Sickle Cell Disease: Routine Screening of Children including TCD

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Sickle Cell Disease: Routine Screening of Children including TCD.

- Outline
- Definition of Sickle Cell disease
- Laboratory Techniques and Sickle Cell disease
- Different Techniques Used
- Trans cranial Doppler scan

Laboratory Techniques and Sickle Cell disease

- Laboratory techniques are test that are carried out on patient specimens with the aim of diagnosing sickle cell disease.
- Blood samples are used for this tests.
- The samples are analysed using cytological, biochemical or molecular techniques.

Definition of Sickle Cell Disease

- First described by Herrick in 1910.
- It is a hereditary chronic heamolytic condition.
- It is caused by a single missense mutation in the Beta globin gene on chromosome 11.
- At the protein level, there is a single amino acid mutation GTG for GAG at codon 6 this results in valine replacing glutamic acid.

Sickle cell disease

- It is the most common genetic disorder in Nigeria.
- It has an incidence rate of 2-3%.
- The sickle cell trait has a prevalence of 20-30%
- This combine to make it a disease of signifant health importance.

Investigations

- New born screening using high performance liquid chromatography HPLC or capillary electrophoresis (CE)
- Full blood count with peripheral blood film.
- Sickling test
- Haemoglobin solubility test
- Haemoglobin Electrophoresis
- Molecular testing: DNA based assays.

New born Screening

- It is performed using high performance liquid chromatography technique or capillary electrophoresis or isoelectric focusing techniques.
- Carried out at birth.
- Samples taken from hill prick placed on filter paper/ blood cards to obtain dried blood spots (DBS).

Justification for NBS for Sickle cell disease

- Early detection of the disease by 3-6 months or earlier.
- Monitoring and management of early complications like sepsis, sequestration.
- Reduction of morbidity and mortality.
- Use of prophylactic measures (vaccination, folic acid, penicillin prophylaxis).

New born Screening Nigeria Experience

- Regional centers for new born screening were set up by the federal government in 2011 by the MDG office.
- The centers were increased to 6 in 2013.
- One for each geopolitical zone.
- FMC keffi, Yenogua, Ebute- meta, Abakalilki.
- Pilot studies had been carried out by different groups before then.
- All centers use HPLC technique.

High Performance Liquid Chromatography (HPLC)

- It is an automated technique.
- The method separates as well as quantifies the haemoglobin fractions present in the sample.

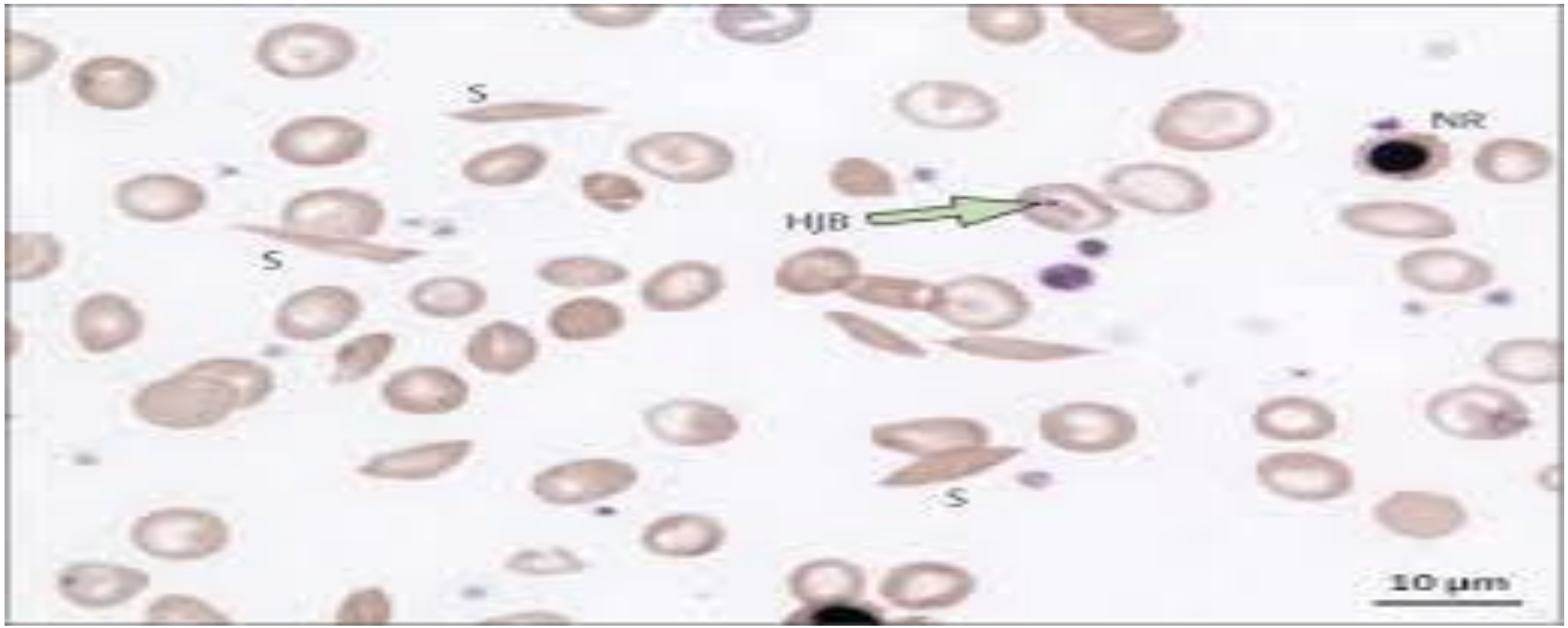
Full blood count and peripheral blood film examination

- Most common test used in the haematology Laboratory.
- Can be used to detect haemoglobinopathies.
- Counts are usually obtained from automated analyzers.
- Anaemia is present in patients with the disease.
- Important to establish steady state haemoglobin packed cell volume for patient.

Full blood count and peripheral blood film examination

- The platelets counts may be normal or elevated.
- Peripheral blood film stained with romanosky stain shows:
  - Target cells
  - Sickle cells
  - Nucleated red cells
  - Polychromatic cells
  - Increased platelets

Peripheral Blood film



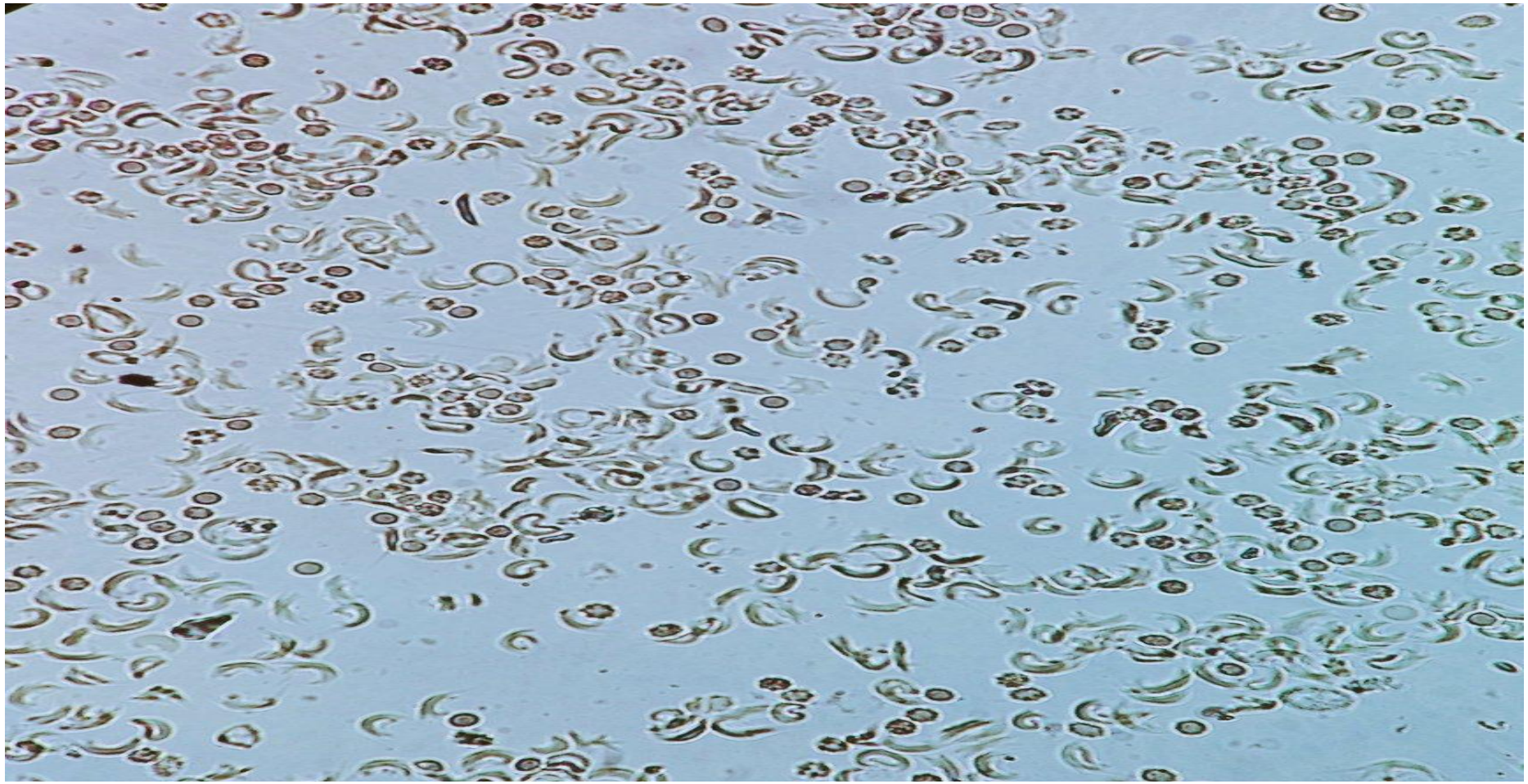
Sickling Test

- This test is used to induce oxygen desaturation and sickling of red cells.
- Fresh blood is collected and mixed with a reducing agent.
- It is used as a screening test as it is unable to differentiate carrier state from disease state.

Sickling Test

- Mix 1 drop of blood with 1 drop of 2% sodium metabisulphite solution on a microscope slide.
- Cover with a cover slip and seal the edge with wax/vaseline. Allow to stand at room temperature for about 30 minutes.
- Examine under a microscope with the dry objective.

Sickling Test



Haemoglobin solubility Test

- Principle:
  - Decreased haemoglobin solubility by deoxygenated S cells leading to formation of a precipitate.
- Method
  - Blood sample is collected
  - There is addition of reducing agent and reducing agent
  - Incubate sample mixture.

Haemoglobin solubility Test

- Samples must be run concurrently with control samples (known positive and negative samples)
- Results
  - Precipitation of the S cells is shown by turbidity of the mixture.
  - Compare sample result with control results.

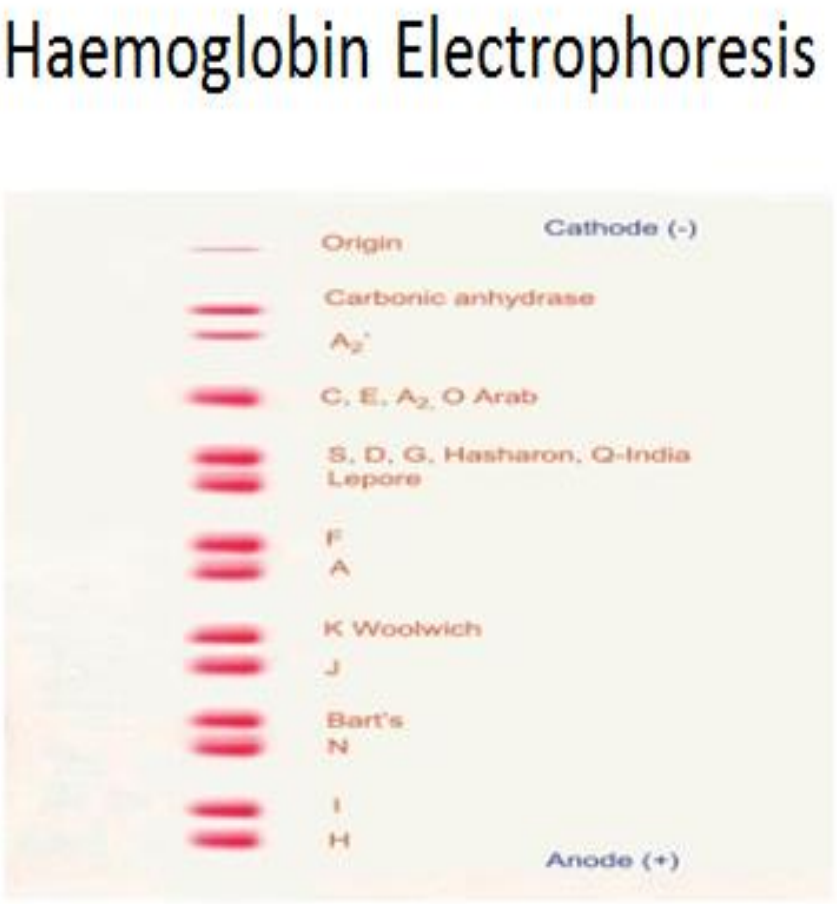
Haemoglobin Electrophoresis

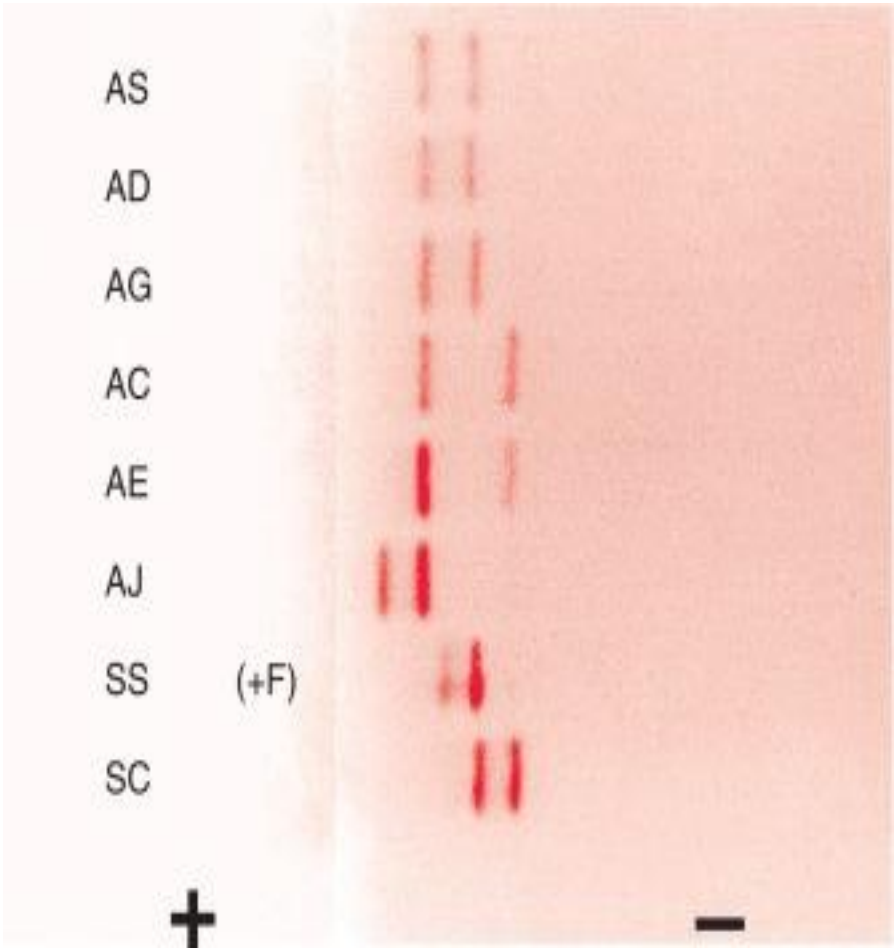
- It is a simple rapid and low cost technique for detecting haemoglobin variants.
- It is carried out using cellulose acetate membrane at a pH of 8.4-8.6.
- At alkaline pH, haemoglobin is a negatively charged protein, and when subjected to electrophoresis will migrate toward the anode (+).

Haemoglobin Electrophoresis

- Structural variants that have a change in the charge on the surface of the molecule at alkaline pH will separate from each other.
- When sample is being run, control samples must be included.

Haemoglobin Electrophoresis





Capillary Electrophoresis (CE)

- It combines two principles of separation of hemoglobins, the electrophoretic mobility in alkaline buffer and the electro-osmotic flow technique.
- High voltage is applied to an in silica glass capillary to prompt hemoglobin molecules to migrate toward a detector of 415-nm wavelength.

Capillary Electrophoresis (CE)

- CE is able to detect and relatively quantify HbF,
  - HbA
  - HbA_2
  - , HbS
  - HbC
  - HbD^{Punjab}
  - HbO^{Arab}
  - HbE
  - Hb Lepore

Trans cranial Doppler Scans

- It is a screening test.
- It uses ultrasound waves to measure cerebral blood flow rates and velocities.
- It is used as a predictor of the risk of stroke.
- The stroke prevention trial in sickle cell disease (STOP) trial concluded that the test help identify children at risk of developing ischaemic stroke.
- The test is useful for children 14 years and below.

Trans cranial Doppler Scans

- The flow velocity measured as and time average maximum mean velocity (TAMV) of 200cm/s was used as cut off.
- The flow velocity above the cut off indicates a 10% risk of developing a stroke within the year. Studies however seem to suggest that using a value of 140cm/s produces better predictive values.
- The test method currently uses both peak systolic velocity (PSV) and time average maximum mean velocity (TAMV) for risk prediction.

STOP Risk Classification and Management Strategy

- Using the TAMV, the following values are used as cut offs
- <170cm/s Normal study
- 170-200cm/s Borderline values requiring frequent monitoring.
- >200cm/s treatment/ intervention required usually transfusions and hydroxyurea therapy.
- Using PSV, the following values are used as cut offs
- <200cm/s Normal study
- 200-250 borderline/conditional values requiring frequent monitoring.
- >250cm/s high risk. Treatment/ intervention required.

Conclusion

- Sickle cell disease is a disease with severe financial and psychological consequences on patients and care givers.
- Early detection and treatment help prolong th lives of the individuals concerned.
- New born screening programs enable early detection.

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ARISE

African Research And Innovative Initiative For Sickle Cell Education

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