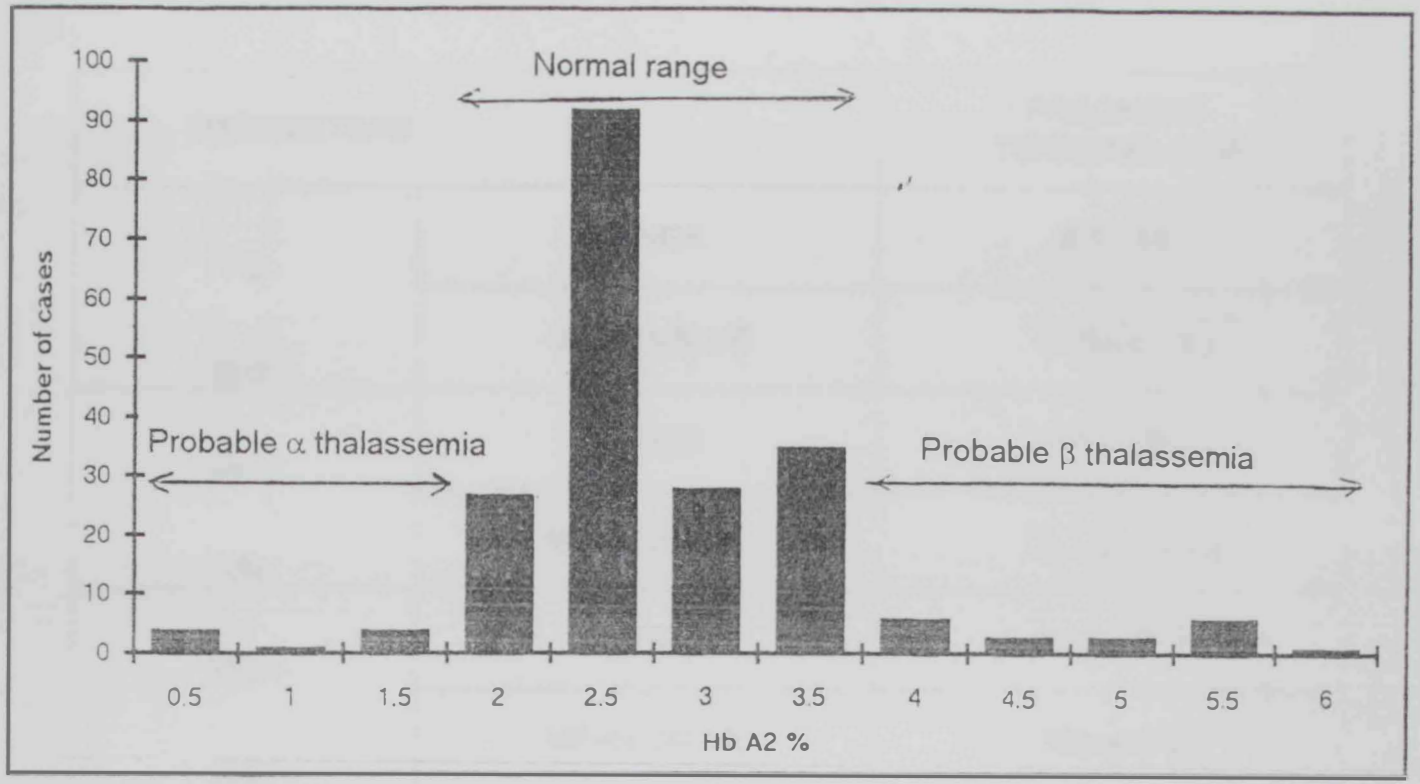

Figure 22: Hb A_2 expression in pregnant females. Only females with normal Hb A_2 (2-3.5%) were taken into consideration and studied for the erythrocyte indices and Hb F expression. Females with low and high Hb A_2 were excluded.

Table 6: The hematological data range and mean values among pregnant female population.

PARAMETERS		PREGNANT TOTAL NO.= 206
Hb (g/dl)	RANGE	8.1 - 15
	MEAN VALUE	11.65(± 1.6)*
MCV (fl)	RANGE	63 - 102
	MEAN VALUE	79.78(± 12.6)
MCH (pg)	RANGE	19.3 - 36.5
	MEAN VALUE	26.6(± 3.2)

* Mean (± S.D.)

a-Hemoglobin Level (Hb)

This parameter showed a difference when compared with the normal and anemic population. Indeed, the amount of hemoglobin in pregnant women was 11.65 g/dl, it came between the normal value observed in the female population which has a mean value of 13.02 g/dl, and the anemic population with a mean value of 10.55 g/dl (**Table 7**). This result is expected in such a physiological state and the slight anemia observed here is most likely due to the increase in blood volume observed during pregnancy.

b-Mean Corpuscular Volume (MCV)

The Mean corpuscular volume in pregnant women ranged from 63 fl to 102 fl (**Table 6**). It appears that this parameter has a heterogeneous distribution. Pregnant females with the lower MCV presented with iron deficiency anemia due to increased demand for iron during pregnancy and lack of supplemental with iron. The one with the highest MCV (102 fl) exhibited a normal hemogram in spite of a high level of regeneration (**Appendix III**). Using the MCV as an erythrocyte parameter, it appears that pregnant women exhibited a slight microcytosis in spite of a normal hemoglobin pattern.

c-Mean Corpuscular Hemoglobin (MCH)

The mean corpuscular hemoglobin observed in this group ranged from 19.3 pg to 36.5 pg with a mean level of 26.6 pg (**Table 6**). This value appears to be normal when compared with the normal population having a mean value of 27.52 pg (**Table 7**). The lowest MCH was observed in the same pregnant females having the lowest MCV. However, the highest MCH is observed in a women with a pregnancy age of 28 weeks who exhibited a normal MCV of 80.6 fl.

Table 7: The hematological data, F cells % and Hb F % range and mean values among normal, anemic , and pregnant female population.

PARAMETERS		Normal Female TOTAL NO.= 127	Anemic Female TOTAL NO.= 120	Pregnant Female TOTAL NO.= 206
F Cell (%)	RANGE	0 - 5	0 - 6	0 - 4
	MEAN VALUE	1.29(± 1) *	1.746(±1)	0.785(± 0.8)
Hb F (%)	RANGE	0.104 - 0.895	0.13 - 1.864	0 - 2.24
	MEAN VALUE	0.485(± 0.2)	0.659(± 0.4)	0.19(± 0.3)
Hb (g/dl)	RANGE	12 - 15.9	6.1 - 11.9	8.1 - 15
	MEAN VALUE	13.02(± 1.38)	10.55(± 1.14)	11.65(± 1.6)
MCV (fl)	RANGE	68.1 - 102.8	48.6 - 98.4	63 - 102
	MEAN VALUE	83.02(± 6.11)	76.03(± 9.42)	79.78(± 12.6)
MCH (pg)	RANGE	20.5 - 36.3	14.3 - 32.1	19.3 - 36.5
	MEAN VALUE	27.52(± 2.59)	24.08(± 3.44)	26.6(± 3.2)

*Mean (± S.D.)

At $P < 0.05$, there is no significantly different between normal females and anemic females, normal females and pregnant females, anemic females and pregnant females, using a t-test for the difference between two means (Sokal and Rohlf,1981; Ronald and Yates,1963).

3-Fetal Hemoglobin Expression

The expression of fetal hemoglobin has been studied in this group by two approaches. The counting of F cells in a fresh blood smear and the quantitation of fetal hemoglobin.

a-Fetal Cells (F cells)

The presence of F cells in blood smears obtained from pregnant females gave a different result. The distribution (**Figure 23**) showed that most females have no fetal cells in their blood smears. However, some patients exhibited a high level of 2%, 3%, and 4% F cells in their blood smears. Most of these patients were pregnant women in advanced pregnancy, and the presence of F cells at this moment could be due to contamination of maternal blood by fetal blood, since it is known that F cells and Hb F as a whole decrease in advanced pregnancy. This is in concordance with our result because most of our sample is formed by pregnant women after mid term and showed a very low level of fetal cells as shown in **figure 23**.

b-Fetal Hemoglobin (Hb F)

The distribution of fetal hemoglobin (Hb F) is depicted in **figure 24**.It appears that this distribution is parallel to what was observed in F cell distribution. Indeed, most pregnant females have no fetal hemoglobin. One female showed fetal hemoglobin reaching 2.24% and also had the highest level of F cells. This woman exhibited a normal hemoglobin pattern having no β thalassemia, but since she was in the last weeks of pregnancy this Hb F level could be explained by a contamination by the fetal blood.

D- β^+ Thalassemic Patients

1-General Characteristics

It is known that β thalassemia is a genetic condition in which the erythrocytic indices are modified and there is a raised fetal hemoglobin and Hb A_2. In order to evaluate the place of environment in the expression of

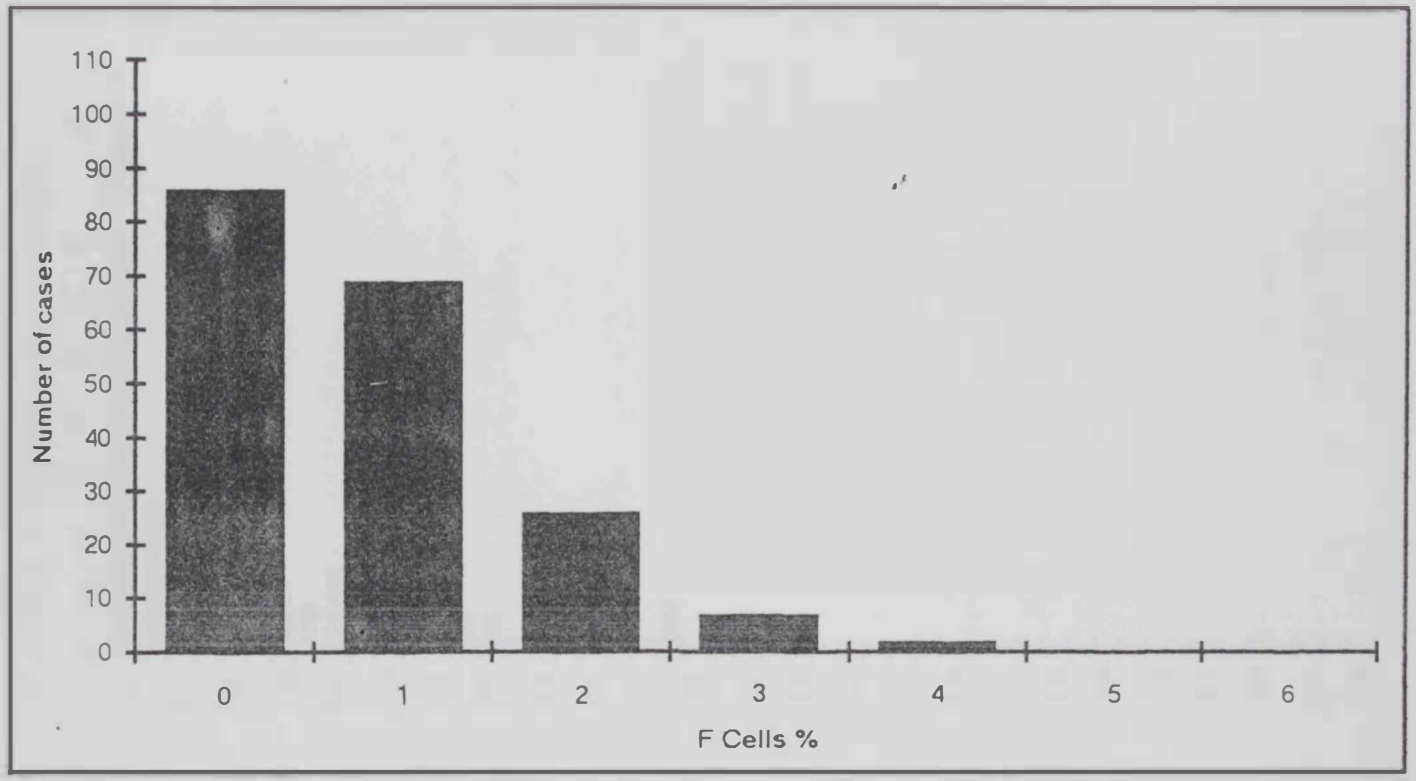

Figure 23: Distribution of F cells % among pregnant female population.

Note that females with 0% F cells are women with advanced pregnancy age over mid term.

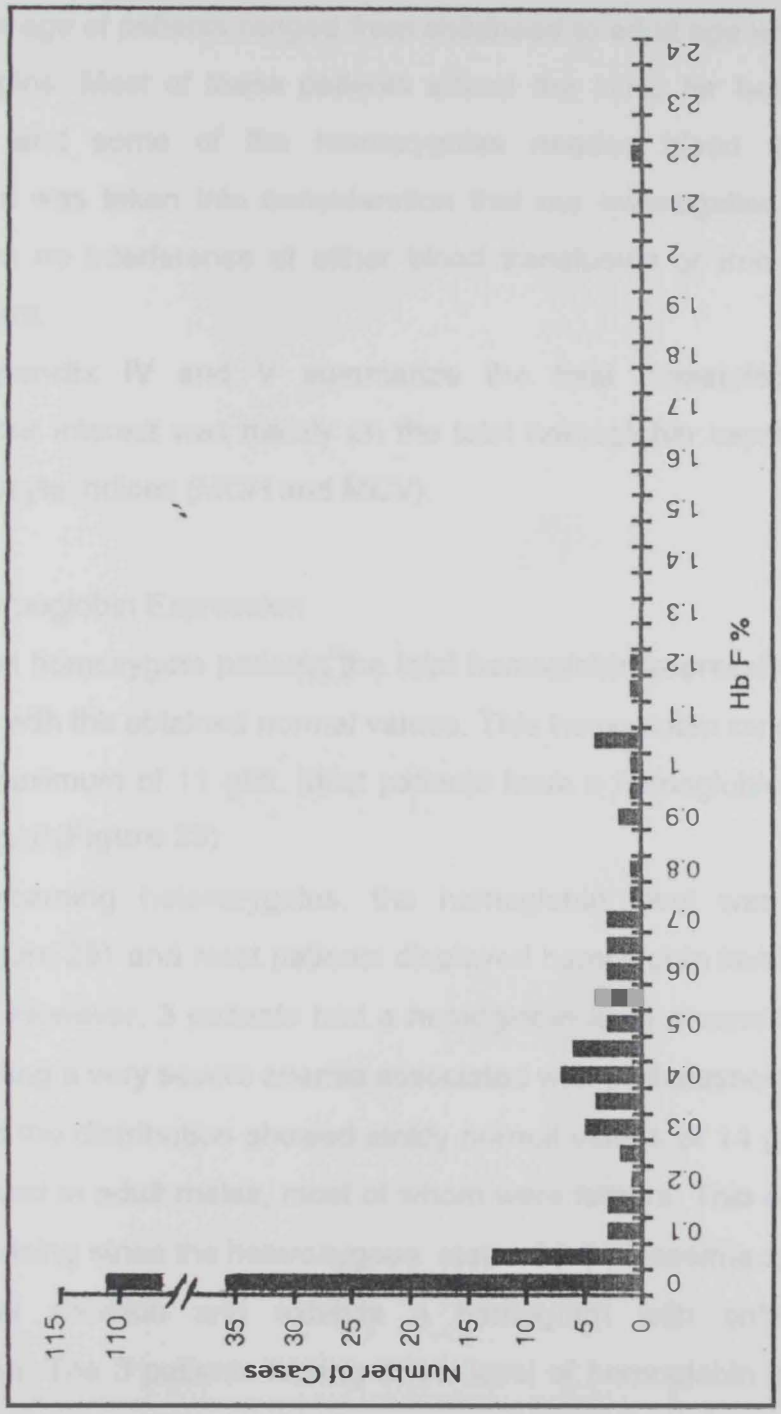

Figure 24: Distribution of Hb F % among pregnant female population.

fetal hemoglobin, β^+ thalassemic patients bearing the same mutation were studied. Indeed, 45 heterozygotes and 20 homozygotes for the β IVSI-5 mutation characterized earlier (**Quaife et al.,1994**) have been studied using the same strategy as for the above samples.

The age of patients ranged from childhood to adult age with different ethnic origins. Most of these patients attend the clinic for hematological problems and some of the homozygotes needed blood transfusion, however, it was taken into consideration that our investigation has been done when no interference of either blood transfusion or iron deficiency were present.

Appendix IV and **V** summarize the total hematological data. However our interest was mainly on the total hemoglobin expression and the erythrocyte indices (MCH and MCV).

2-Total Hemoglobin Expression

In all homozygote patients the total hemoglobin expression was low compared with the obtained normal values. This hemoglobin ranged from 7 g/dl to a maximum of 11 g/dl. Most patients have a hemoglobin level of 9 g/dl to 10 g/dl (**Figure 25**).

Concerning heterozygotes, the hemoglobin level was markedly higher (**Figure 26**) and most patients displayed hemoglobin level of 10 g/dl to 12 g/dl. However, 3 patients had a hemoglobin level around 5 g/dl to 6 g/dl indicating a very severe anemia associated with β^+ thalassemia. On the other hand the distribution showed strikly normal values of 14 g/dl and 15 g/dl observed in adult males, most of whom were fathers. This observation is not surprising since the heterozygous state of β thalassemia approaches the normal situation and exhibits a hemogram with only a slight modification. The 3 patients having a low level of hemoglobin could have other environmental causes of anemia such as parasitic infection or absorption problems.

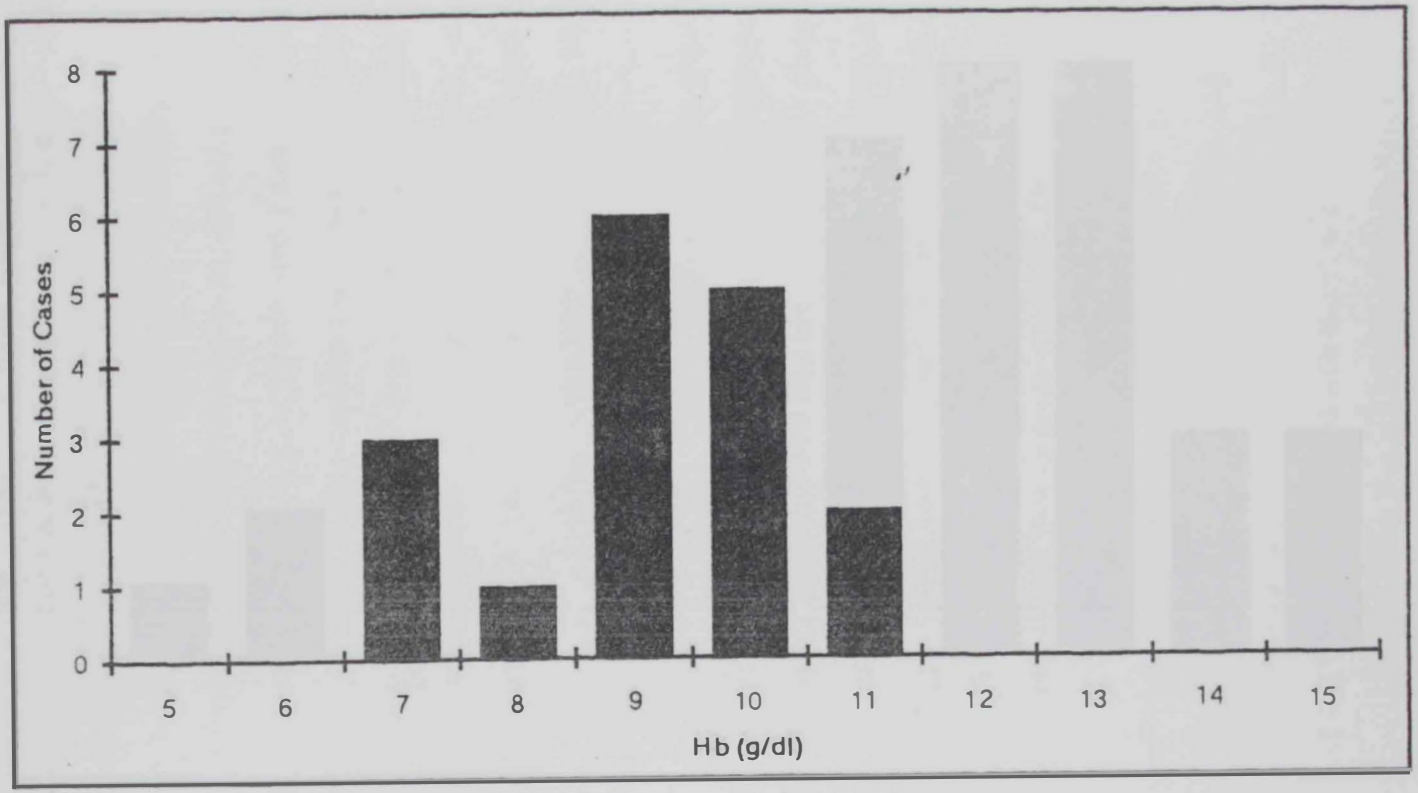

Figure 25: Distribution of Hb (g/dl) among homozygous β IVSI-5 mutation population.

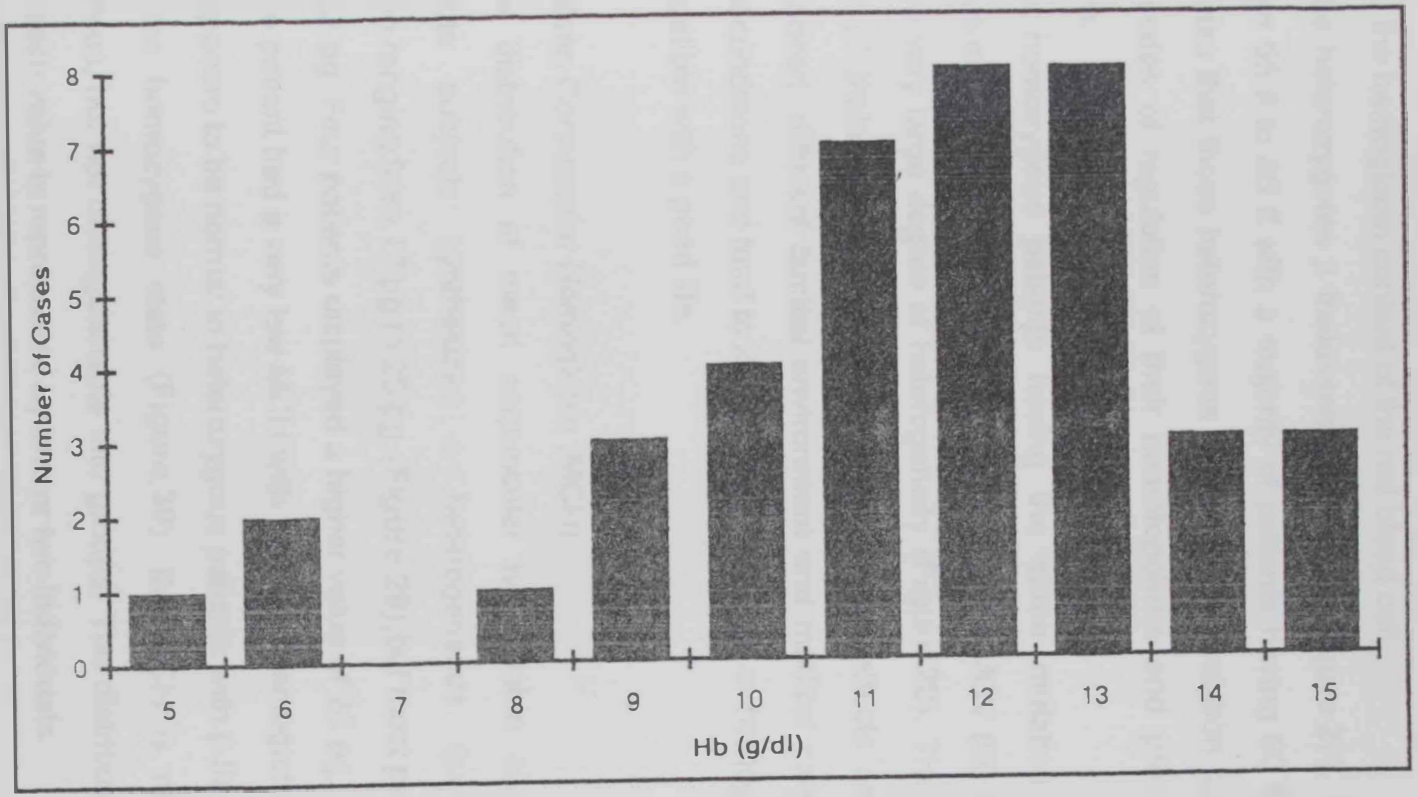

Figure 26: Distribution of Hb (g/dl) among among heterozygous β IVSI-5 mutation population.

3-The Erythrocyte Indices

a-Mean Corpuscular Volume (MCV)

The mean corpuscular volume indicates the average of the cell size present in the blood. It gives an idea of the hematopoietic situation of the patient and the hemoglobin content of the red blood cell.

In the heterozygotes β thalassemia subjects (**Figure 27**), the MCV ranged from 55 fl to 85 fl with a majority of patients having 60 fl to 70 fl. This indicates that those heterozygous for the same mutation could have different modes of regulation of their hematopoiesis and production of hemoglobin.

The homozygous patients having the same mutation on both homologous chromosomes show a wide spread of the MCV (50 fl to 85 fl) indicating a very large degree of heterogeneity (**Figure 28**). This mutation leading to β^+ thalassemia appears to have different effects on different patients. Indeed, different familial environment and medical care improve the clinical conditions and tend to keep a minimum level of hemoglobin and MCV compatible with a good life.

b-Mean Corpuscular Hemoglobin (MCH)

The distribution of mean corpuscular hemoglobin showed that heterozygote subjects synthesized a heterogeneous quantities of hemoglobin ranging from 17 pg to 25 pg (**Figure 29**),but most patients had 19 pg to 22 pg. Four patients displayed a higher value of 26 pg, 27 pg and 28 pg. One patient had a very low MCH with 17 pg of hemoglobin but, this situation appears to be normal in heterozygous patients with β thalassemia.

In the homozygous state (**Figure 30**) the MCH is much more heterogenous, but not distinguishable into groups. The distribution is very wide and each value is represented by one or two individuals.

4-Fetal Hemoglobin Expression

Concerning the β thalassemic samples, it was not possible to study the F cell distribution, these samples were already studied at the phenotype

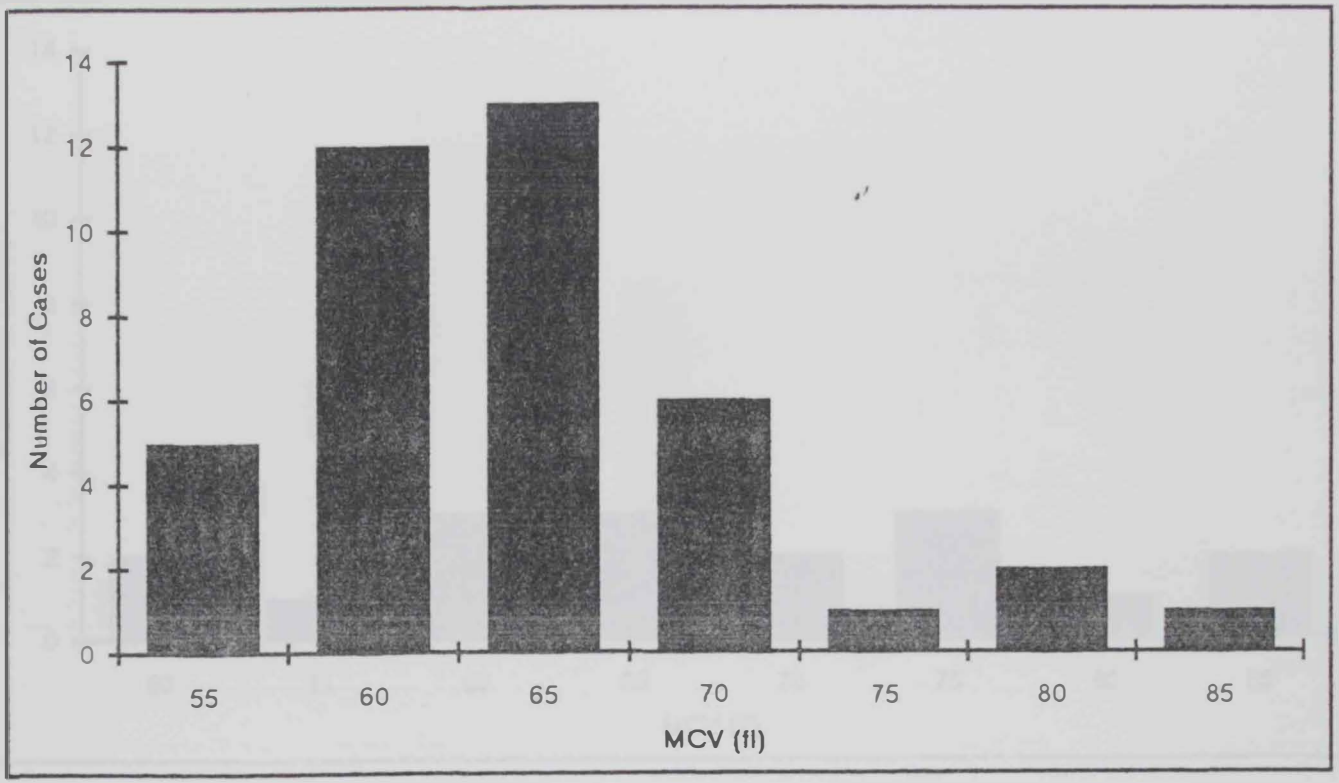

Figure 27: Distribution of MCV (fl) among among heterozygous β IVSI-5 mutation population.

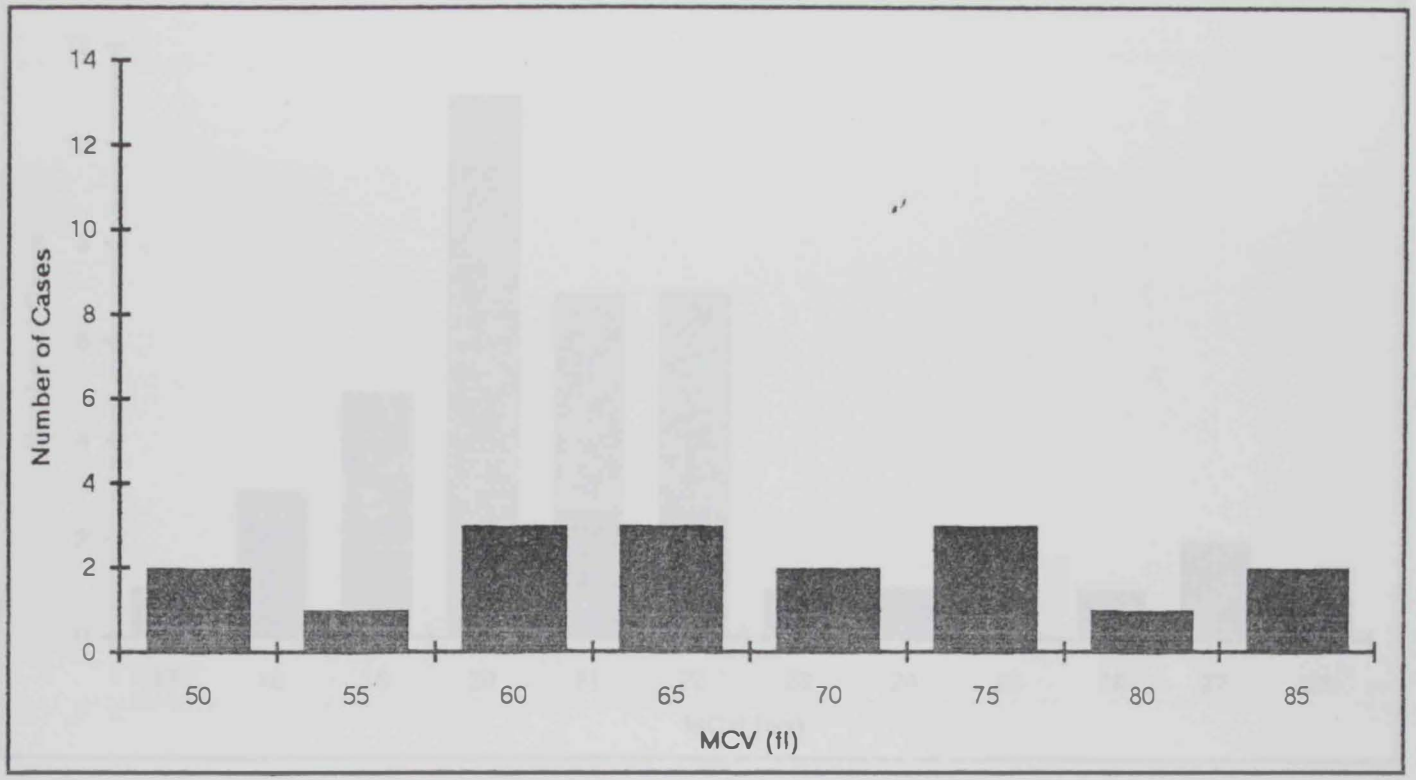

Figure 28: Distribution of MCV (fl) among homozygous β IVSI-5 mutation population.

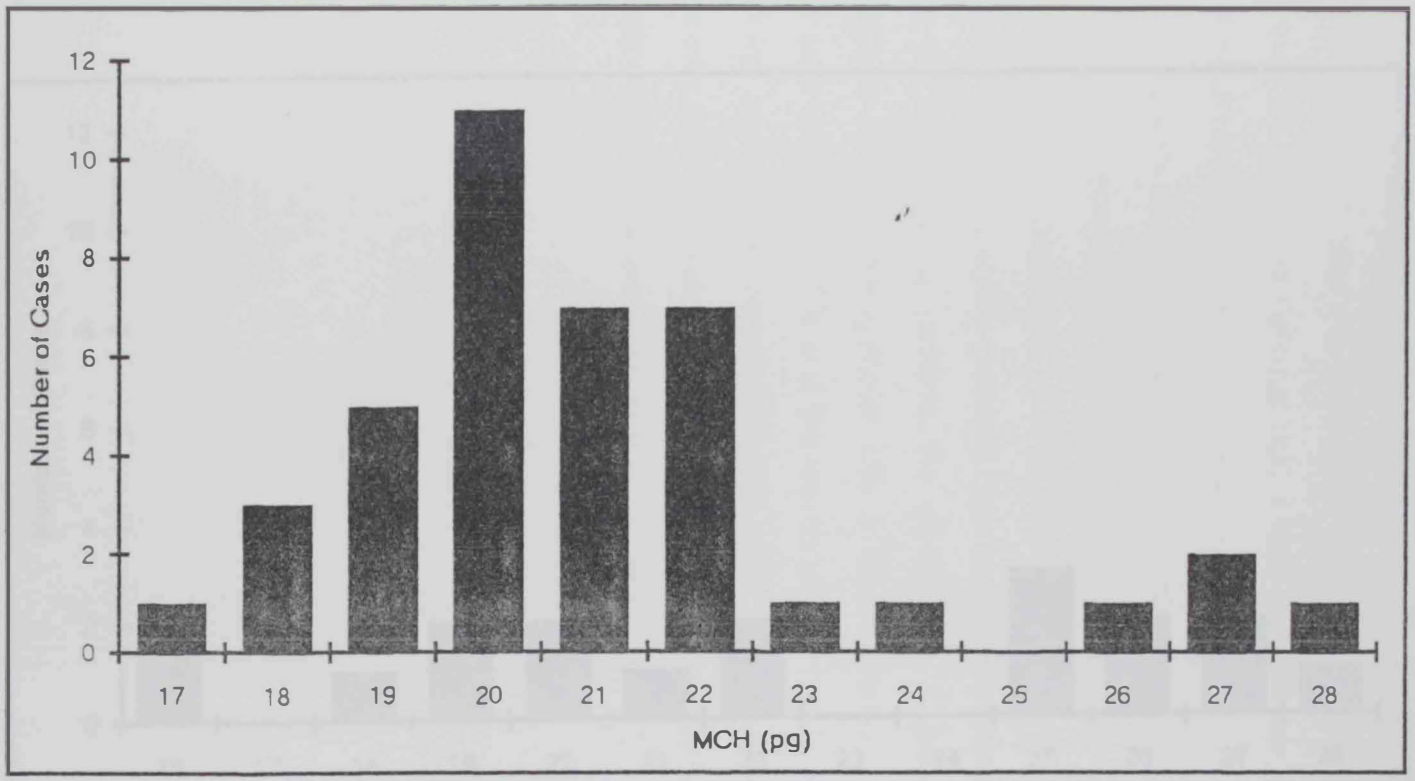

Figure 29: Distribution of MCH (pg) among among heterozygous β IVSI-5 mutation population.

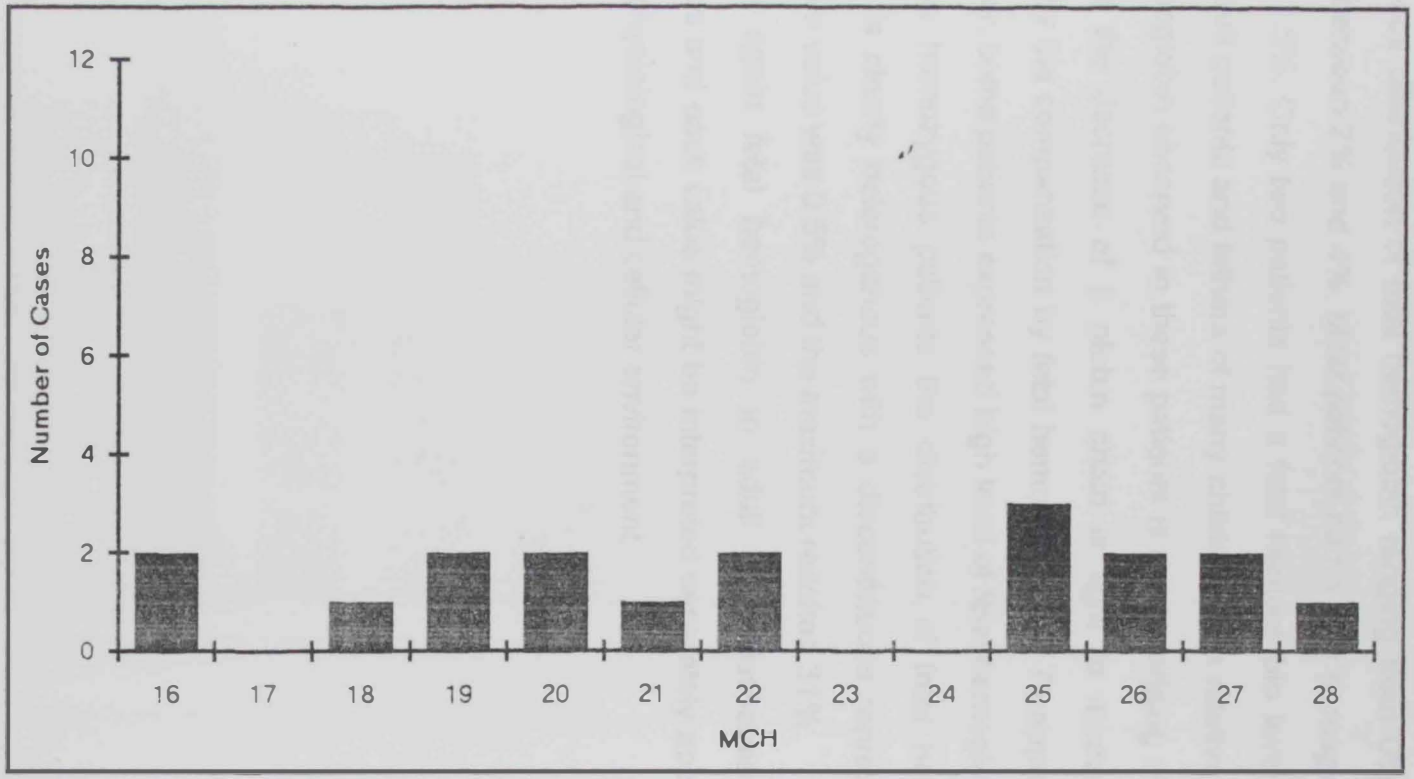

Figure 30: Distribution of MCH (pg) among homozygous β IVSI-5 mutation population.

level in order to determine the type of mutations. The results reported here about the fetal hemoglobin are those obtained from the medical file of each patient.

In the heterozygous state (figure 31) it was observed that there is a heterogeneous distribution of fetal hemoglobin ranging from 0% to 4.5% with a gap between 2% and 4%. Most patients had a fetal hemoglobin level of 0.5% to 1.5%. Only two patients had a fetal hemoglobin level of 4.5%. They are adult patients and fathers of many children. The relative low level of fetal hemoglobin observed in these patients is not surprising since in β^+ thalassemia the decrease of β globin chain is light to moderate, and subsequently the compensation by fetal hemoglobin (Hb F) appears to be low. However, some patients expressed high level of fetal hemoglobin.

In the homozygous patients the distribution of fetal hemoglobin (figure 32) is clearly heterogenous with a discontinuous representation. The minimum value was 0.5% and the maximum reached 31%.

Once again fetal hemoglobin in adult β^+ thalassemia is very heterogenous and each value might be interpreted separately according to the special physiological and cellular environment.

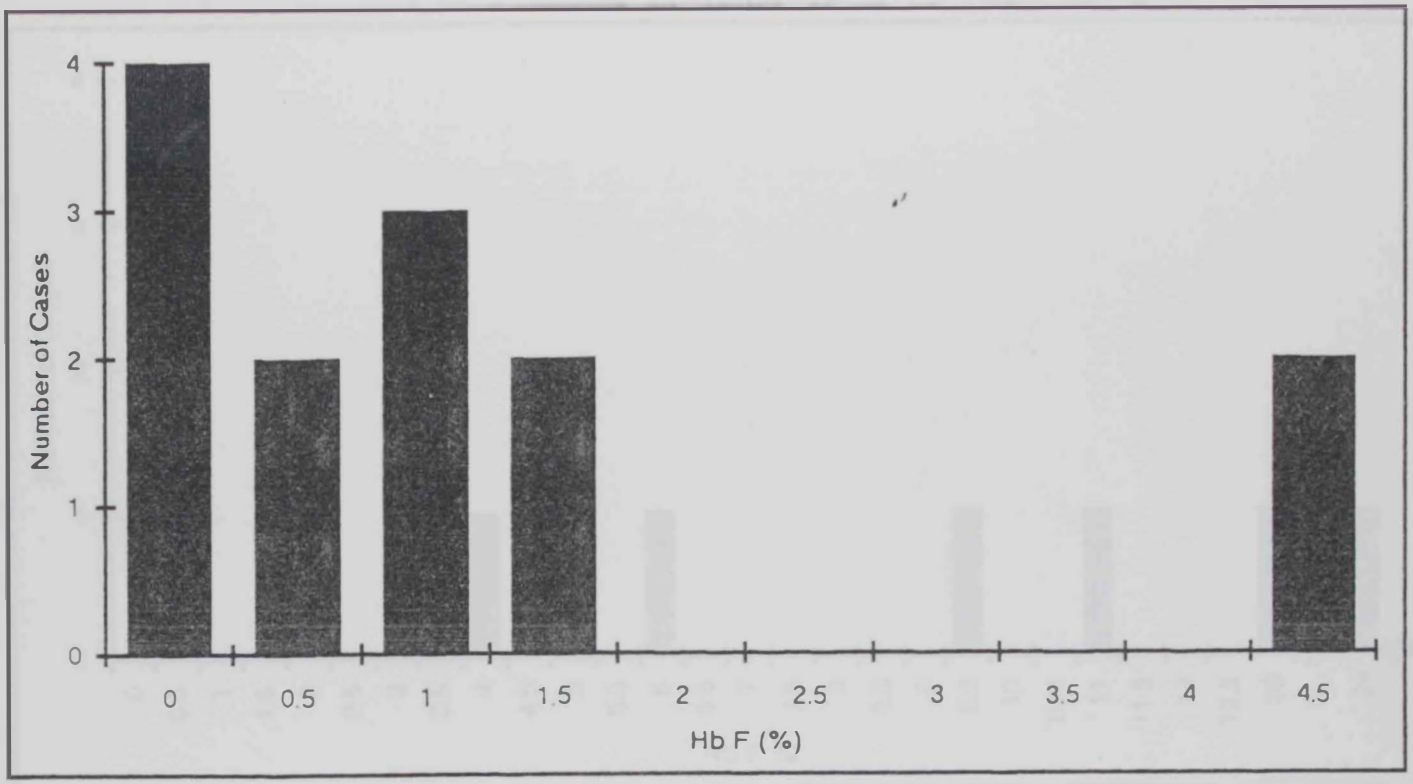

Figure 31: Distribution of Hb F % among among heterozygous β IVSI-5 mutation population.

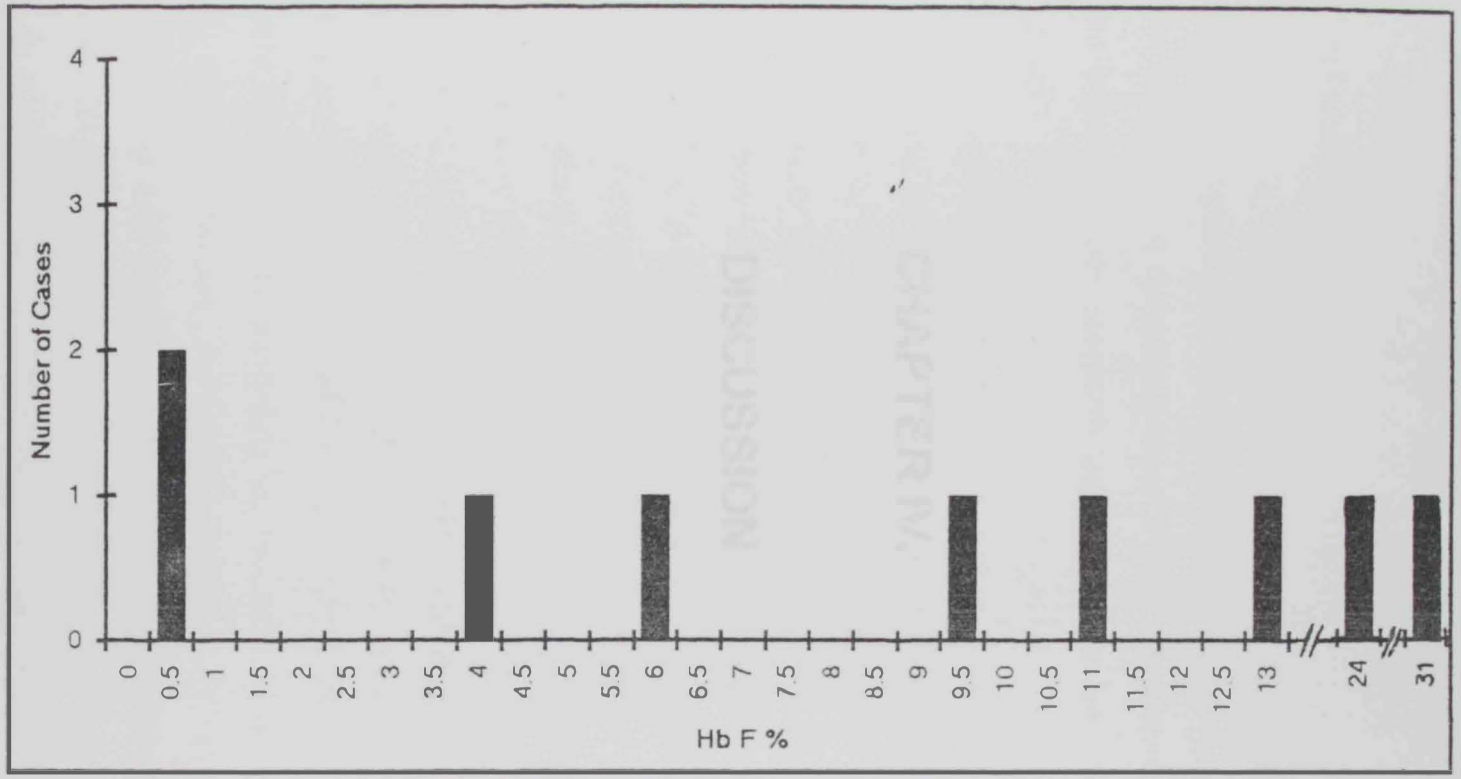

Figure 32: Distribution of Hb F % among homozygous β IVSI-5 mutation population.

CHAPTER IV.

DISCUSSION

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DISCUSSION

Hematological data

The hematological data and fetal hemoglobin expression presented in this work concerned 4 different groups of UAE population: the reference group, the anemic group, pregnant women group, and β^+ thalassemic patients group.

Concerning total hemoglobin expression and the erythrocyte indices, we used the normal values obtained in our normal population as reference. The normal ranges for hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) are similar to those reported in other normal populations. Indeed, the normal hemoglobin was 14.83 g/dl for males and 13.02 g/dl for females, the MCV was around 83 fl, MCH was 27.5 pg. These results are not surprising because there are no physiological conditions leading to changes in hemoglobin concentration or erythrocyte indices.

The anemic population formed of 171 patients with hemoglobin of less than 12 g/dl, exhibited a characteristic pattern of microcytic hypochromic anemia with MCV of 75 fl and MCH 24 pg. The anemia patients were not subdivided according to the cause of anemia. Certainly there are different causes for the anemia such as thalassemia (**Giblett,1969; Klug and Cumming,1986; Weatherall,1990; Emery and Mueller,1992; Olivieri,1992**) or G-6-PD deficiency, and acquired, like iron deficiency or parasitosis (**El Hazmi,1985**). The parameters observed here can not be used as a model for anemic patients since many factors work together to give the picture seen in our work. The determination of the total hemoglobin, decrease in cell volume and the lack of pigment within the red blood cell are certainly due to different factors genetic and environmental.

Most of our patients of this group had a disturbed hemogram and attended the clinic for hematological investigations.

Pregnancy was used as one of the physiological factors leading to changes in hematological data (**Weatherall and Clegg, 1981; Anyaegbunam et al.,1989**). However, this state depends on the age of pregnancy and on the proper conditions of each individual because a large numbers of our samples were obtained from pregnant women after mid term of pregnancy. We did not observe much variation in the hemoglobin concentration. Indeed, the mean value of hemoglobin was 11.65 g/dl which is less than the normal value but higher than that observed in the anemic female population. This value appears to indicate the presence of anemia but this condition is observed in pregnant women and it is most likely due to the simple phenomenon of increased blood volume and increased fetal hemoglobin during pregnancy.

The β^+ thalassemic patients have been chosen in order to evaluate the hematological data and fetal hemoglobin production in a homogeneous group bearing the same genetic mutation characterized at the molecular level and known to us as the β^+ thalassemia (β IVSI-5 G→C) (**Quaife et al.,1994**). This group is characterized by a broad variation in all parameters. We could not measure a mean value and took into consideration the ranges. The total hemoglobin ranges from 7 g/dl to 11.2 g/dl in the homozygous patients and 5.6 g/dl to 15.9 g/dl in the heterozygous patients. This large heterogeneity was observed in different patients of the same family indicating once more that hemoglobin production is under many controlling factors other than genetics.

The erythrocyte indices MCV and MCH are typical of β thalassemia. There is a clear microcytosis in both homozygotes (MCV ranges from 50 fl to 85 fl) and in heterozygotes (MCV ranges from 55 fl to 85 fl). It was established that no iron deficiency was interfering in these patients and the results obtained here are the profiles of β thalassemia only and any other environmental factors leading to a change in the hematological parameters. The patients with an MCV of 85 fl are patients with an increased

reticulocytosis and presenting with a marked poikilocytosis and young cells. The MCH is also variable from one individual to another, but always indicating the typical hypochromia of β thalassemia patients. This hypochromia was remarkably important in some individuals reaching a low level of 17 pg. The quantity of hemoglobin within the red blood cell of patients having the same genetic mutation appears very heterogenous, This leads us to think that the hemoglobin synthesis within the red blood cell is mediated by the genetic background of each patient and the environment in which this background is expressed.

Fetal Hemoglobin Expression

Fetal hemoglobin ($\alpha_2\gamma_2$) in adults persists normally in a small proportion of erythrocytes called F cells. Synthesis of γ chains in these cells in probably completed very early in their mutation and fetal hemoglobin represents only about 10%-30% of the hemoglobin of the fetal cells (**Weatherall and Clegg, 1981**). Population data indicates that among normal individuals, F cells frequency has a skewed distribution (**Pembery et al.,1978; Weatherall and Clegg, 1981**). About 3% of Europeans have more than 8% F cells and family studies have indicated that this is inherited as a heterozygous trait from dominant gene (**Bunn and Forget,1986**). There is also good evidence that the erythrocytes containing fetal hemoglobin have a longer life span and subsequently are selected and protected against hemolysis (**Rapaport,1987**). Other works have demonstrated that fetal hemoglobin production is under the control of many different genetic factors acting in cis with the gamma gene and in trans in other locations (**Bunn and Forget,1986**). However, these works are still incomplete because there are always many exceptions to this rule. This situation gives place to other factors mainly environmental such as hormonal medication and different physiological or pathological conditions.

In our work fetal hemoglobin has been studied to explain some factors involved in its production. We used two different approaches to

quantify fetal hemoglobin. Four groups of populations were examined: normal population and three other groups namely anemic group, pregnant women group, and β^+ thalassemic patients group.

The mean value of F cells obtained in the normal group was about 1.2% according to our technique. This value appears to be in the normal ranges observed in other populations (**Chiewslip et al.,1991**) and will constitute a good reference for the interpretation of our results. There was no big difference between males and females who displayed a mean value of 1.16% and 1.29% respectively. This difference was not statistically significantly different. In the same group the proportion of fetal hemoglobin among the total hemoglobin is also similar in males and females and the mean level was always less than 1% with an approximate value of 0.5%. In this group we did not observe any correlation between fetal cells and fetal hemoglobin production and it was failing to support the belief that in subjects with high fetal cells we observed a high level of fetal hemoglobin (**Weatherall and Cegg,1981**).

In the anemic group there were more F cells in the blood smear and the average F cell was around 2%. It is evident that some patients had no fetal cells in their blood but most of them displayed a different proportion of fetal hemoglobin. In this group also there was no difference between males and females. The mean level for F cells in anemic males was 1.93%, in females it was 1.746%. This difference was not significant and both males and females were considered to have the same range. The conclusion from this group is that fetal hemoglobin continued to be expressed in anemic conditions and may be caused by genetic or environmental factors.

Pregnancy was considered a physiological nongenetic condition in which fetal hemoglobin could be present in adult blood (**Anyaegbunam et al., 1989**). In fact, pregnant women had a heterogenous distribution of fetal cells with a mean value of 0.785%. This result is interpreted as a normal value. We did not observe any abnormal rise in fetal cells production most likely because of the age of pregnancy. Parallel to Fetal cells the fetal hemoglobin proportion was also normal expressed heterogenously but with

a mean value of 0.19%. The results obtained from the normal females and the pregnant women indicate that there is no sex linked genetic factors modulating the fetal hemoglobin expression as reported in some papers (**Morris et al.,1991; Dover et al.,1992**).

The β^+ thalassemic patients displayed a marked differences in fetal hemoglobin expression. Unfortunately the fetal cells were not reported in this work because it was not possible to do this study when patients attended the clinic. The results of fetal hemoglobin are only those that we obtained from the patients files. The fetal hemoglobin concentration was very heterogenous and there were a large variations from the heterozygous to the homozygous patients. In the heterozygous group the range was narrow and limited (0%-4.5%). These differences in expression are not the result of genetic factors since we are dealing with the same mutation (β IVSI-5 G→C). Some other factors are responsible for fetal hemoglobin expression and could reflect hormonal, cellular and / or environmental variations. However, the homozygotes patients exhibit a large variation from 0.5% to 31%. The big differences observed between patients with an identical genetic defect indicates that other factors could be involved and give a different fetal hemoglobin expression. Indeed the patients with high level of fetal hemoglobin (such as 24% and 31%) are young female children who have a delay in the fetal hemoglobin switching leading to a long persistence of fetal hemoglobin which is not observed in the other patients. This large difference in expression within a homogenous genetic population and sometimes within the same family imposes the problem of fetal hemoglobin regulation. How can patients having the same genetic mutation express different amount of fetal hemoglobin?.

Many suggestion could be provided:

Since the fetal hemoglobin is a factor of improvement of the thalassemia condition and clinical symptoms as shown by **Bordin et al.,1989; Dover and Charache,1989; Powars et al.,1989; Franklin,1990; Al Awamy et al.,1991**, it could be understood that if there is a good follow up of the patient and good care there is no need for fetal hemoglobin production.

Surprisingly, this hypothesis is untrue since many patients exhibiting a rised in the level of fetal hemoglobin suffer from their disease (**El Hazmi,1982; Bayoumi et al.,1988; Awaad and Bayoumi,1993**). However, patients with low level of fetal hemoglobin support their thalassemia. These controversial observations lead us to assume that fetal hemoglobin production could be under several determinants such as:

-Genetic determinant which regulate the gamma chain production, this depends on the integrity of the gamma gene and its environment.

-The selection of erythrocyte containing fetal hemoglobin (F cells) which survive longer than the other cells (**Bunn,1991**) and by this fact give rise to fetal hemoglobin.

-A nongenetic factor regulating the chain association with the heme in the cytoplasm.

CHAPTER V.

CONCLUSIONS

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CONCLUSIONS

The production of fetal hemoglobin is under the control of many factors, the majority classified as genetic and the nongenetic factors. The nongenetic factors are physiological factors (i.e. pregnancy, and rapid erythroid regeneration) and the acquired disorders. The genetic factors are the hemoglobinopathies which can be divided into the structural variants (i.e. sickle cell hemoglobin) and disorders of synthesis (i.e. β thalassemias).

Samples of normal (nonanemic) subjects, anemic subjects, pregnant women and a group of β^+ thalassemic patients of UAE population bearing the same mutation were studied for fetal hemoglobin expression and erythrocytic indices. The expression of fetal hemoglobin has been studied by two approaches. The counting of F cells in a blood smear and the quantitation of fetal hemoglobin. The hematological data of the samples have been provided by Al-Ain hospital.

From this study we managed to establish a normal range reference. Also we conclude that fetal hemoglobin expression (fetal hemoglobin and F cells) is very heterogeneous and not related to the sex. Furthermore, the environment plays a large role in fetal hemoglobin expression and on the hematological indices as well.

CHAPTER VI.

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CHAPTER VII.

APPENDIX

Appendix I: The general characteristics and hematological data among normal population.

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
118	F	43	14.1	93	30.8	0	0.130
500	F	ad. *	13.6			1	0.104
7	F	ad.	13	75	27.1		0.117
30	F	33	14	86	30		0.134
484	F	40	12.4	77	27.4	0	0.137
65	F	22	13.1	70.4	22	1	0.145
136	F	38	12.4	88.1	28.7	1	0.156
435	F	3	12.3	74.9	24.2	0	0.170
45	F	19	13.5	89	29.7		0.188
388	F	20	13.3	79.4	25.1		0.195
112	F	80	12	89.4	28.3	0	0.205
140	F	33	12.2	89	30.9	1	0.211
74	F	40	12.5	81.5	26.7	0	0.226
161	F	30	13.9	87	28.6	2	0.228
123	F	11	12.2	74.5	24.3	1	0.238
69	F	28	13.7	85.2	28.3	0	0.269
108	F	ad.	12	86.4	28.1		0.271
423	F	11	12.4	69.7	22	1	0.272
478	F	22	12.6	79	26.4	2	0.273
153	F	22	13.5	83.4	27.4	0	0.277
445	F	33	12.2	77.8	25.1	2	0.277
469	F	12	13.1	85.6	27.4	0	0.282
148	F	6	12.4	80.5	25.8	4	0.295
47	F	29	12.9	89.7	29.9	3	0.300
54	F	ad.	12.1	83.8	27.8		0.301
85	F	32	12.5	91.5	29.9	1	0.302
129	F	ad.	13.2	70.7	21.9	3	0.309
86	F	40	13.5	83.6	26.6	0	0.319
101	F	ad.	13.7	77.8	25.6	1	0.322
410	F	15	13.2	80.6	26.2		0.324
187	F	20	12.3	91.4	29.4	2	0.328
99	F	ad.	13.5	86	27.8	1	0.343
485	F	ad.	12.3	94.5	30.6	1	0.353
195	F	28	14			1	0.359
257	F	ad.	14.8	85.6	28.3	0	0.375
405	F	43	13.1	89.5	24.2		0.379
15	F	10	13.5	84	24.2		0.387
416	F	32	12.3	84.8	27.7	2	0.396
151	F	25	12.6	80.4	26.6	2	0.398
230	F	35	12.4	84.8	28.7	3	0.406
474	F	25	12.2	78	28	0	0.410
350	F	22	13.5			1	0.422
443	F	20	13.6	81.8	26.2	0	0.430
119	F	27	14	92.3	20.5		0.436
211	F	60	12.7	85.3	36.3		0.450

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
501	F	ad.	12			1	0.471
83	F	27	12.6	79.6	25.3	0	0.478
483	F	30	13.1	86	32	1	0.484
135	F	9	12.7	80.9	26.8	2	0.497
302	F	15	12.4	85.3	28		0.510
363	F	29	12.4	82.4	26.8	2	0.522
152	F	30	14.3	81.6	26.1		0.534
182	F	70	15.9			1	0.537
437	F	24	14	84	27.2	1	0.542
124	F	33	14.3	87.9	28.7	1	0.547
291	F	11	13.5	83.5	27.6	2	0.552
312	F	ad.	12	84.5	27.5	3	0.557
381	F	30	12.7	85.4	28.4		0.566
98	F	20	13	90.9	29.8	1	0.569
243	F	30	13.2	91.2	30.9	1	0.580
399	F	3	13.7	80.4	26.4		0.580
207	F	7	12.7	78.5	25.6		0.584
427	F	48	12.7	72.1	35.3	1	0.586
91	F	ad.	12.2	78.2	26.3	1	0.599
409	F	30	12.9	85.4	28.3		0.614
348	F	36	12.7	81	26.6	2	0.616
82	F	25	13	86.6	27.7	1	0.623
351	F	22	13.4			1	0.630
289	F	30	12.2	73.5	24	41	0.632
49	F	ad.	12	87.5	28.5	1	0.644
224	F	32	14.1			1	0.646
422	F	2.42	12.3	86.3	27.9	0	0.652
439	F	32	13.8	81	26.2	5	0.658
273	F	1	12.2	79.3	26.3		0.664
154	F	31	14.1	87.2	28.6	0	0.667
360	F	62	14.6	92.2	30.5	3	0.675
453	F	ad.	13.4	82.1	26.6		0.676
327	F	ad.	14	86.4	28	3	0.699
102	F	24	12.4	89.8	29.6	1	0.708
205	F	13	12.6	79.8	26.4	3	0.710
460	F	0.67	13.5	76	24.3		0.715
417	F	7	12.8	76.8	24.9	1	0.728
318	F	ad.	13.4	79.2	25.2	1	0.736
309	F	ad.	13.5	85.9	28.6	3	0.746
347	F	56	12.9	84.2	26.6	0	0.778
294	F	25	14.4	85.1	27.2	1	0.791
221	F	42	12.9			0	0.793
324	F	ad.	13.3	91.5	29.2	3	0.827
455	F	ad.	13.4	85.7	28.1		0.828
320	F	ad.	12.8	85.6	28.4	2	0.831
362	F	30	14.1	83.9	27.1	0	0.833
353	F	20	13.4	79.2	25.4	2	0.846

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
406	F	30	12.9	89.5	29.5		0.847
106	F	ad.	12.4	74.3	24.3	1	0.848
266	F	14	14.1			2	0.892
14	F	40	14.1	81	29.5		0.895
451	F	37	12.9	91.6	30.9		
262	F	40	12.9	95.2	31	2	
459	F	3	12.8	88.6	28.7		
434	F	ad.	12.3	87.2	27.8	0	
196	F	30	13.1			2	
303	F	ad.	13.9	90.2	29.5		
55	F	ad.	12.3	90.7	29.4		
160	F	33	13.2	86.6	28.1	0	
449	F	13	13.2	74.1	23.5	1	
226	F	70	13	82.1	28.2	0	
42	F	26	12.9	81.9	26.6	0	
323	F	ad.	13.7	88.6	29.1	2	
201	F	ad.	13.5			3	
332	F	29	14.5	94.2	30.2		
97	F	35	13.4	84	27.4		
4	F	ad.	12.8	83	28.9	3	
313	F	ad.	13.3	77.6	24.6		
142	F	21	12.6	68.1	21.4		
203	F	ad.	13			5	
438	F	33	13	79.5	25.5	1	
137	F	18	12.5	81.6	26.3	1	
359	F	19	12.8	95.1	31.8	1	
223	F	17	14			3	
370	F	31	14.9	86.1	28.2	1	
361	F	33	13.3	90.4	29.3	0	
59	F	22	13.5	75.3	24	0	
404	F	0.01	15.6	102.8	33.5		
26	F	30	12.8	83	31		
28	F	30	12.7	84	28.3		
68	F	ad.	12.8	74.8	24.3	0	
150	F	26	13.2	85.5	27.7	2	
9	M	ad.	17.2	79	30		0.250
31	M	30	14.6	80	29.6		0.510
21	M	37	13.9	85	30.7		0.640
8	M	ad.	14.8	87	30.8		0.860
93	M	24	12.5	74.1	22.8	1	0.108
32	M	ad.	13.4	89	26.8		0.108
115	M	40	14.2	82.7	26.4	0	0.110
494	M	25	18			1	0.118
498	M	45	17.3			2	0.118
131	M	40	13.3	87.9	29.8	3	0.120
17	M	5	12.6	62	22.9	0	0.122
287	M	ad.	12.6	98.3	32	1	0.126

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
5	M	ad.	17.3	86	31.5	3	0.132
111	M	ad.	16.3	94.6	31.2	2	0.135
390	M	36	17.3	83	23.9		0.142
389	M	48	15.6	87.8	28.8		0.145
35	M	29	15.3	89.2	29.7	0	0.164
341	M	ad.	16.9	81.2	26.2	2	0.168
479	M	ad.	16	79.1	25.5	1	0.169
468	M	12	13.4			0	0.170
436	M	7	13.8	79	25.5	2	0.171
278	M	46	16.1	78.3	25.4	0	0.178
66	M	45	15.2	77.6	24.3		0.178
392	M	32	15	90.6	29.5		0.179
61	M	ad.	15	92.1	29.9		0.180
259	M	ad.	15.3	81.2	26.6	0	0.181
67	M	8	13.5	79.1	25.1		0.181
487	M	30	16	90	29.5	1	0.189
81	M	ad.	14.8	88.3	28.2		0.195
110	M	ad.	15.2	82	26.6	0	0.199
476	M	17	13.8	76	24.4	2	0.202
75	M	60	16.2	82.8	27.3	0	0.207
379	M	26	12.9	61.2	18.9		0.207
492	M	ad.	14.5	81.2	26.9	1	0.209
475	M	55	19.4	91.2	29.7	1	0.213
490	M	ad.	16.3	84.7	27.8	2	0.215
414	M	25	15.1	84.4	27.9		0.216
76	M	ad.	16	82.6	27	1	0.218
141	M	13	14.1	82.7	26.8		0.220
116	M	45	16.4	81.6	26.9	1	0.228
84	M	ad.	13.1	80.5	26.3		0.234
70	M	20	14.5	78.7	25.1	0	0.238
171	M	ad.	13.4	83.4	27.2	1	0.238
40	M	3	13.8	79.8	25.4	0	0.239
488	M	22	14.5	81.2	26.2	1	0.240
166	M	53	14.4	84.7	27.4	0	0.242
345	M	35	13.2	86.3	28.2	0	0.250
79	M	ad.	17	91.3	29.7		0.251
279	M	43	14.7	79.8	25.8	1	0.256
90	M	ad.	17.6	83.4	27.1	0	0.265
77	M	26	17	93.3	30.9	1	0.265
128	M	25	14.6	86.7	28.8	3	0.272
60	M	ad.	17.5	83	26.5	0	0.273
130	M	30	13	76.9	25.2	1	0.278
2	M	37	13.9	86	30	4	0.278
496	M	ad.	16.7			1	0.302
380	M	24	14	89.3	24.2		0.302
36	M	70	16.2	81	26.3	2	0.303
126	M	40	16	88.6	29.5		0.303

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
73	M	50	12.6	64.7	19.6	0	0.306
165	M	25	15.4	87.4	29.1	0	0.311
256	M	45	18.2	96.5	32.4	0	0.314
493	M	4	13.2			1	0.314
413	M	8	13.9	77.5	23.1		0.314
504	M	23	16.7			0	0.317
272	M	45	15.5	90.3	29.5	0	0.321
431	M	ad.	16.6	84	26.9	1	0.322
194	M	60	12				0.326
276	M	14	13.3	76.1	24.5	1	0.330
252	M	50	13.9	80.8	26	0	0.336
491	M	11	14.1	82.4	27	1	0.340
393	M	29	15	83	29.5		0.347
12	M	ad.	15.1	82	30.2		0.348
391	M	ad.	17.3	83	23.9		0.349
57	M	30	16.2	89.9	29.2	0	0.350
248	M	11	14.1		33.6	1	0.356
261	M	28	16.1	87.4	25.4	4	0.356
264	M	ad.	14.5			1	0.359
344	M	30	13.8	89.3	29.2	1	0.364
275	M	11	12.2	74.3	24.5	2	0.375
342	M	ad.	13.8	85.6	28.1	4	0.378
339	M	12	14.1	75.1	24.4	2	0.380
127	M	28	13.5	91.7	30.6		0.383
378	M	46	14.7	79.7	26.3		0.384
333	M	45	14.1	81.2	26.2	1	0.397
447	M	10	13.1	79.7	24.8	0	0.398
349	M	40	12.6			1	0.405
301	M	10	12.3	80.9	25.5	3	0.408
471	M	ad.	14.7	83.2	27.1	1	0.411
470	M	25	16.2			0	0.415
499	M	36	12.8			1	0.417
486	M	40	15.5	92.3	20.2	1	0.418
13	M	36	15.8	83	30		0.419
1	M	ad.	16.1	91	34.7	0	0.422
158	M	38	15.6	79.6	26.2	1	0.428
186	M	28	14.3	78.7	25	2	0.428
281	M	54	12.9	69.9	21.6	3	0.432
430	M	33	14.6	90.6	29	2	0.436
163	M	ad.	12.9	83.5	27.4	0	0.441
104	M	ad.	16.2	86.4	28.3		0.443
330	M	0.5	12			1	0.448
411	M	4	14	80.8	27		0.448
89	M	ad.	16.5	91.2	29.5		0.467
263	M	35	17.2			1	0.499
314	M	ad.	16.3	86.7	28.3		0.500
178	M	40	15.7	95.8	3.5	4	0.507

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
331	M	65	12 7	81 7	26 1	0	0 511
260	M	ad	14 9	88 1	25.3	4	0.511
307	M	35	12			4	0 514
39	M	45	14	84 5	27 2	0	0 516
441	M	ad	16.7	88 2	29.1	2	0.523
147	M	36	15.4	95 3	30.2	1	0 525
220	M	31	13.7	81	25 6	4	0.525
244	M	45	18	90 3	30.3	4	0.526
241	M	ad	15.4	83.6	28	1	0.530
188	M	0 01	14 9				0.536
242	M	7	13.5	77 4	25.6	0	0.540
63	M	37	13.9	85 3	28.1		0.541
181	M	ad.	15.4	66 9	20.9	0	0.546
506	M	17	16 4			1	0.549
265	M	35	15 2	81 6	26	1	0.554
80	M	28	14 4	94.8	30.9	0	0.564
95	M	48	15.5	87 1	27 9		0.574
463	M	ad.	12.7	84 4	27.5		0.579
193	M	ad.	15	89 9	28.9	1	0.592
176	M	8	12.4	81	26.3	1	0.594
296	M	25	15.3	86.2	27.8	1	0.594
366	M	25	15.9	80.2	25.9	1	0.603
185	M	55	14 5			0	0.606
271	M	25	14 9	77 6	25.1	1	0.606
183	M	37	12.9	85 4	28.5	2	0.610
444	M	42	16.9	87 7	28.3	2	0.622
295	M	50	13.8	89	29.3		0.630
105	M	ad.	16.5	87.9	28.8		0.643
457	M	5	12.9	80.6	26.9		0.655
329	M	ad.	17.6			3	0.658
222	M	11	13.9	83	27	1	0.667
173	M	ad.	14.6	65.5	20.6	0	0 670
177	M	42	16	80.9	26.5	0	0.672
246	M	ad.	15.1	86.7	28.6	1	0.678
107	M	ad.	15.2	84.9	28.5	3	0.691
368	M	50	17.7			2	0.703
133	M	38	16.5	88.7	28.9		0.709
374	M	20	14.2		30.3	3	0.711
398	M	38	13.2	72.1	22.4		0 714
464	M	ad.	14	86.6	28.8		0.725
219	M	ad.	17.7	84 8	27.7	0	0.737
218	M	52	14.4	84 8	28.4	0	0.738
270	M	ad.	16.2	91	30.3	2	0.748
343	M	ad.	12.5	84 1	26.9	0	0.751
424	M	41	17	89.1	29.6	2	0.783
401	M	85	13.2	83.3	25.8		0.785
6	M	ad.	17 2	83	30 7	41	0.788

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
245	M	35	16.1	93.9	31.4	1	0.705
146	M	42	16.2	91.2	30.4		0.820
315	M	32	16.1				0.821
316	M	10	12.3	83.6	27.3	3	0.822
58	M	38	15.1	95	31		0.822
20	M	3	12.7	82	27.6		0.825
125	M	ad.	18.1	85.1	28	0	0.850
502	M	30	17.1			0	0.856
157	M	15	14.7	83.3	22.3	1	0.866
452	M	3	12.2	80.2	26.6		0.866
505	M	25	14.5			1	0.882
172	M	53	14.1	99.8	33.8	0	0.890
467	M	17	13.4	81.7	26.7		
96	M	31	15	82.1	26.1		
317	M	50	12	71.8	21.9	3	
169	M	33	14.6	92.5	29.5	0	
415	M	35	16.5	88.5	28.8		
217	M	ad.	14.9	87.3	29.7		
206	M	8	12.3	79.2	26.4	2	
376	M	30	12.7	81.1	26.2	2	
156	M	46	15.5	88.9	28.6	1	
29	M	ad.	15.3	83	29.9	3	
503	M	30	15.8			1	
325	M	ad.	16	81.5	26	1	
277	M	30	16.8	93.5	31.1	3	
44	M	45	15.7	81	27	1	
458	M	5	12	74.9	24.3		
466	M	35	16.4	86.6	28.4		
235	M	13	14.2		24.4	1	
10	M	ad.	16	80	29.5	44	
159	M	30	15.3	86.9	28.5		
305	M	30	15.2	89.4	29.7	2	
268	M	26	12.4			0	
179	M	50	14.2	79	24.7	0	
267	M	22	13.2			0	
462	M	13	13.3	84.9	28.2		
92	M	1	12.9	71	22.2	0	
394	M	17	15	83.6	27.4	1	
371	M	ad.	15.3	90.8	30.5	4	
143	M	70	12.4	80.4	25.7		
94	M	32	17.4	88.2	38.9	1	
227	M	25	13.7	80.5	25.7	0	
228	M	45	15.2	78.8	25.8	1	
149	M	58	16.4	91.3	29.9	1	
465	M	ad.	15.3	81.3	25.8		
138	M	11	12.4	77.3	24.6	1	
216	M	11	13.7	85	28	1	

Number	Sex	Age (year)	Hb (g/dl)	MCV (fl)	MCH (pg)	F cells (%)	Hb F (%)
197	M	0.03	17.4				
229	M	45	12.6	86.2	27.5	1	
52	M	17	13.7	77.2	24.7	1	
33	M	ad.	15	94.7	30.5	1	
198	M	11	14			0	
225	M	39	14.2	78.3	25.5	2	
37	M	30	16.2	84.1	27.7	0	
254	M	49	13.9	80.1	25.8	0	

* Note
ad adult

Appendix II : The general characteristics and hematological data among anemic population.

Number	Sex	Age (year)	Hb (g/dl)	MCV (ft)	MCH (pg)	F cells (%)	Hb F (%)
311	F	ad.*	10.4	68.1	21.5	2	0.13
365	F	35	11.9	91.2	30.1	1	0.139
174	F	40	10.4	83.6	27.3	1	0.155
204	F	39	8.5				0.167
357	F	ad.	11.9	77.6	24.6	0	0.176
403	F	80	10.4	83.4	25.3	6	0.185
418	F	60	11.6	87	29.2	2	0.194
145	F	13	11.1	70.1	21.6	2	0.196
192	F	25	8.9	84.2	27.6		0.202
334	F	65	9.4			5	0.204
213	F	70	8.8	86.6	27.5	1	0.208
358	F	22	11.9				0.211
232	F	27	9.1	72.1	22.3	3	0.215
283	F	ad.	9	73	23.3	0	0.227
233	F	26	10.4	74.7	23.6	1	0.231
375	F	55	10.8	93.3	31	0	0.232
3	F	ad.	11.1	70	23.9	1	0.233
408	F	25	11.4	75.8	25.3	3	0.236
38	F	24	6.1	54.7	15.9		0.238
356	F	ad.	9	73.4	23.2		0.238
88	F	ad.	9.9	69.4	21.7		0.242
355	F	ad.	11.9	93.7	30.9	1	0.247
419	F	17	10.7	71.1	22.3	1	0.248
237	F	30	11.7	88	29.6		0.251
372	F	ad.	11.5	75.2	23.2	5	0.267
319	F	ad.	9.8	75.8	23.5		0.274
170	F	32	11.7	89.4	23.9	1	0.28
234	F	32	10.8	83.8	21.6	0	0.289
442	F	ch.*	8.9				0.296
299	F	43	11.3	80.9	26		0.323
236	F	30	9.9	66.2	20.1		0.33
113	F	20	9.8	76.3	23.7	1	0.351
167	F	26	10.8		25.2		0.371
202	F	35	11.1			0	0.371
184	F	32	11.7	89.4	28.9		0.374
120	F	30	11.4	73.6	24.1	1	0.375
214	F	11	9	69.4	22	1	0.382
56	F	24	9.9	66.1	20.4	2	0.393
364	F	40	10.6	69.4	21.8		0.402
480	F	35	11			4	0.414
103	F	ch.	11	76.1	25.2		0.418
144	F	25	8.2	48.6	14.3	0	0.422
72	F	18	11.6	91	29.3	0	0.426
78	F	30	11.9	80.2	25.9	0	0.438
209	F	28	11.2	82.6	27	3	0.44
433	F	ad.	11.5	82.1	26.5		0.449
48	F	8	11.4	81.8	26.9		0.457
298	F	ad.	11.2	88	28.4	2	0.459
34	F	4	11.3	81.7	26.7		0.465
352	F	19	9.1			1	0.467
255	F	ad.	11			0	0.488
495	F	19	10.9				0.489
336	F	10	9.2	78	25.6	4	0.493
190	F	ad.	11	79.7	25		0.496

Number	Sex	Age (year)	Hb (g/dl)	MCV (ft)	MCH (pg)	F cells (%)	Hb F (%)
122	F	26	11.5	94.3	32.1	0	0.499
215	F	1.08	11	70.5	21.3		0.504
249	F	25	10.8	78.2	25.5		0.504
448	F	26	11.7	85.2	27.9	2	0.505
210	F	45	10.4	84.5	27.8		0.512
247	F	ad.	11			0	0.519
46	F	27	7.1	59.7	17.7		0.554
27	F	ad.	10.6	82	27.4		0.567
62	F	22	11.6	76	24.3	2	0.575
19	F	38	10.1	82	28.9	2	0.576
24	F	ad.	11.4	81	24.1	1	0.591
251	F	65	10.9	86.6	27.7		0.601
100	F	38	11.2	75.5	23.7	2	0.603
310	F	ad.	11.5	77.8	25.1	1	0.614
472	F	0.83	10	59.7	18.5		0.618
155	F	40	11.3	75.5		2	0.629
456	F	ad.	11.3	75.5	23.8	6	0.663
354	F	14	10.7	66.3	20.4		0.683
477	F	ad.	9.7	75	26	1	0.709
412	F	35	11.7	85.5	28.3	5	0.733
384	F	50	11	69.3	21.5	0	0.738
387	F	35	9.8	65.7	20.4	2	0.751
121	F	ad.	11.1	66.9	20.8	2	0.768
300	F	28	10.6	71.1	22.2	2	0.786
454	F	ad.	7.6	55.2	28.2		0.812
284	F	ad.	11.1	84.4	26.7	1	0.819
385	F	32	11.7	75.5	25.1	0	0.827
322	F	ad.	11.2	78.6	24.7		0.866
25	F	30	11.8	75	26.8	2	0.877
426	F	ad.	11.4	68.5	21.6	2	0.962
64	F	27	7.4	58.8	17.2		0.967
41	F	ad.	10.7	87.7	28.5	1	0.987
274	F	30	9.7	65.6	20.2	1	0.998
22	F	10	11.7	67	24	3	1.005
269	F	ad.	11.2			1	1.014
304	F	ad.	8.8	63.8	19.4		1.079
373	F	0.67	10.7	65.6	20.3	1	1.114
396	F	ad.	11	69.5	22.1	6	1.131
420	F	20	10.9	57.6	17.9	5	1.137
293	F	50	11.3	66.8	20.7	1	1.169
231	F	70	7.8	76.9	24.3	0	1.173
397	F	ad.	10.9	78.4	25.1	4	1.26
164	F	4	11.6	79.2	26.1	1	1.316
139	F	25	11.7	89.6	29.7	1	1.37
421	F	ad.	11.5	87	27.3	2	1.373
43	F	26	11.2	76.8	24.3		1.422
191	F	70	11.3			1	1.513
386	F	20	11.8	83.1	26.9	1	1.528
50	F	20	10.6	75.7	23.8	4	1.576
87	F	38	9.8	65.6	20.4	0	1.684
429	F	36	10.6	73.9	23.5	2	1.728
297	F	ad.	10.8	82.7	26.8	1	1.751
407	F	24	11.6	98.4	30.3	1	1.794
450	F	30	11.3	72.9	23.2	3	1.809
286	F	26	9.9	74.3	24.2	3	1.855

Number	Sex	Age (year)	Hb (g/dl)	MCV (ft)	MCH (pg)	F cells (%)	Hb F (%)
199	F	26	11.8			1	1.864
132	F	ad.	9.9	71.6	22.4	1	
134	F	ch.	10.7	70.1	22.5		
208	F	ad.	11.1	58.9	18.7	3	
250	F	65	11.9	73.8	22.9		
258	F	ad.	10.1	80.9	25.4		
280	F	11	11.9	79.8	25.8	4	
292	F	40	11.9	72.3	22.8	0	
428	F	50	10.4	71.7	22.3		
473	F	60	11	80	22.1	2	
481	F	80	11.6			2	
11	M	45	8.7	70	22.8		0.121
51	M	45	9.8	70.8	22.7		0.132
482	M	ad.	11.2	63	22.8	2	0.142
432	M	50	11.2	74	23.2	3	0.231
114	M	63	9.6	87.3	29		0.234
71	M	15	10.2	77.9	24.7	0	0.244
461	M	1.08	6.9	52.7	15.9		0.247
377	M	62	10.1	74	23		0.247
180	M	40	11.3			1	0.258
395	M	ad.	10.9	80	25.9		0.261
446	M	80	11.7	69.3	21.4	1	0.269
382	M	5	11.6	82.1	26.9		0.277
162	M	2	9.6	72.3	23.3	1	0.304
285	M	3	10.8	74.1	24	1	0.308
117	M	0.5	11.3	66.8	21.8		0.31
328	M	62	10.7			2	0.325
383	M	4	10.9	68.2	21.4		0.368
402	M	2	11.5	82	27		0.391
400	M	32	9	84.8	27.1		0.414
425	M	4	11.4	68.5	21.6	1	0.432
489	M	33	8.4	86.9	26.2	1	0.438
367	M	50	11.9	62.2	18.9	6	0.455
253	M	60	11.5	82.4	27.4	0	0.467
53	M	45	8.4	71.9	22.9		0.48
340	M	54	9.9	72.7	22.3	4	0.495
200	M	44	10.7				0.544
306	M	62	9.6	72.6	21	3	0.58
238	M	30	10	68.4	21.9		0.615
497	M	48	11.9			1	0.625
282	M	8	10.5			2	0.63
189	M	18	5.8				0.644
175	M	ad.	10.8	75.7	24.6	0	0.732
288	M	62	11.1	73.7	22.9	1	0.744
440	M	ch.	7.9	84.8	28.9	2	0.758
308	M	45	11.5			1	0.803
239	M	20	9.4	89.5	29.8	2	0.892
168	M	10	8.6	76.2	24.8	0	0.914
346	M	32	11.6	73.2	22.6	4	0.936
335	M	70	10.6	91.6	29.5	6	0.975
240	M	13	10.4	57.4	18.5	1	1.015
369	M	ad.	11	87.2	28.4	2	1.094
212	M	18	7.4	60.3	18.1		1.157
321	M	62	9.6	72.6	21	1	1.256
109	M	0.25	10.2	80.4	26.5		1.307

Number	Sex	Age (year)	Hb (g/dl)	MCV (ft)	MCH (pg)	F cells (%)	Hb F (%)
326	M	0.66	10.7	76.1	24.6	3	1.368
16	M	30	9.5	69	23.5		1.382
290	M	62	10.7	73.6	22.8	4	1.746
338	M	0.42	10.4	74.1	23.8	1	1.785
18	M	60	9.3	75	24.7		1.793
337	M	2	10.8	84.5	27	3	
23	M	35	10.7	88	30.2	4	

* Note:
ad. : adult
ch. .child

Appendix III: The general characteristics and hematological data among pregnant females population.

Number	Age of Pregnancy (week)	Hb (g/dl)	MCV (fl)	MCH (pg)	F Cell (%)	Hb F (%)	Hb A2 (%)
20	35	11.8	87.6	28.5		0.4003	2.00
64	32	10			1		2.00
31	40	13.3			0	1.2225	2.02
142	32	12.1	72.6	23.4	1	0	2.03
89	36	8.2			3	2.2448	2.04
16	28	12.1	80.6	36.5		0.3015	2.05
27	36	11.7			1	0.3889	2.05
114		12.9	86	29.1	2	0	2.06
70	39	13.2				0.4816	2.06
139	28	11	73.1	23.9	2	0	2.07
63	39	14.6			0	0	2.07
75	34	11.1	70.4	22.9		0.5782	2.09
243	34	10.5			2	0	2.10
15	40	11.7	83	26.7		1.0926	2.11
224	34	11			3	0	2.11
235	32	9.4			0	0	2.11
3	38	12.7					2.12
115	33	9.8			1	0	2.12
2	34	12.8					2.12
36	38	10	70	23.7	0	0.3533	2.12
32	32	10.6	76.9	25.3	1	1.0659	2.12
219	34	13.1			1	0	2.14
34	33	11	79	24.4	3		2.17
213	30	11.4			2	0	2.17
244	37	12.4				0	2.17
210	36	8.1	66.2	20.5	2	0	2.18
169	29	10.6	73.8	24.1	2	0	2.18
109	34	10			0	0	2.18
134	36	8.3	70.5	22.6	2	0	2.18
90	27	11.9			1	0.4253	2.20
93	37	12.9			0	0.1584	2.21
196	15	8.2	63	19.3		0	2.21
167	34	10.3	77.3	35.1	0	0	2.22
178	30	12.2			0	0	2.22
135	36	9.8	71.7	23.2	1	0	2.22
225	31	10.2			0	0	2.24
205	24	11.7	83.5	27.7	1	0	2.24
113		10.7	72.5	23.5	1	0	2.26
234	34	9.8			0	0.1	2.26
242	28	10.3			1	0	2.28
92	38	11.1			0	0.4088	2.28
240	26	10.2			2	0.09	2.29
30	38	10.7			0	0.6711	2.29
162	35	9			1	0.9348	2.33
86	37	12			1	0.7419	2.33
120	29	10.9	81.7	26.1	1	0	2.34
160	32	10.5			0	0	2.34

Number	Age of Pregnancy (week)	Hb (g/dl)	MCV (fl)	MCH (pg)	F Cell (%)	Hb F (%)	Hb A2 (%)
198	27	8.8	74.3	24.2	1	0	2.34
230	37	9.8			1	0	2.35
230		9.8			1		2.35
207	39	10.2			4	0	2.35
165	36	10.7	78.2	26.3	1	0	2.35
71	35	11.5			0		2.35
38		12.5			0	1.0717	2.36
191	37	11			3	0	2.36
247	38	13.5			0	0	2.37
154	28	11.7			0	0	2.37
251	36	11.4			3	0	2.37
67	38	13.7				0.5382	2.37
176	39	13.9			0	0.07	2.37
164	35	13.5			1	0	2.38
257	40	12.9			2	0	2.39
151		14			1	0	2.40
112	32	11.8	79.9	26	1	0	2.40
201	30	9.3	65	20.4	0	0.07	2.40
177	30	11.4			0	0	2.40
132	26	13.6	90.1	30.8	1	0.5144	2.41
110	26	12.1			0	0	2.41
157	36	14.6			1	0	2.42
145	36	12.7	86.4	29.2	1	0.9129	2.42
152	33	11.4			1	0	2.42
246	24	12			2	0	2.43
68	32	13.6				0.6544	2.44
214	30	10.5			0	0	2.44
149	38	14.8			1	0.6571	2.44
202	28	11.3	75.2	24.7	0	0	2.45
150	26	12.4			2	0	2.45
233	37	12			1	0	2.46
129	30	13	91.2	30.5	1	0	2.46
232	34	13.7			1	0	2.46
195	38	11.6	90	30.7	0	0	2.47
236	30	10.6			1	0	2.47
130		10.8	81	26.6	2	0	2.47
138	39	11.1	82.3	27.4	1	0	2.48
237	39	13.3			2	0	2.48
175	27	12.1			1	0.05	2.48
80	32	13.4			0	0.6244	2.48
88		12.3			0	0.5086	2.48
184	30	10.5	73.7	24	1	0	2.49
166	27	11.7	94.8	31.8	1	0	2.50
66	38	13			1	1.0503	2.50
78	38	11.5			2	0.3318	2.50
136	30	9.9	81.5	27.3	1	0.7273	2.50
159	31	14			1	0.3629	2.50

Number	Age of Pregnancy (week)	Hb (g/dl)	MCV (fl)	MCH (pg)	F Cell (%)	Hb F (%)	Hb A2 (%)
227		11.3			0	0	2.51
209	34	11.5			1	0	2.51
10	30	10.5			0	0.2831	2.51
255		12.2			2	0	2.52
163	37	13.6			1	0	2.52
146	28	11.2	89.6	29.8	0	0.6474	2.52
212	37	10.7			1	0	2.53
13	29	13.7				0.4074	2.53
111		12	87.6	28.7	1	0	2.54
131	31	11.1	82.3	27.5	0	0	2.54
239	31	13.4			3	0	2.55
158	34	11.2			2	0.4417	2.55
74	38	12.3	86.4	28.8		0.8109	2.56
133	30	10.8	81.1	27.1	1	1.0417	2.56
81	28	13.7			1	0.4555	2.56
161	36	9.6			1	0	2.56
91	35	13.2			1	0.2232	2.56
147		10.4	77.7	25.8	0	0	2.56
256	38	14.4			2	0	2.57
148	38	12.9			0	0	2.58
223	36	13.5			2	0.09	2.59
228	31	12			0	0	2.60
228		12			0		2.60
204		11.4			1	0	2.61
211	35	12			1	0	2.63
140	26	11.2	102	28.7	2	0	2.63
73	35	14.5				0.4865	2.64
102	34	11.8			0	0	2.65
84	40	10.5			0	0.4589	2.65
126	26	11.2	94.2	31.7	2	0.3	2.66
65	36	11.7				0.394	2.67
194	36	10.5	77.1	25.6	2	0	2.68
143	34	11.6	82.6	27.9	0	0	2.68
248	35	11			1	0	2.69
208	28	9.6	80.2	26.5	4	0	2.70
199	32	9.2	66.7	21.1	0	0	2.70
83	8	10.5			0	0.4942	2.70
185	29	11.8	81.2	27.2	0	0	2.71
6	28	11	84.2	27.6	0	0.4293	2.71
172	36	13.9			0	0.065	2.72
200	34	12	87.2	28.9	1	0.055	2.72
144	36	11.3	91.1	30	0	0	2.73
222	29	11.1			1	0	2.73
127	30	11.4	77.5	25.3	2	0	2.74
94	4	11.3			0	0.2667	2.75
189	30	10.1	79.8	26.5	2	0	2.75
26	26	10.9	82	29.5	3	1.1991	2.76
180	36	11	80.5	28.5	1	0	2.77

Number	Age of Pregnancy (week)	Hb (g/dl)	MCV (fl)	MCH (pg)	F Cell (%)	Hb F (%)	Hb A2 (%)
226	38	14.2			0	0	2.77
85	39	11.9			0	0.5667	2.79
203		11.1	77.6	25.3	1	0	2.80
183	33	11	82	27.2	0	0	2.80
197	36	11.6	91.7	30.4	0	0	2.81
216	32	13.6			0	0	2.82
182	32	9.5	84.4	27.5	1	0	2.82
108	28	10.2			0	0	2.83
220	24	13.6			0	0	2.85
119		12.1	79.6	26.3	0	0	2.86
186	32	11.9			0	0	2.86
33	32	11.5	86	28.5	0		2.88
62	36	13.9				0.5579	2.88
125	34	13.2	95.2	32.2	1	0	2.89
79	34	12			1	0.4601	2.89
218	38	11.6			0	0.05	2.90
193	34	12.5			1	0	2.90
155	36	11.4			1	0	2.90
241	39	11			2	0	2.91
99	33	9			0	0	2.93
221	28	11.7			1	0	2.93
105	22	12.4			0	0.05	2.96
121	35	13.2	83.8	28.5	0	0.19	2.96
123	23	11.9	81.4	29.4	1	0.16	2.97
173	31	9.5			0	0	2.98
8	35	13.5			0	0.3278	2.98
39	39	12.5			0		2.99
107	29	11.2			0	0	3.00
50	21	14			1	0.6339	3.00
116	27	10.3	76.5	25	1	0.05	3.02
82	25	12.5			0	0.7308	3.05
190	30	11.3	94	31.1	1	0.05	3.06
28	36	12.9			0		3.06
231		11.6			0	0.1	3.07
44	36	14			0	0.4254	3.09
53	36	11.3			1		3.11
156	27	13.6			0	0	3.12
54	20	9.7			1	0.3294	3.14
55	39	14.4			2	0.9926	3.14
229	36	13.4			0	0	3.17
229		13.4			0		3.17
258	27	11.1			0	0	3.18
253	30	15			1		3.18
252	28	10.3			2	0	3.19
97	36	12			0	0	3.20
52	31	12.3			1		3.22
187	38	12.3			0	0	3.23
41	34	13.1			0		3.25

Number	Age of Pregnancy (week)	Hb (g/dl)	MCV (fl)	MCH (pg)	F Cell (%)	Hb F (%)	Hb A2 (%)
60	34	10.2			0		3.31
43	38	12.5			0	0.592	3.32
42	35	9.6			0		3.33
58	37	11 5			0		3.34
40	38	9.8			0		3.34
57	22	12.7			1		3.35
179	36	13.3			0	0.05	3.36
104	38	9.5			0	0	3.38
56	20	12.3			0	0.7684	3.39
96	36	9.5			0	0.3571	3.39
106	32	13.7			0	0	3.40
103	36	11 3			0	0	3.42
45	29	12			0		3.44
249	34	12.2			1	0	3.45
100	30	14 7			0	0.05	3.46
238	30	12.3			1	0 11	3.47

Appendix IV: The general characteristics and hematological data among among homozygotes β IVSI-5 mutation population.

Number	Age (years)	ORIGIN	Hb (%)	MCV (fl)	MCH (pg)	HbA2 (%)	Hb F (%)	Ferritin
F5 1		pakistani				2.8	13	
F5 2		pakistani				2.7	11.2	
F6 3	9mon.*	Iranian	9.1	65	21.1	7.8		365
F9 3	3.5	pakistani	7	88.3	28.6	2.4	31	589
F9 4	1.8	pakistani	7.1	63.8	20.3	4.4	9.5	24
F15 3	10	Balochi	10.5	85.7	27.8	2.4	0.8	4.5
F15 4	7	Balochi	11.2	77.4	25.4	3.3	6.4	5.7
F15 6		Balochi	10.1	58.8	18.7	4.8		10.5
F15 7		Balochi	9.9	52	16.3	4.1		12.4
F15 8		Balochi	10	52	16.4	4.5		12.4
F17 3		Balochi	9.3	80.1	26.9	2.5	> 10	245
F17 4		Balochi	8.1	78	25	2.5	4.2	> 1000
F19 3			9.8	81.5	27.3	2.6	24	622
F19 4			9.6	72.4	22.3	5.2		38
F19 5			11.1	63.2	19.9	5.3	0.5	19
F20 4		Balochi	3.7	67.3	20.6	5		159
F20 7		Balochi	9.7	68.2	22.7	3.6		18
F25 3			10.8	79.4	26.5			
F27 3		Omani	7.6	72.9	25.1	2.6		
F27 4		Omani	10.7	60.7	19.4	3.2		

* Note:
mon. : months

Appendix V: The general characteristics and hematological data among heterozygotes β IVSI-5 mutation population.

Number	Age(years)	Origin	Hb(g/dl)	MCV(fl)	MCH(pg)	HbA2(%)	Hb F(%)	Ferritin
F5 3		pakistani				6.1	0.5	
F5 4		pakistani				4.7		
F6 1		Iranian	15.8	73.1	22.8	4.5		102
F6 2		Iranian	12.3	69.9	21.7	5.3		44
F9 1		pakistani	14.2	58.7	18	4.5	0.5	32
F9 2		pakistani	14.2	58.7	18.3	4.8	1.6	32
F15 1		Balochi	15.2	70.9	22.5	4.6		7.1
F15 2		Balochi	12.2	61.9	20	5		9.7
F15 5	6	Balochi	14.6	85.5	28.3	4.6	1.4	4.1
F17 1		Balochi	15.9	7.9	25	5		102
F17 2		Balochi	12.2	65.1	20.5	5		88
F17 5		Balochi	12.5	68.3	22.4	2.2		6
F17 6		Balochi	13.2	74.4	24.6	4.5		14
F17 7		Balochi	12	65.3	20.6	4.9		5
F17 8		Balochi	9.9	62.9	20	2.4	1.6	14
F17 9		Balochi	11.6	62.9	19.8	4.8		3
F19 1			12.6	64.9	20.7	5.3	1	37
F19 2			10.1	58.4	19	5.1	1	< 1
F19 6			5.6	61.7	20	5.2	4.8	23
F19 7			10	64.5	20	5.4	0	13
F19 8			6.7	77.4	27.1	3.1	0	8
F19 9			13.1	65.8	21	5.3	0	13
F19 10			8.5	67.2	21.6	2.1	0	
F20 1		Balochi	11.2	63.9	20.9	4.8		36
F20 2		Balochi	11.1	67	21.4	7.3		9
F20 3		Balochi	10.3	83.9	27.8	2.9		981
F20 5		Balochi	6.5	56.7	18.7	8.2		40
F20 6		Balochi	9.1	71.8	23.4	4.9		46
F25 1			13.1	66.9	21.4			
F25 2			13.8	69.1	22.1			
F25 4			11	66.4	20.7			
F25 5			11.5	80.4	26.3			
F25 6			10.3	61.8	19.5			
F26 1			13.9	60.9	19.2		4.5	
F27 1		Omani	12.8	63.2	20	4.2		
F27 2		Omani	13	62.8	20.1	3.6		
F28 2		Omani	9.9	58.1	17.7	4.3		4
F29 1		UAE	15.1	69.1	22.3	4.7		49
F30 1		Balochi				4.8		
F33 1		Iranian	12	68.3	21.6	4.3		
F38 1	64	Balochi						
F40 1			11.8	64.7	19.4	3.7		
F42 1			13.2	68	21	2.3	< 2	
F43 1	50	UAE	13.4	73.6	22.3	4.1		292
F43 2	35	UAE	11.7	70.4	22	4.8		16