= Haematology 435 =

Hemoglobinopathies Practical

إن أحسنا فمن الله عزوجل, وإن أخطأنا فمن أنفسنا والشيطان.
نظراً لشمولية العمل لمحاضرات الطلاب والطالبات بإمكانكم اعتماده كمصدر للمذاكرة.

➢ **Color Codes:**

- Pink: Girls' notes. Blue: Boys' notes. Red: Important Notes. Gray: Extra notes.

➢ **Done by:** **Sara Alkhalifah, Alanood Alsalman, Deema Alfaris, Moneera Alhosseni, Raghda Alqassim, Rifan Hashim, Abdulaziz Alshalan.**

➢ **Revised by:** **Sara Alkhalifah**

➢ **References:**

- Girls&Boys Doctors Slides and Notes.

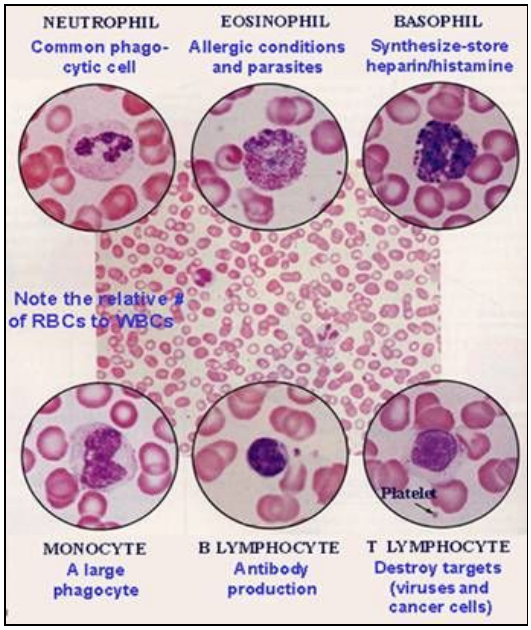
➢ **Correction file:** (HERE)

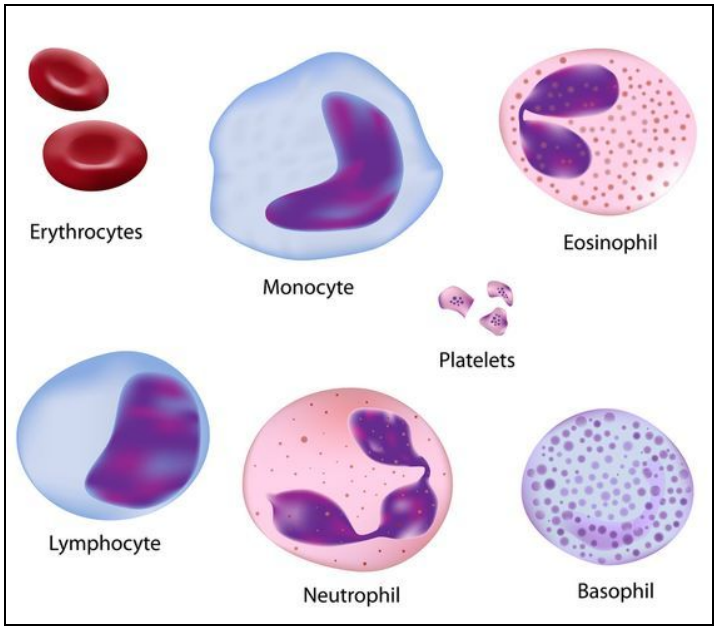
***We recommend revising Thalassemia and sickle cell anemia before the OSPE exam**

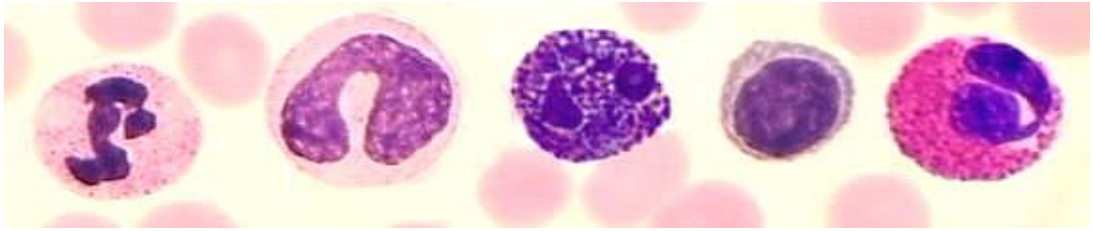
Normal hemoglobin: Recap (extra)

Haemoglobin type	Newborn	% in Saudi adult
Hb A	**15-40%**	**95.0%**
Hb A2	**< 0.3%**	**3.5%**
Hb F	**60-85%**	**1.5%**
Hb Bart's	**< 0.5%**	_

- HbC ($\alpha 2 \beta 2$ 6 GLU→ LYS)
- HbS ($\alpha 2 \beta 2$ 6 GLU→ VAL)
- HbE ($\alpha 2 \beta 2$ 26 GLU→ LYS)







1) neutrophil, 2) monocyte, 3) basophil, 4) lymphocyte, 5) eosinophil

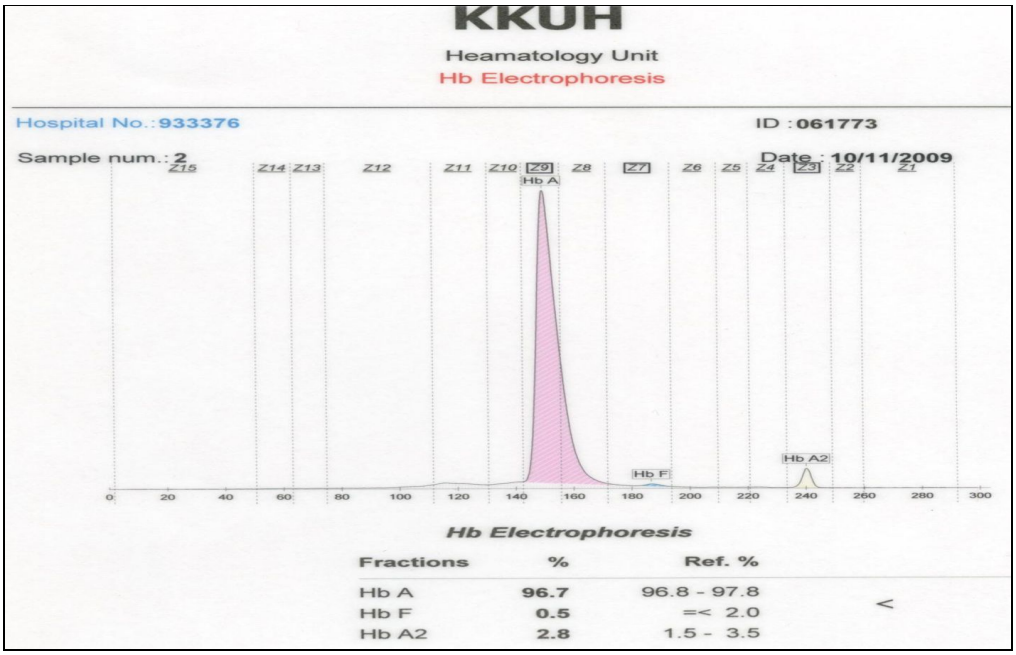
Try to differentiate between different types of WBCs (you might be asked about it in the exam)

Clinical and haematological abnormalities important notes

- **HbA2 is more than 3.5% in Beta thalassemia, and less than 1.5% in alpha thalassemia.**
- **The most accurate Hb test is Hb electrophoresis.**
- **In electrophoresis if HbS is present:**
  - **more than 45% indicates homozygous sickle cell anaemia.**
  - **less than 45% indicates sickle cell trait.**
- **Anisocytosis: significant variation in RBCs size.**
- **Poikilocytosis: abnormal shape of RBCs.**
- **Target cells: RBCs with a dark centre surrounded by a light band that again encircled by a darker ring.**
- **Microcytic hypochromic: RBCs are smaller and paler than normal.**
- **Further investigation for blood smear: Hemoglobin electrophoresis[1], genetic study and family study.**
- **Further investigations for Hb electrophoresis: genetic study and family study.**

[1] Its very important to order Hb electrophoresis for any Hb disorders.

CASE 0 normal:

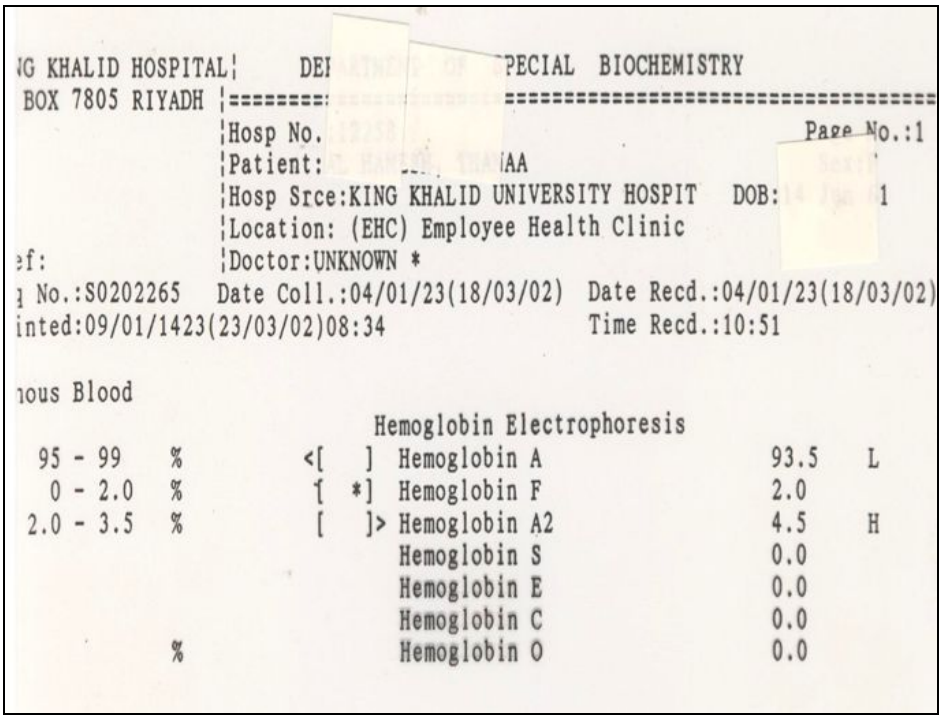


	Results	Normal	Comment
Hb A	**96.7%**	**95 -97 %**	**normal**
Hb F	**0.5 %**	**0.5-1.5%**[2][3]	**normal**
Hb A2	**2.8%**	**1.5-3.5 %**[4] **<1.5 in α thalassemia** **>3.5 in β thalassemia**	**normal**
The results indicate normal Hb			

- **In order to get a full mark:**

1. **Write each Hb result and the normal range (Hb A, Hb F, Hb A2)**
2. **Abnormal Hb if present e.g. HbS.**
3. **Explain results if needed**
4. **Write the diagnosis**

[2] Even 0% is normal.
[3] Medscape reference <2%
[4] When the result is on the borderline for example: when Hb A2= 3.6%. It is also considered normal.



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ious Blood

                                Hemoglobin Electrophoresis
  95 - 99    %          <[   ]  Hemoglobin A                 93.5    L
   0 - 2.0   %           [  *]  Hemoglobin F                 2.0
 2.0 - 3.5   %           [   ]> Hemoglobin A2                4.5     H
                                Hemoglobin S                 0.0
                                Hemoglobin E                 0.0
                                Hemoglobin C                 0.0
             %                  Hemoglobin O                 0.0
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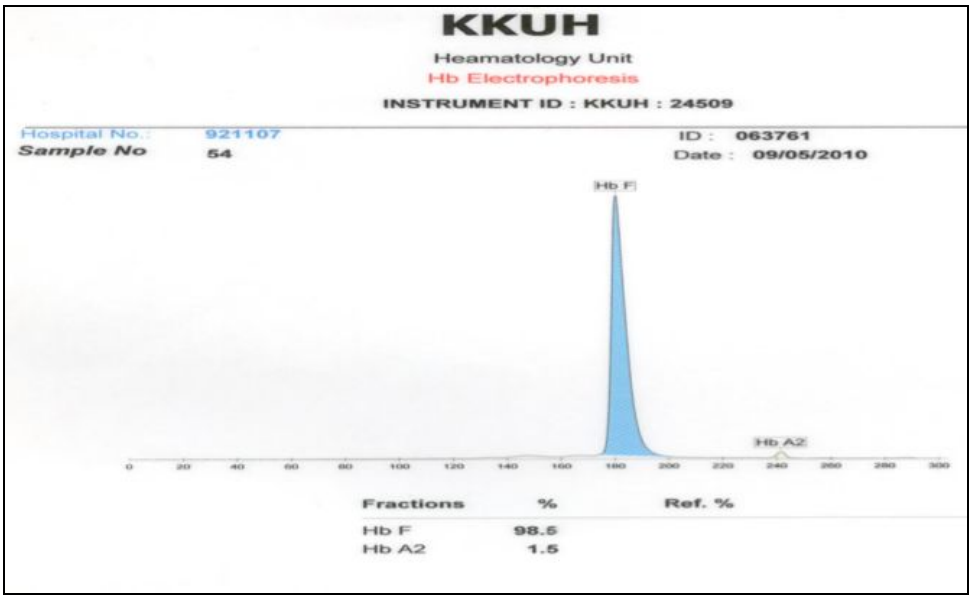
Comment on the following:

1. **Normal hemoglobins (A, A2, F)**
2. **Percentage of each**
3. **Abnormal Hb**

Hemoglobin A2 is raised and Hb A is low which indicates β thalassemia

CASE 1:

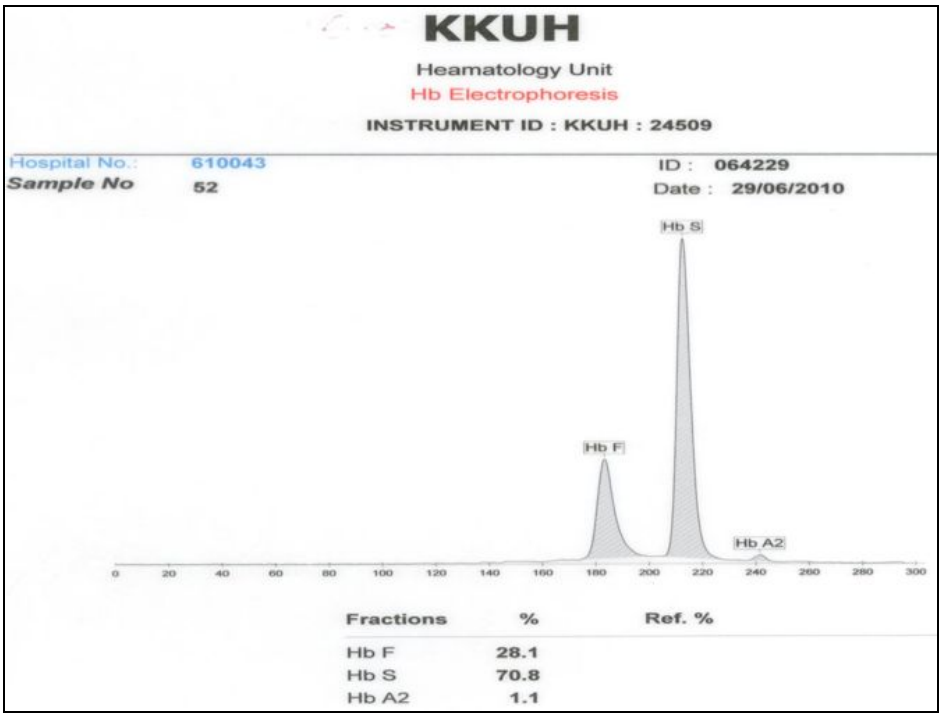


	Results	Normal	Comment
Hb A	**0.0%**	**95 -97 %**	**Absent**
Hb F	**98.5%**	**0.5-1.5%**	**Increased**
Hb A2	**1.5%**	**1.5-3.5 %**	**Normal**[5]
Diagnosis: Hereditary persistence of HbF[6]			

[5] Exclude Thalassemia when Hb A2 is normal!

[6] **Hereditary persistence of fetal hemoglobin** is a benign condition in which significant fetal hemoglobin (hemoglobin F) production continues well into adulthood.

Case 2:

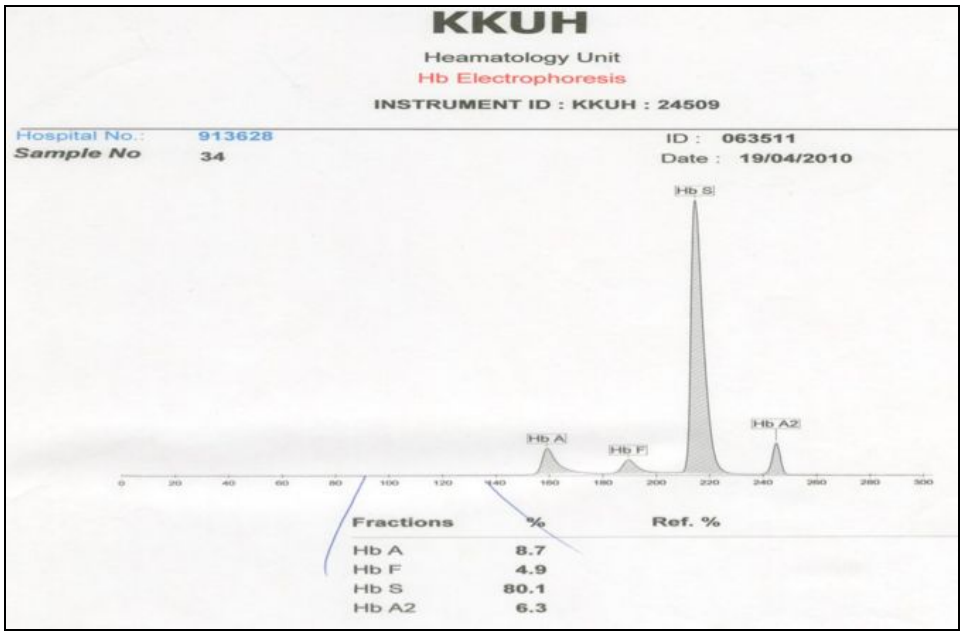


	Results	Normal	Comment
Hb F	**28.1%**	**0.5-1.5%**	**High**
Hb S	**70.8%**	**0.0%**	**Present**[7]
Hb A2	**1.1%**	**1.5-3.5 %**	**Low** (indicates α thalassemia)
Diagnosis: α thalassemia, sickle cell anemia			

- **What would be your diagnosis if Hb A2 was 4.5 and everything else was the same?**
  - **B thalassemia and sickle cell anemia**
- **What further investigations will you do?**
  - **Family and genetic study.**

[7] Check percentage of Hb S, if >45% → anemia, if <45% → trait

Case 3:

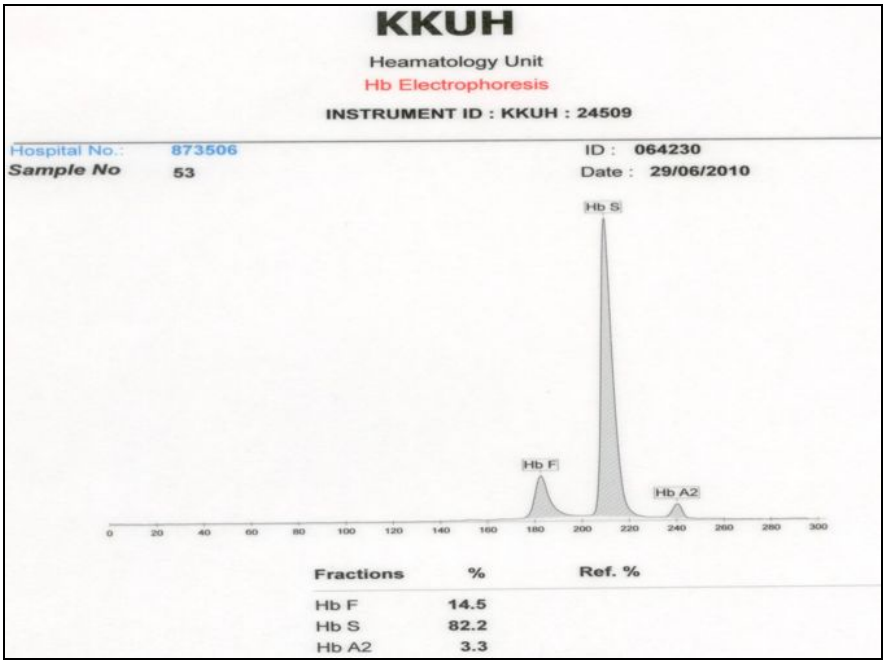


	results	normal	comment
Hb A	**8.7%**[8]	**95 -97 %**	**Low**
Hb F	**4.9%**	**0.5-1.5%**	**High**
Hb S	**80.1%**	**0.0%**	**Present**
Hb A2	**6.3%**	**1.5-3.5 %**	**High**
Diagnosis: β thalassemia, sickle cell anemia			

- **How to diagnose sickle cell anemia:**
  - **If Hb S is <45 the diagnosis will be sickle cell trait**
  - **If Hb S is >45 the diagnosis will be sickle cell anemia**
- **What further investigations would you do?**
  - **Family study, Genetic study**

[8] Presence may be due to blood transfusions. For correct results, electrophoresis should be done before blood transfusion! Otherwise the results may appear normal or indicate a different disease.

Case 4:

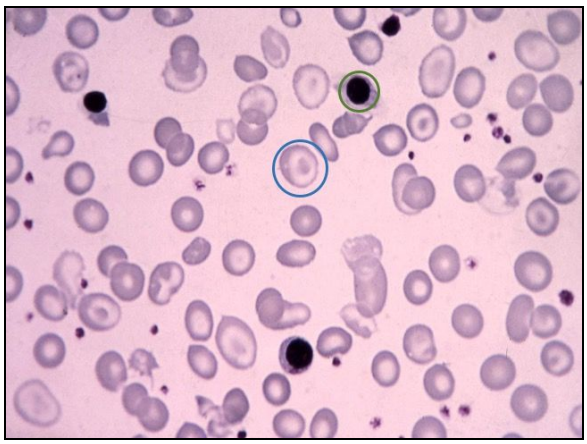


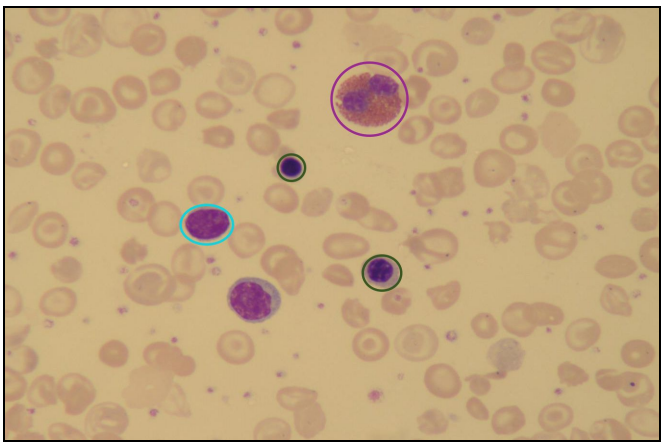
	results	normal	comment
Hb A	**0.0%**[9]	**95 -97 %**	**Absent**
Hb S	**82.2%**	**0.0%**	**Present**
Hb A2	**3.3%**	**1.5-3.5 %**	**Normal**
Hb F	**14.5%**[10]	**0.5-1.5%**	**Raised**
Diagnosis: Sickle cell anemia			

[9] Even if HbA is absent comment on it.
[10] Hb F is raised in 5-15% of patients with sickle cell anemia.

Case 5:

A one-year-old child came to hospital presenting with weight loss, pallor, and abdominal distension. The doctor ordered a blood smear and it is shown down below.





- **What are your findings?**
  - **Nucleated RBCs**[11] *
  - **Target cells**[12][13] *
  - **Anisocytosis**
  - **Poikilocytosis**
  - **Hypochromic microcytic RBCs**
  - **Eosinophil** *
  - **Lymphocyte** *

- **What is your diagnosis?**
  - **β Thalassemia**[14]

- **What further investigations would you do?**
  - **Hemoglobin electrophoresis, Family study, genetic study, serum iron**[15] **and TIBC**

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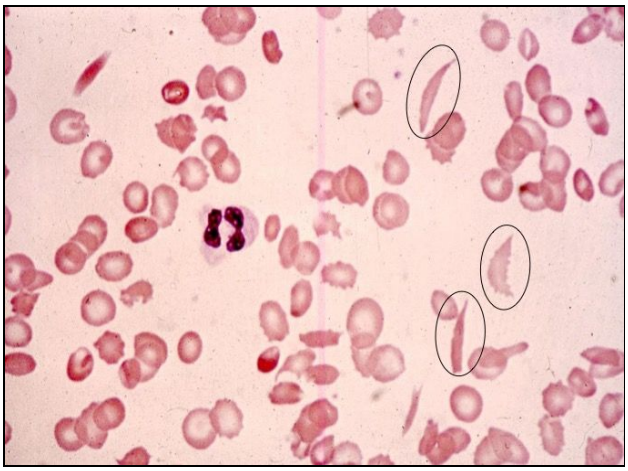
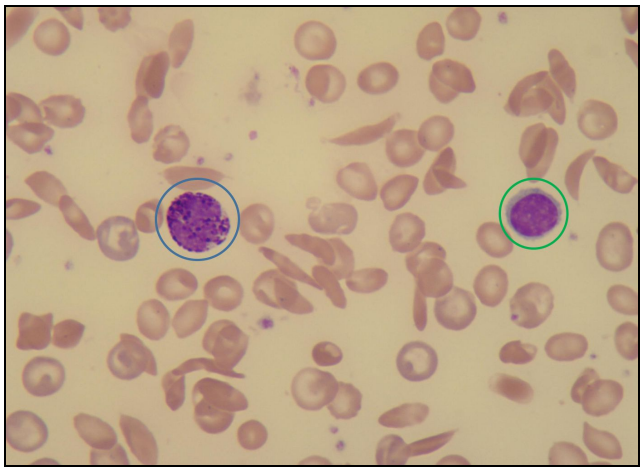
[11] Indicates β thalassemia
[12] Indicates α and β thalassemia (best sign)
[13] Fewer target cells are seen in iron deficiency anaemia
[14] How do you differentiate between iron deficiency anaemia and thalassemia? Answer is in the table in Dr's notes
[15] Serum iron will be low in the beginning and high after transfusions (oral Iron chelation therapy with Deferiprone 'ferriprox')

Case 6:





- **What are your findings?**
  - **Sickling cells**
  - **Target cells**
  - **Basophil** *
  - **Lymphocyte** *
  - **Reticulocytes**

- **What is your diagnosis?**
  - **Sickle cell disease**[16][17][18]

- **What further investigations would you do?**
  - **Hemoglobin electrophoresis**[19]
  - **Genetic study, family study**

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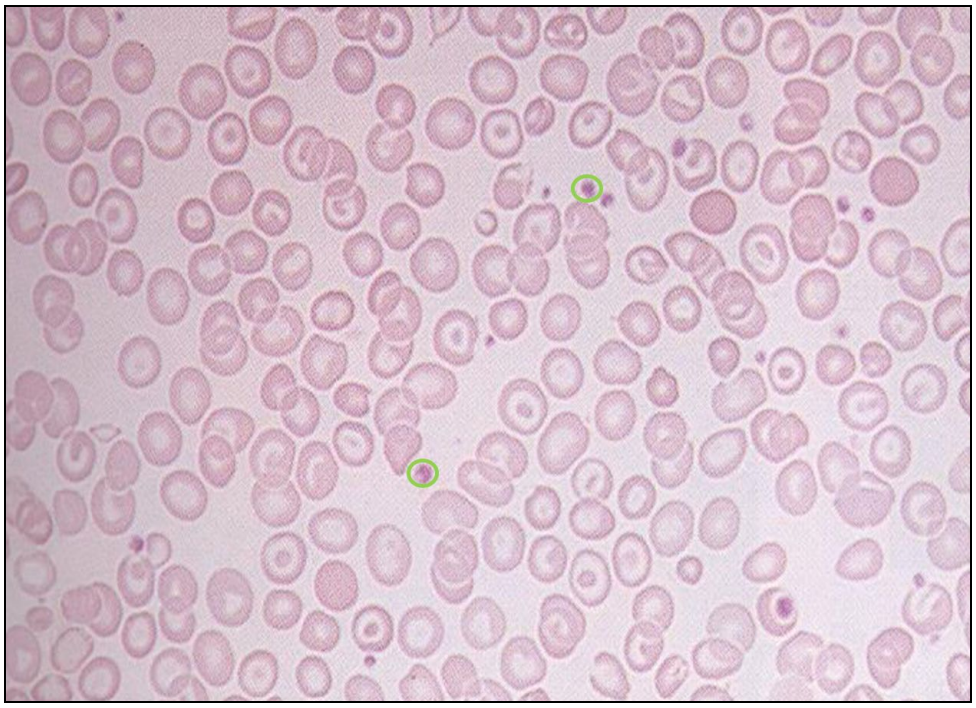
[16] **Don't write trait or anemia**

[17] Usually normal MCV, MCH. Target cells may be present (they're not limited to thalassemia).

[18] Prevalent in 3 regions in Saudi Arabia: southern (Jaizan) northern (Khaibar, AlOla) and the east (including other countries of the gulf like Qatar)

[19] To know the percentage of sickling cells (to determine whether it's sickle cell anemia or sickle cell trait)

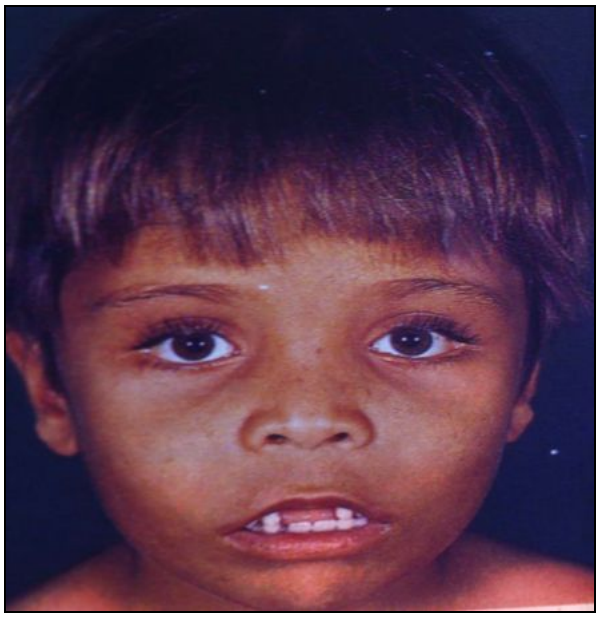
Case 7:

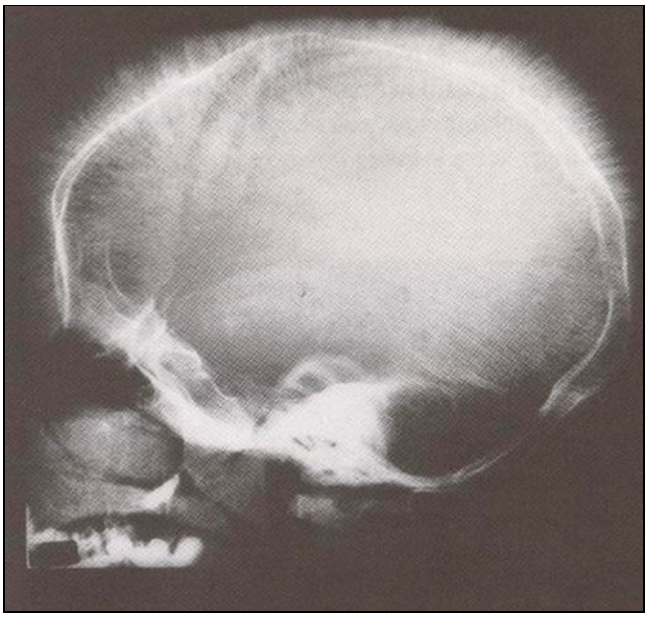


- **What are your findings?**
  - **Anisocytosis**
  - **Poikilocytosis**
  - **Target cells**
  - **Microcytic hypochromic**
  - **Platelets***
- **What is your diagnosis?**
  - **α thalassemia**
- **What further investigations would you do?**
  - **Hb electrophoresis**
  - **Genetic study, family study**
  - **Serum iron and TIBC[20]**

[20] Note that it can be difficult to distinguish α thalassemia from iron deficiency anemia by blood smear only! That is why iron tests and Hb electrophoresis are important for the diagnosis of α thalassemia.

Case 8:





- **What are your findings?**
  - **First image: Thalassemia face**
    - Forehead bossing (protrusion)
    - Prominent maxilla
    - Widened space between eyes
    - Nose depression
  - **Second image: Hair on end appearance**[21] [22] (crewcut appearance)

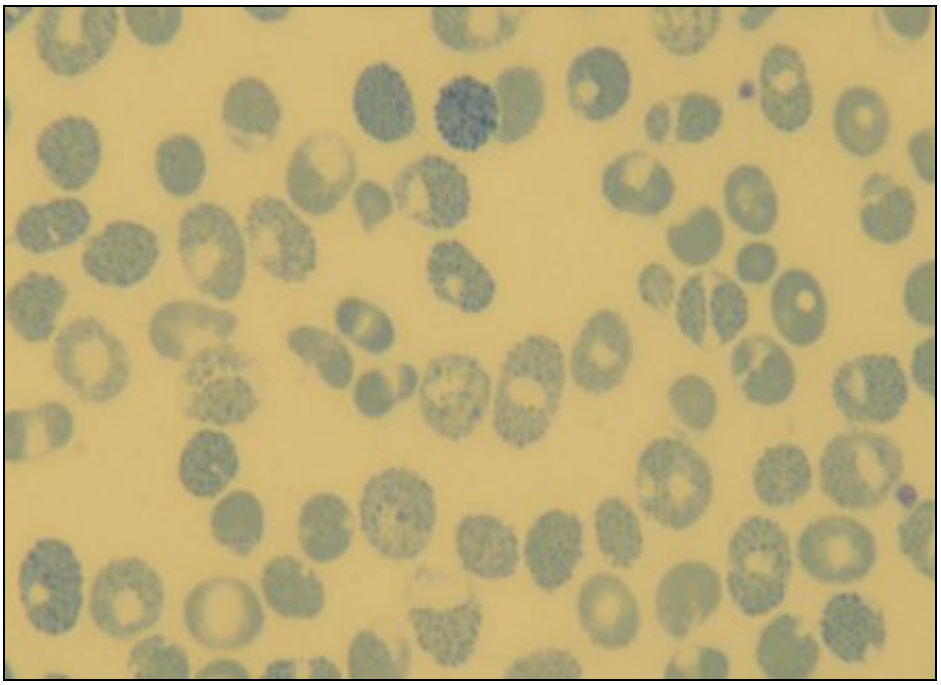
- **What is your diagnosis?**
  - **β Thalassemia major**

- **What further investigations would you do?**
  - **CBC**
  - **Hemoglobin electrophoresis**
  - **Genetic study, family study**

[21] Other causes for hair on end appearance, sickle cell disease, hereditary spherocytosis, iron deficiency anaemia

[22] Due to expansion of the bone marrow

Case 9: Read it just in case



- **What's the name of this test?**
  - **Blood smear (film)**

- **What are your findings?**[23]
  - **Golf Ball appearance**

- **What is the stain used to diagnose this disease?**
  - **Supravital stain**

- **What is your diagnosis?**
  - **Hb H disease**

- **This chronic haemolytic anaemia results from the inheritance of both the (α+) and (α0) thalassaemia alleles, leaving one functioning α-globin gene per cell.**
- **α-globin chains are produced at very low rates, leaving a considerable excess of β-chains, which combine to form tetramers β4 (Hb H).**

[23] Note that Heinz bodies are positive in HbH disease and G6PD deficiency

Dr's notes:

Note that Heinz bodies are positive in HbH disease and G6PD deficiency
Note that iron overload occurs when serum ferritin is greater than 500

Type	Features	Alleles
β thalassemia minor	Mild anaemia	β^+/β β^0/β
β thalassemia intermediate	Occasional transfusions depending on severity	β^+/β^+ β^0/β^+
β thalassemia major	• Transfusions are needed + iron chelation by deferrioxamine (IV). • Many target cells and nucleated RBCs	β^0/β^0 Can present with Beta+ in which there is 10% synthesis

Incidence rates in Saudi Arabia:

- Highest incidence of alpha thalassemia is in Jaizan
- Highest incidence of hydrops fetalis is in AlHofuf
- Highest incidence of beta thalassemia is Najran
- Highest incidence of sickle cell anaemia is in AlQateef

Note the following regarding sickle cell disease:

- Acute infection with parvovirus and low folic acid in sickle cell patients causes transient aplastic crisis owing to reticulocytopenia
- SCD patients get osteomyelitis caused by Salmonella (but the most common organism cause of osteomyelitis is Staph.Aureus)
- HbS, B-thalassemia & G6PD deficiency patients are resistant to malariae
- SCD patients cannot become pilots or live in high altitudes

B chain has 146 amino acids, the replacement of glutamic acid by another amino acid causes abnormal Hb (positions of glutamic acid are 6,7,26 and 121) what you need to know is:

1. HbS (Valine instead of Glutamic acid at position 6) **how?** By a single point mutation:

DNA base for glutamic acid is Guanine Adenine Guanine (GAG) > in HbS it becomes Guanine Thiamine Guanine (GTG)

2. HbC (common in west Africa, Lysine instead of Glutamic acid at position 6)
3. HbE (common in south east Asia, Lysine instead of Glutamic acid at position 26)
4. Hb O ARAB (Lysine instead of Glutamic acid at position 121)
5. Hb D PUNJAB (India and Pakistan, Glutamine instead of Glutamic acid at position 121)

Consequences of HbS:

1 Chronic hemolytic anemia

2 Microvascular occlusion LEADING TO INFARCTION (mostly BONE) Mainly Head of femur or infarction of periosteum of bone cause Hand-foot syndrome

Note that hypersplenism traps platelets, neutrophils and even red cells. Hence, those with hypersplenism need blood transfusions and sometimes splenectomies (5+ Y/O)

This patient has β thalassemia trait, if her husband has β thalassemia trait as well: Prenatal thalassemia diagnosis is mandatory how? Via taking chorionic villi biopsy

- Within 6-12 weeks of gestation: vaginal approach
- 12+ weeks of gestation: transabdominal approach

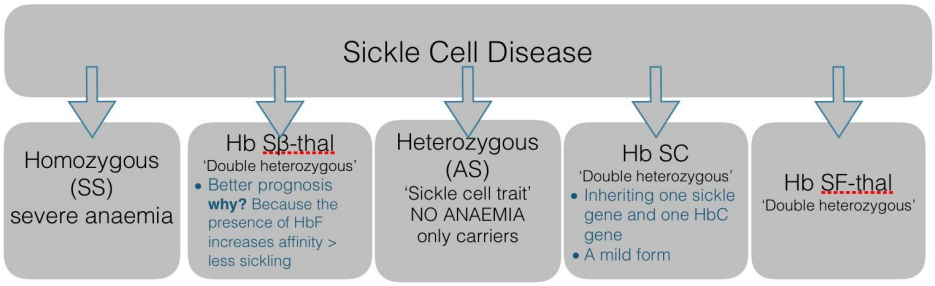
Chorionic villus sampling:
form of prenatal diagnosis to determine chromosomal or genetic disorders in the fetus. It entails sampling of the chorionic villus (placental tissue) and testing it for chromosomal abnormalities, usually with FISH or PCR.

إيش فحوصات الزواج؟

1. CBC
2. Virology (HBV, HIV, HCV)
3. Hemoglobin electrophoresis
4. Reticulocyte count

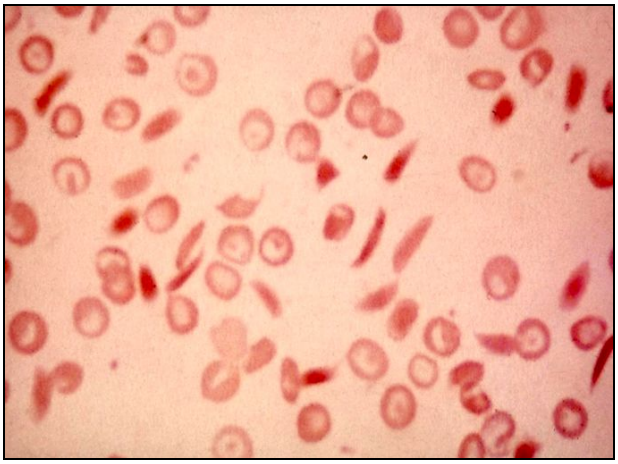
How to differentiate thalassemia from IDA

Iron Deficiency Anaemia	Thalassemia
Low red blood cells	High RBC count
Normal	High HbA2 in β thalassemia Low HbA2 in α thalassemia
Heterogenous RBCs	Homogenous RBCs
Low MCH and MCV	Very low MCV and MCH



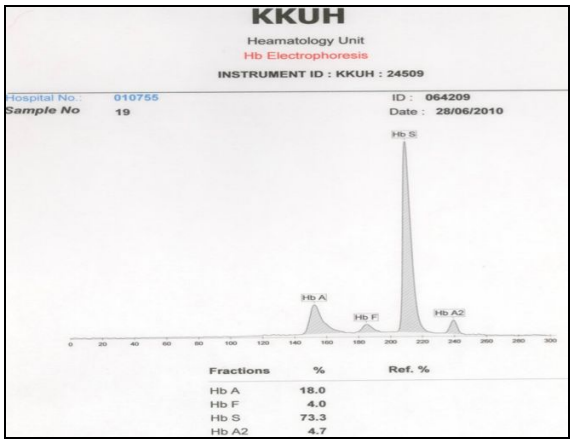
Check your understanding

1-A 4-year-old child came to the hospital, he presented with pallor, jaundice, and swollen ulcerated feet. The doctor ordered a blood smear and it is shown down below.



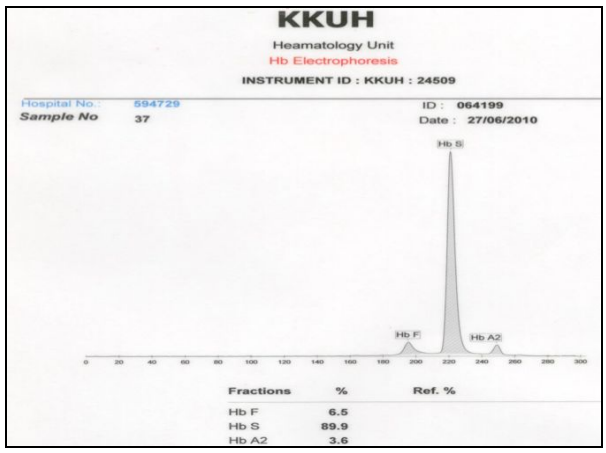
- **What are your findings?** Sickling cells, target cells, and reticulocytes.
- **What is your diagnosis?** Sickle cell disease
- **What further investigations would you do?** Hb electrophoresis, family study, and genetic study.

2- A 2-year-old child came to the hospital with severe bony deformities and jaundice. The doctor ordered Hb electrophoresis and it is shown down below.



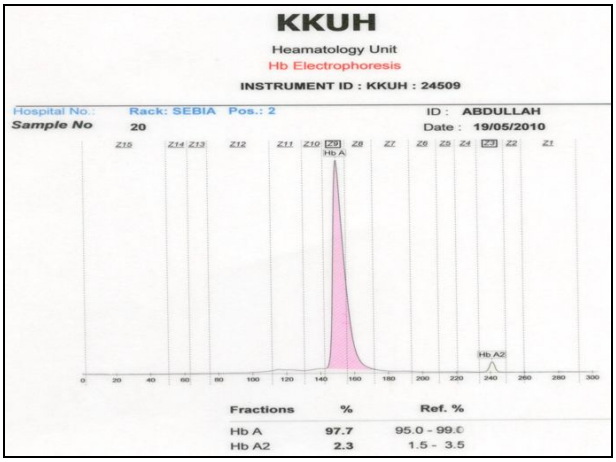
- **What are your findings?** Low Hb A (normal 95 -97 %), raised Hb F (normal, 0.5-1.5%), HbS is present (normal, absent), Hb A2 is raised (normal, 1.5-3.5%)
- **What is your diagnosis?** Sickle cell anemia and β thalassemia
- **What further investigations would you do?** Family study and genetic study.

3- A 5-year-old child came to the hospital with jaundice, pallor, and hepatosplenomegaly. The doctor ordered Hb electrophoresis and it is shown down below.



- **What are your findings?** Absent Hb A (normal 95 -97 %), raised Hb S (normal, absent), normal Hb A2 (normal, 1.5-3.5 %)[24]
- **What is your diagnosis?** Sickle cell anemia
- **What further investigations would you do?** Family study and genetic study.

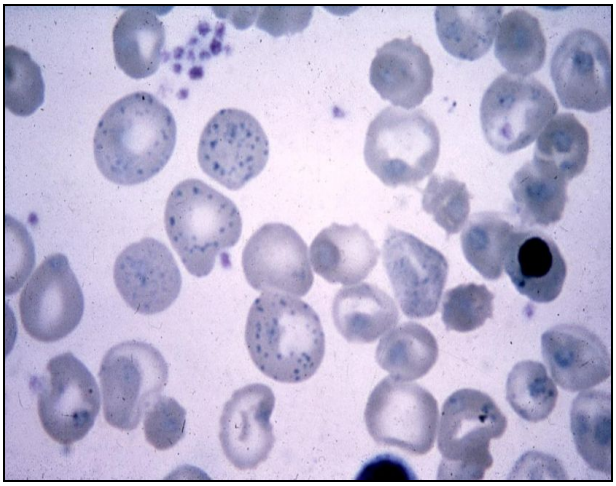
4- A 46-year-old female patient came to the clinic complaining of dizziness and tiredness. Hb electrophoresis is shown down below.

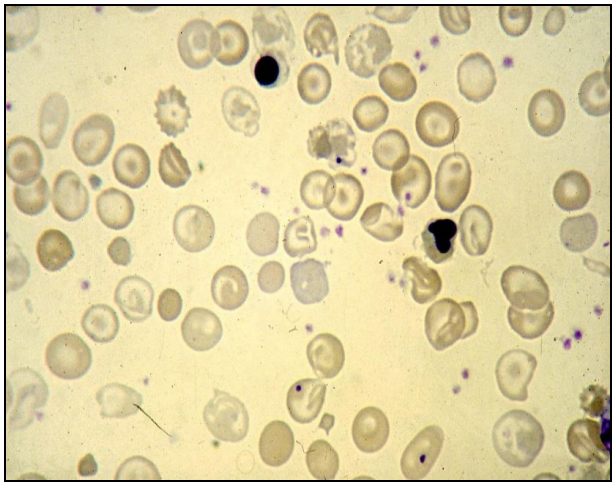


- **What are your findings?** Hb A is normal (95 -97 %), Hb A2 is normal (1.5-3.5 %), Hb F is absent[25]
- **What is your diagnosis?** مافيها الا العافيه

[24] If it's around the borderline it is considered normal.
[25] It is normal for Hb F to be absent

5- A 5-year-old child came to the hospital presenting with hepatosplenomegaly and jaundice. The doctor ordered a blood smear and it is shown down below





- **What are your findings?**
  - **Nucleated RBCs**
  - **Target cells**
  - **Anisocytosis**
  - **Poikilocytosis**
  - **Hypochromic microcytic RBCs**

- **What is your diagnosis?**
  - **β Thalassemia**

- **What further investigations would you do?**
  - **Hemoglobin electrophoresis, Family study, genetic study, serum iron and TIBC**