RED CELL METABOLISM

RBCs has No: nucleus, ribosomes, protein synthesis, lipid synthesis, no oxidative metabolism or mitochondria

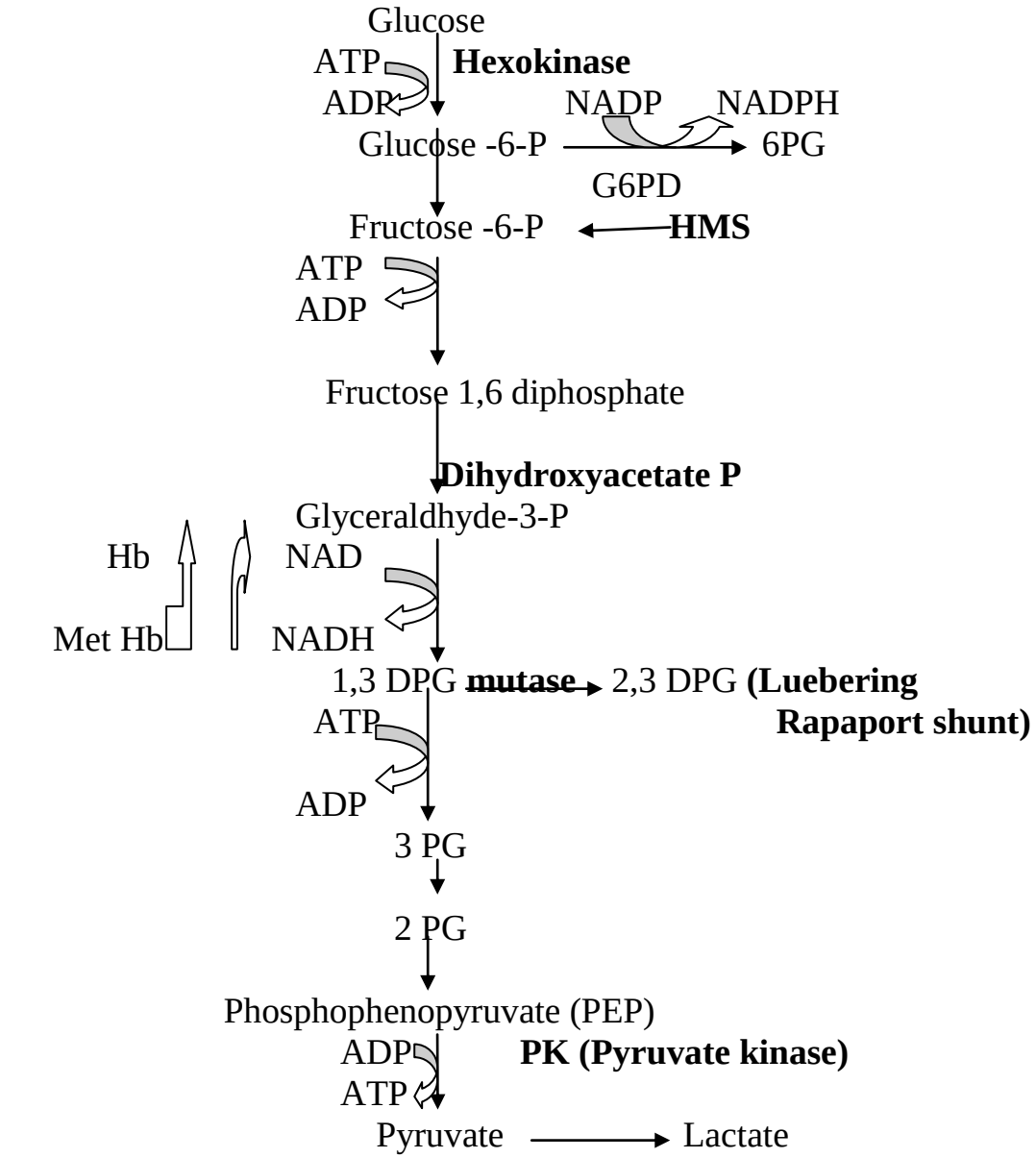
So, **the energy required for RBCs** is produced Anaerobically by :

Glycolytic pathway (90%)

Pentose Phosphate shunt (10%)

1-Glycolytic Pathway (Embden Meyerhoff Pathway)

Glucose → Lactate



Glycolytic Pathway:

Glucose enter RBC: either passively

or through specific transport ptn. called ptn. 4.5

Conversion of glucose → pyurvate by anaerobic glycolysis is accompanied by production of:

NADH

2 ATP

2,3 DPG

2 ATP:

- Regulate Na+/K+ pump
- Maintain RBCs : flexibility & normal shape.
- ↓ ATP due to a defect in this pathway → ↓RBCs life span & EVH.

NADH:

It is the main reducing power under N conditions.

Met Hb $\xrightarrow[\text{NADH}]{\text{Met Hb reductase}}$ Hb

N: amount of NADH produced= amount consumed during reduction of pyruvate to lactate, so when it is used for met Hb reduction → pyurvate accumulate.

2-3 DPG:

- Present in higher concentration in RBCs than other cells.
- Binds to β chain of Hb A→ low affinity → gives O2 easily to tissues → shift of O2 dissociation curve to the right.
- All Hbs can combine e' it except Hb F (x β chain).
- It is formed by side chain pathway only found in RBCs **(Luebering Rapaport shunt)**
- 1,3 DPG $\xrightarrow{\text{mutase}}$ 2,3 DPG

- A block below its formation (e.g: PK def.) → ↑2,3 DPG → shift of O2 dissociation curve to the right → O2 is given easily to tissues patient tolerate anaemia.

2-Pentose (Hexose) Monophosphate shunt (HMS)

G6P —G6PD→ 6-Phosphogluconate+ NADPH
NADP

It produces the reducing power of RBCs in the form of NADPH.

RBCs need their reducing power to overcome oxiditive stresses w' may cause:

Oxidation of RBCs membrane ⟶ rigidity.

,, ,, Hb → met Hb → Heinz bodies ppt.

This pathway provides 10% of Energy of RBCs.

It works under oxiditive stresses w' ↑in: Infections,
diet & drugs.

Functions of NADPH:

- Reduction of oxidized glutathione (GSSG) into reduced glutathione (GSH) {functioning form} in the presence of glutathione reductase.
- HMP is much rapid than E.M & can be accelerated up to 30% fold when oxidizing substances accumulate.
- The rate of NADPH formation depends on:
  G6PD enzyme
  rate of oxidation of glutathione.

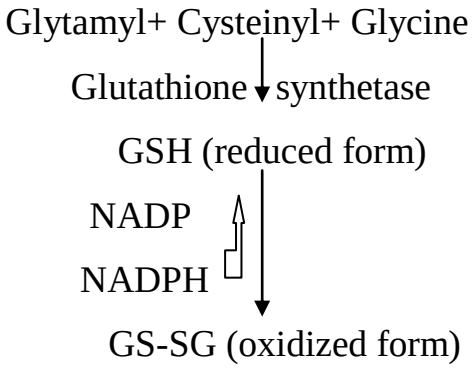
Glutathione Cycle:

Glutathione:

It is synthesized by normal RBCs.

It protects Hb & membrane protein from oxidation.

Cycle:



Reduced Glutathione :

Protection of Hb & RBCs from oxidation (met Hb → Hb).

,, ,, RBCs proteins.

Detoxification of H2O2.

Disorders of RBCs metabolism

1-Herediatry disorders of **glycolytic pathway.**

2- Disorders of **oxidation reduction system**.

3- ,, ,, **HMS.**

4- ,, ,, **nucleotide metabolism.**

Enzyme deficiency may be due to:

- Failure of synthesis
- Abnormal structure (can't react e' substrate).
- ,, ,, (,, ,, ,, activator or inhibitor)
- Unstable molecule (disappears rapidly from RBCs).

I- Disorders of Glycolytic pathway

(Embden-Mayerhof pathway)

Pyruvate Kinase (PK) deficiency:

Phosphoenolpyruvate (PEP) $\xrightarrow{PK}$ Pyruvate

ADP ATP

So, in PK:

↓ glycolytic rate

↓ ATP production

↑ 2,3 DPG ⟶ patient can tolerate anaemia

PK isoforms (isoenzymes):

L form: in liver

M form: in muscles

Inheritance: AR

C/P:

- Presented at infancy, childhood or at adult life.
- Anaemia : 4-10 g/dl , but patient can tolerate it (due to ↑2,3 DPG)
- It improves as child grows (Hb A)
- It ↑ e' infections.
- Jaundice, neonatal jaundice.
- Gall stones, cholecystitis, biliary colics.
- HSM
- Skeletal changes.

Lab diagnosis:

I-3 evidences

II-CBC:

N morphology, or shrunken, irregularly contracted due to ↓ATP.

↑ Retics.

III-Special tests:

i- Autohaemolysis test: 3 tubes

1-patient blood only ⟶ haemolysis

2- ,, ,, + glucose ⟶ ,,

3- ,, ,, + ATP ⟶ no haemolysis

Corrected by ATP & not glucose.

ii- Osmotic fragility :

Fresh sample ⟶ ↓ O.F due to ↑retics (resist osmosis)

Incubated sample (24 hs) ⟶ ↑O.F due to ↓ ATP.

iii-↑2,3 DPG, shift of O2 dissociation curve to the right.

iv- Enzyme assay:

By spectrophotometer , usually affected persons have 5-20% of N, but sometimes ↑ due to:

↑ retics

WBCs (ve 200 times Pk activity than RBCs).

Ttt:

- Folic acid
- Transfusion
- Splenectomy
- cholycystectomy

II-Disorders of Oxidation reduction system

Failure to generate sufficient reducing power to overcome oxidant stresses → Met Hb formation → ppt of denatured Hb (Heinz bodies) → RBCS membrane rigidity.

Pathway of met Hb reduction.

A- Enzymatic reduction:

- NADH met-Hb reductase
- NADPH ,, ,, ,,

B- Non enzymatic reduction:

- Methylene blue
- Ascorbic acid
- Reduced glutathione

Abnormalities in met Hb reduction

A-NADH met-Hb reductase

It reduces met-Hb formed under N conditions.

Met Hb —(met Hb reductase; NADH → NAD)→ Hb

Its deficiency: congenital methaemoglobinaemia.

C/P:

Cyanosis since birth due to ↑ met-Hb , but:

- no shortening of RBCs life span (no haemolysis).
- no Heinz bodies formation
- patient lives N.

Diagnosis:

By enzyme assay ↓

D.D:

Other conditions causing cyanosis since birth: e.g:

- Cyanotic heart diseases

- Pulmonary diseases.
- Abnormal Hb (Hb M).
- Exposure to oxidizing compounds (e.g: nitrites).

B- NADPH-met-Hb reductase

- Reduced met-Hb formed under oxidizing stresses.
- Its deficiency causes nothing (no cyanosis, no H.A), as NADPH can function through:
- Reduced glutathione
- Or ascorbic acid

III-Disorders of HMP & Glutathione

Defects in pentose shunt or glutathione metabolism, impair the ability of the red cell to defend against oxidants e' oxidation of its Hb & proteins.

The most common cause is G6PD def.

Causes:

1-G6Pd def. sex linked.

2-Glutathione reductase def. AR

3-Glutathione synthetase def. AR

4-Glutamyl- ctsteine synthetase def. AR.

Pathophysiology of oxidant cell injury due to GSH depletion:

It occurs in steps:

- Oxidation of Hb → met Hb
- Further oxidation → sulf Hb
- Intracellular ppt. of oxidized Hb & its aggregation as Heinz bodies.
- Attachment of Heinz bodies to cell membrane → membrane rigidity.

G6PD deficiency (Heinz body H.A)

Introduction

- It is the most common enzymatic disorder of RBCs in humans associated e' H.A.
- More than 400 G6PD variants r known according to their biochemical properties.
- Many of them r not ass. e' clinical disorders.
- Others r ass. e' H.A.
- Different biochemical forms of the enzyme exhibited, distinguished by different electrophoretic mobility, kinetic properties.

- Variants divided 5 classes according to the residual enzyme activity based (WHO).
- Mediterranean and African (A-) variants - by far the most clinically significant.
- Enzyme activity scarcely detectable in the Mediterranean type but close to normal in the African variant.

G6PD isoenzymes:

1- G6PD B:

Normal enzyme activity.

Found in 70% of American blacks.

2-G6PD A+:

Mutant form but e' N activity

Faster in electrophoresis than B

Found in 20% of American blacks.

3- G6PD A-:

Its mobility as A+

Deficient variant e' low activity: 5-15% of N activity.

Ass. e' haemolysis: older cells r sensitive to oxidant stress.

4-G6PD Mediterranean:

Mobility as B.

Low activity: 5-15% of N activity

Entire population of cells (old & new cells) r sensitive to oxidant stresses ⟶ haemolysis

5- G6PD Canton:

Activity is markedly reduced

Relation between enzymes & haemolysis:

1-G6PD B:

½ life 60 days, ↓e' RBCs aging

Yet older cells have sufficient enzymes to overcome oxidant stresses.

2-G6PD A- :

½ life 13 days

Old cells: haemolysis under oxidant stresses

3- G6PD Mediterranean:

G6PD low even in new cells, so all cells (old & new) r sensitive to haemolysis under oxidant stresses.

WHO working groups

- Class I: severely deficient, associated with chronic nonspherocytic hemolytic anemia
- Class II: severely deficient (1%-10% residual activity), associated with acute intermittent hemolytic anemia (G6PD Mediterranean)
- Class III: moderately deficient (10%-60% residual activity) - intermittent hemolysis usually ass. with infection or drugs (G6PD A)

- Class IV: normal activity (60%-150%)
- Class V: increased activity (>150%)

Genetics:

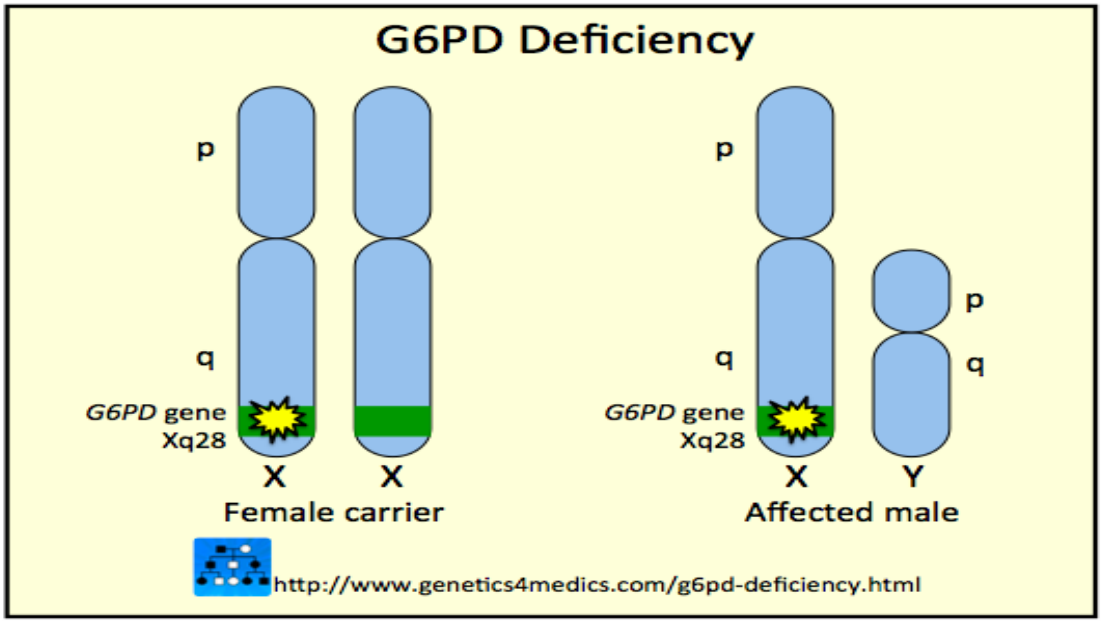
Gene coding for G6PD enzyme is located on X chromosome.

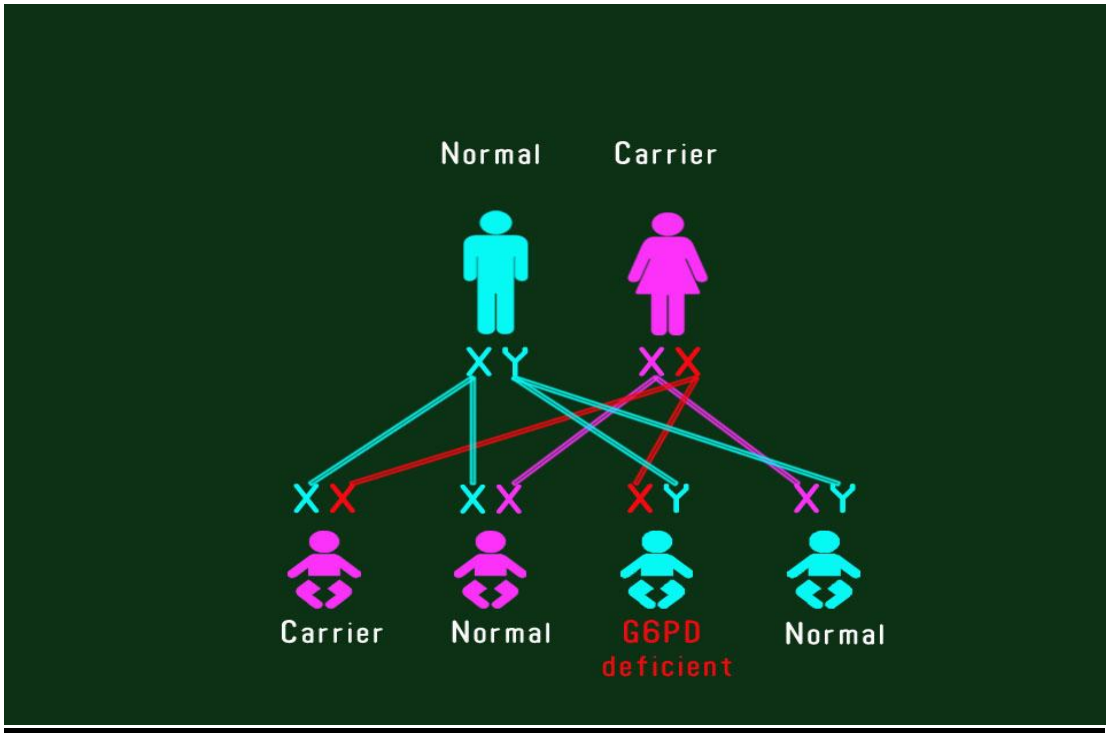
Male: has 1 X chromosome: 1 G6PD variant.

Female: has 2 X chromosomes: 2 G6PD variants ; homozygous or heterozygous (carrier).

According to Lyon hypothesis:

1 X chromosome is active & the other 1 is inactive, so female carrier may be N or abnormal according to gene carrying G6PD is active or not.





C/P:

3 ways of presentation:

1-Acute haemolysis:

drugs: antimalarial

favism

infection induced haemolysis

2-Chronic non spherocytic H.A

3- Neonatal jaundice

I-Acute H.A:

A- B-Acute drug (or infection)haemolysis:

Infection → severe bacterial infection→ ↑H2O2 (oxidant stress)

Drugs (oxidant drugs: e.g:antimalarial, sulphonamides, antibacterial, analgesics)

After 3 days → IVH

Varying severity according to:

- Type of drug
- Dose of drug

- Duration of therapy
- Type of deficiency
- **C/P:**
- Signs appear after 3 days of antimalarial drugs
- Anaemia, jaundice, haemoglobinaemia, haemoglobinuria (dark urine)
- Hb uria continues 24 hs then stops
- Self limited
- Retics are ↑ to overcome the condition as it contains more enzyme activity

C-Favism:

Fava beans contain oxidant stresses

C/P:

H.A don't always follow fava beans in G6PD def.

This depends on:

Dose to body weight ratio (↑ in children)

Type of beans (more when eaten raw).

Hs or days after ingestion of beans IVH

It is common presentation in children

In between attacks: patient is completely normal

II-Neonatal jaundice (NNJ):

- Severe jaundice 2-40 days after birth & needs exchange transfusion.
- The condition is aggravated in neonates due to lack of other reducing powers as peroxidases & catalases & also due to liver immuaturity

C/P:

- Jaundice occurs on day 2-40 & has higher bilirubin than Rh incompatibility

- Anaemia : 7-8 gm/dl
- Retics: 5-20%

Prognosis: good if kernictrus is avoided

III- chronic H.A

Diagnosis of G6PD def.:

1-History:

Drugs, infection, ingestion of fava beans.

Family history.

2-During attacks:

IVH (see before)

3- CBC:

In between attacks: no abnormalities.

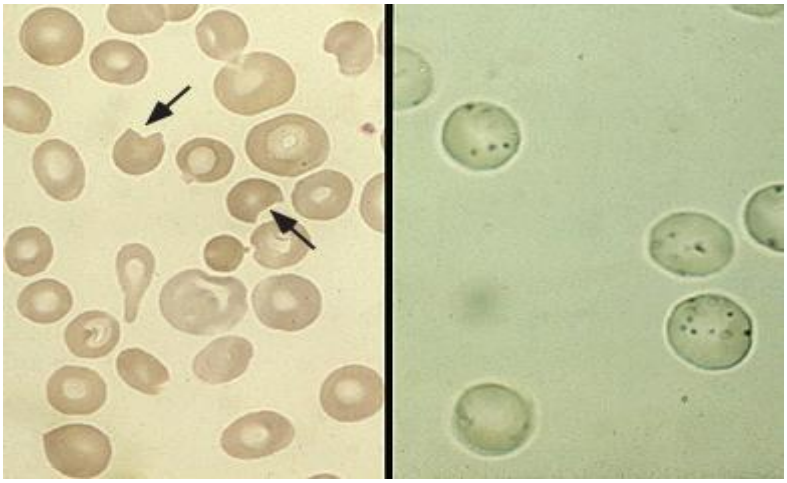
During attacks:

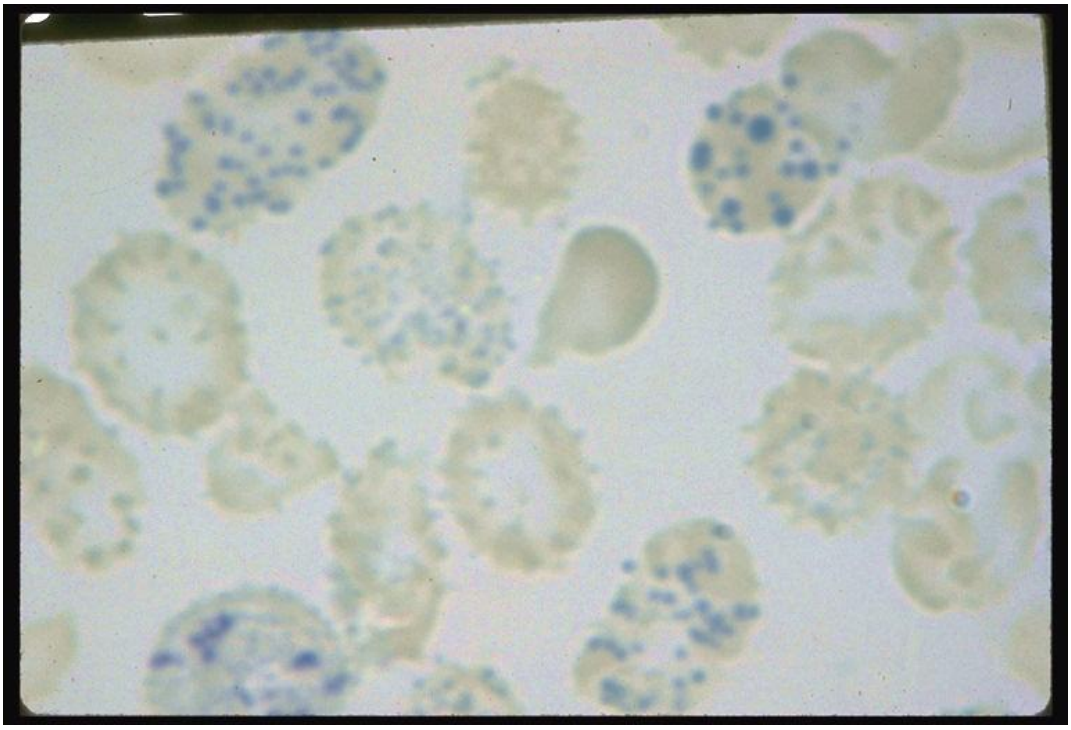
NNA, ↑retics.

Heinz bodies detected by supravital stains

Bite cells.

Peripheral Blood Smear





4-Screening tests: (G6PD test)

They r semi quantitative but reliable.

- Briliant cresyl blue discoloration
- Met Hb reduction
- U.V spot test.

In vitro:

Hb $\xrightarrow{\text{oxidant stress}}$ met Hb

+MB: N shunt : met Hb ⟶ Hb

: abnormal shunt: met Hb remains as it is.

Inability of reduction of met Hb to Hb in the presence of methylene blue (MB).

Screening tests depend on the fluorescent properties of NADPH.

5- Enzyme assay:

If screening tests +ve⟶ quantitative assay of G6PD activity by spectrophotometeric assay.

Should be done 3 weeks after acute attack when retics count return N as retics contain ↑ enzyme activity ⟶ false results.

6- DNA analysis by PCR

Diagnosis of female heterozygous:

To prevent carriers & consanguinity by:

Gene analysis by PCR

Ascorbic cyanide test

Enzyme assay.

Ttt:

Prophylactic:

Avoid ppt agents.

If fever : give paracetamol not aspirin.

Vit E in high doses protects against haemolysis.

Curative:

Any offending agents should be stopped.

Ttt underlying infections

In mild cases: observation for progress of anaemia.

In severe cases: Emergency packed RBCs transfusion (10-20 ml/kg).

IV- Disorders of nucleotide metabolism

1-Pyrimidine 5' nucleotidase (P5N) deficiency

Nucleotides —P5'N→ degeneration

AR

Congenital H.A of moderate to severe degree (non spherocytic H.A (NSHA).

Ch by:

Basophilic stippling accumulation of polyribosomal aggregates & mitochondrial fragments.

± Splenomegaly.

± mental retardation.

Diagnosis:

- Blood film
- Enzyme assay
- ↑Pyrimidine nucleotides

2-Adenosine Deaminase (ADA)

- If ↑ hereditary non spherocytic H.A. AD
- If ↓ immune deficiency AR
- **Diagnosis:**
- Enzyme assay
- **Ttt:**
- Transfusion
- BMT