Appendix

1.Preparation of Blood Film:

For performing differential leucocytes count and to comment on morphology of RBC,Platelate,WBC a thin blood film was made as follows:

Using glass capillary tube, a small drop of blood was placed on the slide, 1cm from one end in the mid line.

The slide was placed on a flat surface and held down firmly.

With the thumb and fore finger at opposite ends, as quickly as possible, the spreader holed at an angle of 45^{o},was placed just in front of the drop and drawn back to touch the drop of blood to allow the blood to spread all the way along the contact line between the two slides.

The spreader was pushed forward smoothly and rapidly, maintaining contact between two slides.

Blood film formed should be 3-4cm long. Evenly spread with no rigid tails.

The film was allowed to dry before labeling with a pencil at the thick end of the blood film.

The film was placed on a staining rack, flood with Leishman's stain and leaved for 3 min to fix.

Twice as much buffered distilled water was added.

The blood film was leaved to stain for 10 min.

Stain was washed off with tap water.

The back of the slide was cleaned and stand upright to dry .

2. Sysmex:

Procedure:

1-The reagent needed was checked.

2-the power switch was turned on .self auto rinse, and background check will be automatically performed and the vend (vend for analysis) will appear, whole blood mode was selected.

Sample number: inputted by pressing sample number then number of sample was entered. Then enter key was pressed.

Sample was mixed sufficiently.

The tube was seted to the sample probe, and in that condition the start switch was pressed.

When the LCD screen display analyzing the tube was removed.

After that the unit executes automatic analysis and the result was displayed in the LCD screen.

The result was printed out.

Appendix3: Haemoglobin Electrophoresis:

Preparation of Haemolysate:

1-Two volumes of washed packed red cells were lysed in one volumes of distilled water.

2-Then one volume of carbon tetra chloride was added.

3-The tube was shaked vigorously for approximately one min.

4-The tube was centrifuged at 3000 rpm for 30 min.

5-The supernatant was transferred to a clean sample container.

Procedure

1. With the power supply disconnected the electrophoresis tank was prepared by placing equal amounts of TEB buffer in each of the outer buffer compartments.

2. Two champers was wicked in the buffer and placed one along each divider/bridge support.

3. Cellulose acetate was soaked by lowering it slowly into reservoir of buffer. Cellulose acetate was leaved to soak for at least 5 min before use.

4. The sample well plate was filled with 5 micro liter of each diluted sample or control and covered with a 50 mm cover slip or a 'short' glass slide to prevent evaporation. a second sample well plate was loaded with Zip-prep solution.

5. The applicator tips were cleaned immediately prior to use by loading with Zip-prep solution and then they was applied to a blotter.

6. The cellulose acetate strip was removed from the buffer and blotted twice between two layers of clean blotting paper.

7. The applicator was loaded by depressing the tips into the sample wells twice and this first loading was applied onto some clean blotting paper. The applicator was reloaded and the samples were applied to the cellulose acetate.

8. The cellulose acetate plates were placed across the bridges, with the plastic side uppermost.

9. Two glass slides were placed across the strip to maintain good contact. Electrophoreses was done at 350 V for 25 min.

10. The cellulose acetate was transferred to Ponceau S, fixed and stained for 5 min.

11. Excess stain was removed by washing for 5 min in the first acetic acid and for 10 min in each of the remaining two. It was blotted once, using clean blotting paper and leaved to dry. Diace JV 2001

Appendix 4: Sickling Test:

Procedure:

- 2 drops of the freshly prepared reagent was added to 2 drop of anticoagulated blood on a slide.
- The slide and cover glass was sealed with petroleum jelly/ paraffin wax mixture, or with nail polish.
- Sickling take place almost immediately in sickle cell anemia and should be obvious in sickle cell trait within 1 h.

Appendix 5:

Sudan University for Sciences and Technology

College of High graduate studies

Ethnic and Regional Distribution of Sickle Cell Anemia in Patient Referring to Pediatrics Hospitals of Khartoum State

Pt-name: …… No ( )

Age: ...……

Gender: male ( ) female ( )

Father Tribe: …….

Mother Tribe: ……

Original home: ……..

Residence: …….

History of other disease in family:

……

Clinical feature: ……...

History of blood transfusion (latest date): ……

Phone number: ……

Results:

RBC: ……

Hb: ……..

PCV: ……

MCV: …….

MCH: ……..

MCHC: ……

WBC: ……..

PLT: ……

Peripheral blood picture:

……..

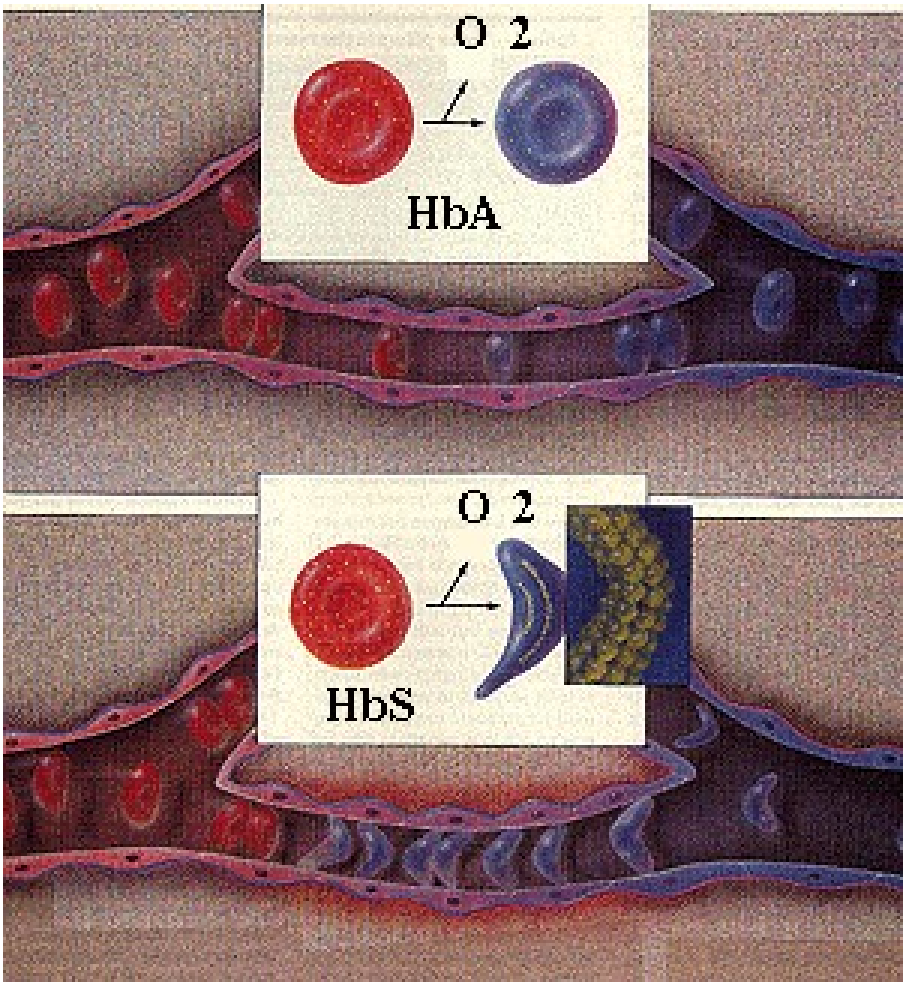
……..

Hb Electrophoresis:

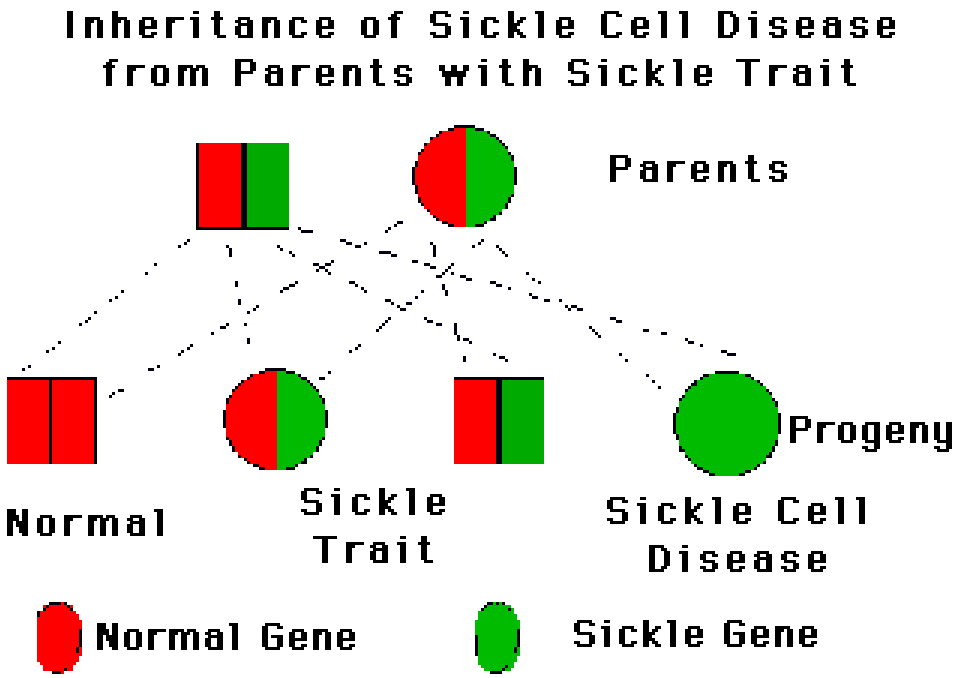
AA ( ) AS ( ) SS ( )

Appendix 6:

Induction of red cells sickling.



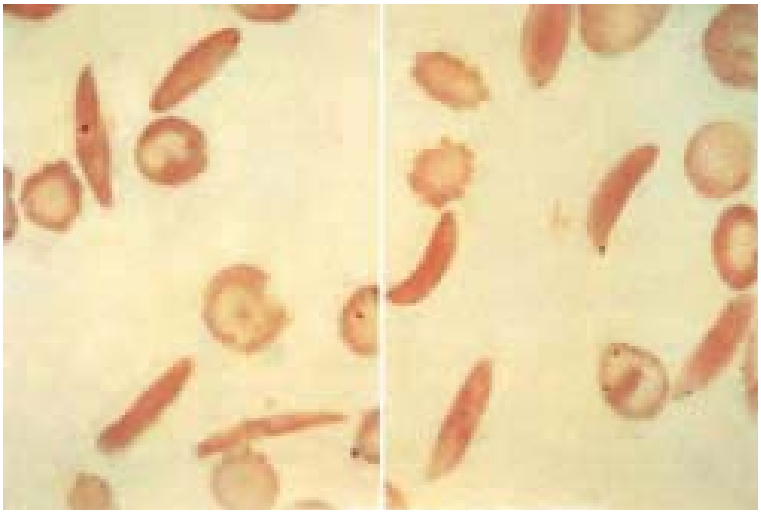
Appendix 7:


Inheritance of Sickle Cell Disease
from Parents with Sickle Trait
Parents
Progeny
Normal
Sickle
Trait
Sickle Cell
Disease
Normal Gene
Sickle Gene

Appendix 8:

Peripheral blood film from patient with sickle cell anaemia.

Showing sickled erythrocytes



Appendix 9:

Fully Automated Hematology Analyzer (sysmex)