CASE

1

Classical Galactosemia

A 4-month-old male child presents with the history of repeated loss of consciousness and refusal to feed specially milk and milk-containing diet. On examination, baby was found to be mild icteric and bilateral cataract* was detected. Liver was palpable below costal margin.

Following are the results of various laboratory investigations:

- Blood sugar: 72 mg/dl (normal random blood glucose = 80–140 mg/dl)
- Plasma-free galactose: 129 mg/dl (normal = <20 mg/dl)
- Serum uric acid: 8.4 mg/dl (normal = 2.5–7.2 mg/dl)
- Blood lactic acid: 4.8 mmol/L (normal = 0.5 to 1.0 mmol/L)
- RBC galactose-1-phosphate level: 54 mg/dl (normal = <1 mg/dl)
- RBC galactose-1-phosphate uridyltransferase activity was absent.

QUESTIONS

Q.1. What is the probable diagnosis in this case? What is the biochemical defect in this disease?

Q.2. Explain the biochemical reason for the above clinical signs and symptoms.

Q.3. What is the urinary finding in this case?

Q.4. What is the treatment regime suggested for this child?

Explanations

Ans.1. This child is suffering with **classical galactosemia** which is due to deficiency of enzyme: **'Galactose-1-phosphate uridyltransferase' (GaliPUT).** This enzyme is responsible for conversion of galactose-1-phosphate to glucose-1-phosphate. It is an autosomal recessive disorder. The pathway of galactose metabolism is illustrated in Fig. 1.

Ans.2. Biochemical explanation of clinical signs and symptoms is as follows:

a. **Hepatomegaly*:** Lack of this enzyme impairs galactose metabolism and results in accumulation of **galactose-1-phosphate** in liver which results in **hepatomegaly.**

b. **Recurrent hypoglycemic attack:** Accumulated galactose-1-phosphate inhibits glycogen phosphorylase enzyme which is manifested as **recurrent hypoglycemic**

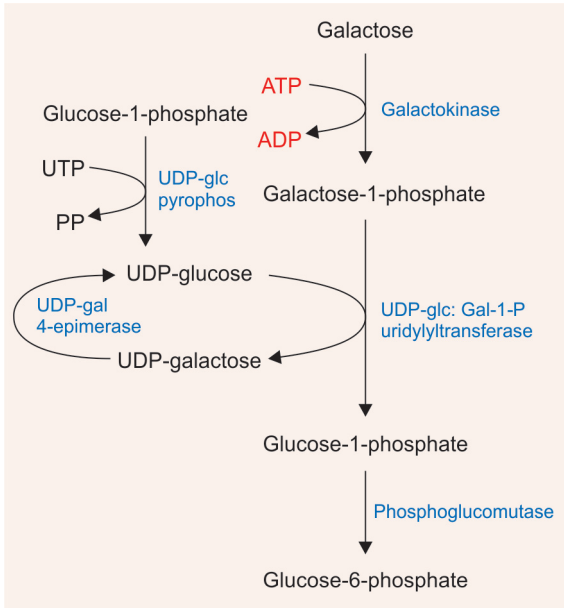

Fig. 1: Biochemical pathway involved in galactose metabolism

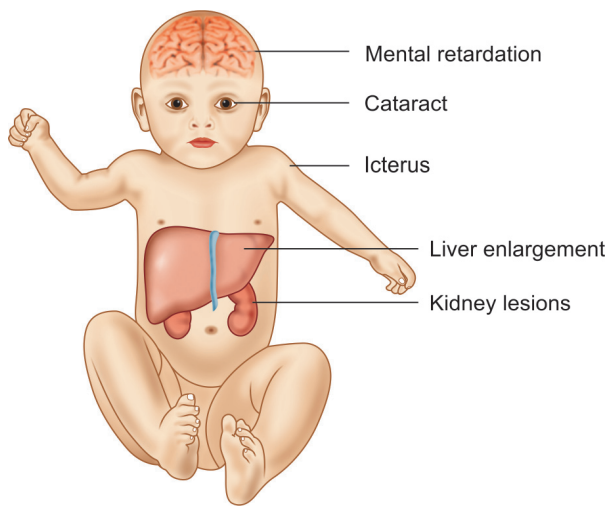

Fig. 2: Key manifestation of classical galactosemia

episodes. This is due to the fact that glycogen phosphorylase is the key enzyme in the liver to supply glucose in blood by glycogenolysis at the time of need. Once inhibited, this enzyme does not run the glycogenolysis and results in recurrent hypoglycemia.

c. **Cataract*:** Cataract in the baby is due to reduction of unmetabolized galactose in the lens of the eye in **polyol pathway**, where **aldol reductase** converts galactose to

galactitol which initiates cataract formation. Cataract is bilateral and in most of the cases, cataract is mild and transient and is reversible provided dietary treatment with lactose- and galactose-free diet, starts early within 20 days of life.

Ans.3. Galactose is not metabolized in galactosuria. This results in higher level of galactose circulating in blood which gets filtered out in the urine.

Benedicts test done in urine of such patients gives positive result as galactose is a reducing carbohydrate.

Ans.4. Treatment of this child is lactose and galactose-free diet. This regime should be started within 10 days of life. Delay in starting the treatment results in organ damage and low IQ. Despite adequate treatment from an early age, children with classic galactosemia remain at increased risk for developmental delays, speech problems and motor dysfunction.

Childhood **apraxia* of speech** and **dysarthria*** require expert **speech therapy.** Cataract may require surgery. If left untreated, galactosemic babies are prone to develop *E. coli* infection and may land up in sepsis and death.

Other important points related to this case:

- *Food items need to be avoided in galactosemic baby*: Breast milk, cow milk, casein and whey-containing food, medications having galactose and lactose to be avoided in galactosemia.
- Prenatal genetic diagnosis is possible in cultured amniotic fluid for **galactose-1-phosphate uridyltransferase enzyme assay.**

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Word Meaning

Hepatomegaly: Enlargement of liver.

Cataract: A disease which causes dense and cloudy lens of eye which impairs vision.

Apraxia: Inability to perform certain task.

Dysarthria: Difficulty in articulation of speech.

CASE

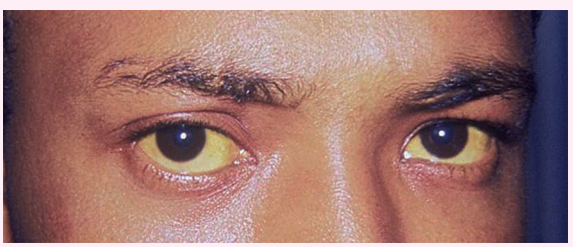
2

Glucose-6-Phosphate Dehydrogenase Deficiency

A 23-year-old African boy is brought with fever, chills and rigors*; on examination, spleen was palpable, **antimalarial drug primaquine** was given. After two days, patient visit again with history of fatigue, breathlessness, pale skin, passing dark-colored urine. He also reported having abdominal discomfort.

Following are the results of various laboratory investigations:

- Hemoglobin: 8 g% (normal = 12–15 g%)
- Total bilirubin: 5.3 mg% (normal = 0–1.00 mg/dl)
- Conjugated bilirubin: 3.3 mg/dl (normal = 0–0.4 mg/dl)
- Unconjugated bilirubin: 2.0 mg/dl (normal = 0–0.6 mg/dl)
- SGPT: 65 IU/L (normal = 15–45 IU/L)
- SGOT: 148 IU/L (normal = 15–45 IU/L)
- Blood urea: 48 mg/dl (normal = 15–45 mg/dl)
- Serum creatinine: 0.82 mg/dl (normal = 0.6–1.2 mg/dl)



Jaundice with icteric sclera

QUESTIONS

Q.1. Explain the possible reason for such a presentation in this male.

Q.2. What is the biochemical basis of the signs and symptoms?

Q.3. Mention the factors which may precipitate the attack of **acute hemolytic anemia (AHA)** in patient having underlying G6PD deficiency. What is **favism**?

Q.4. Why the deficiency of G6PD is more common in geographical areas where there is high prevalent of malaria?

Q.5. What is the treatment of this disorder?

Explanations

Ans.1. This patient is suffering with **glucose-6-phosphate dehydrogenase (G6PD) deficiency.** G6PD is protein having 516 amino acid and approximately 140 mutation is known in it. G6PD is the rate-limiting enzyme of HMP shunt pathway which is the major pathway responsible for production of NADPH. This results in inadequate amount of NADPH production in HMP shunt pathway.

Most of the cases are due to **sporadic mutations*** and in inherited conditions, it is inherited as **X-linked disease.** The *G6PD* gene is located on the long arm (q) of the X chromosome (Xq28).

Most common **enzyme deficiency associated with hemolytic anemia** is **G6PD deficiency** which is responsible for 96% cases of enzyme deficient hemolytic anemia. Less than 4% cases are due to deficiency of **pyruvate kinase** and less than 1% cases of enzyme deficient hemolytic anemia are due to **phosphoglucomutase** deficiency.

Ans.2. Intake of **primaquine** (an antimalarial drug) has precipitated an attack of hemolytic anemia in this patient who is presenting with breathlessness and dark colored urine.

This patient has underlying deficiency of glucose-6-phosphate dehydrogenase which has compromised the production of NADPH. Deficiency of NADPH does not maintain **glutathione in reduced state** which is necessary to protect the cell's hemoglobin and is important to maintain the membrane integrity. This protects the RBC against highly reactive oxygen radicals **(oxidative stress)**.

Ans.3. Precipitating factors for **acute hemolytic anemia** (AHA) in G6PD deficiency are:

a. Drugs
b. **Fava beans**
c. Certain infections.

Drugs

Hemolytic anemia episodes can result from exposure to certain drugs like **antimalarial, antipyretics and sulfa drugs.** Detail lists of drugs responsible for precipitation of acute episode of hemolysis in patient having G6PD are as follows: Primaquine, Sulfacetamide,

Fig. 1: Primaquine tablets precipitate hemolysis in G6PD deficiency

Norfloxacin, Acetanilid, Cotrimoxazole, Dapsone, Doxorubicin, Furazolidone, Methylene blue, Moxifloxacin, Nalidixic acid, Naphthalene, Niridazole, Nitrofurantoin, Pamaquine, Pentaquine, Phenazopyridine, Phenylhydrazine, Rasburicase, Sulfanilamide, Sulfapyridine, Thiazolesulfone, Toluidine Blue.

Fava Beans

Acute hemolytic anemia in G6PD-deficient people can develop after eating fava beans. This is known as **favism.** The chemicals, known as **vicine and convicine**, found within fava beans that trigger acute hemolytic anemia episodes in G6PD-deficient people.

Fig. 2: Fava beans

Typical Presentation of a Child having Hemolytic Episode

The symptoms begin 2 to 3 days after drug intake or infection but the time of onset of symptom is lesser in **favism.**

A child may have a slightly elevated temperature within 24–48 hours and can become irritable and unruly, or subdued and lethargic. Nausea, abdominal pain and diarrhea may develop. Urine may become noticeably dark and can appear red, brown or even black. Affected children may become pale and their resting heart rate may be high **(tachycardia).** Jaundice can also develop and the liver and spleen may become enlarged.

Ans.4. G6PD deficiency affects individuals of all races and ethnic backgrounds. Approximately 400 million people worldwide suffer from G6PD deficiency. **The highest prevalence rates are found in Africa, the Middle East, certain parts of the Mediterranean, and certain areas in Asia.**

The geographic distribution of G6PD deficiency correlates strongly with the distribution of malaria. It is proved in research that *G6PD* gene mutation conveys **protection from malaria** in these regions. The specific manner how **G6PD deficiency protects against malaria is** not fully understood but possibility is that this protective quality is linked to the inability of malaria to grow efficiently in G6PD-deficient cells.

Ans.5. Treatment is symptomatic and care to be taken to avoids precipitating factors. Before prescribing any of the drug enlisted above (in answer 3) drug history need to be explored and any previous episode of hemolysis need to be ruled out.

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Word Meaning

Chills and rigors: Feeling of cold and shivering.

Sporadic mutations: Mutation which is not inherited from parents, rather is acquired.

Tachycardia: Rapid heart rate.

Palpable: Which can be felt by hand.

Oxidative stress: State of excess free radical/reactive oxygen species in the system.

Lethargic: Feeling of weak and sluggish, not interested in doing anything.

CASE

3

Hereditary Fructose Intolerance

A 3-year-old boy is brought in an unconscious state. He had headache, dizziness and a history of intake of **sugarcane juice** followed by weakness. On examination, hepatomegaly was noticed. Baby was icteric and knee joint tenderness was observed.

Following are the results of various laboratory investigations:

- Blood glucose: 40 mg/dl (normal random blood glucose = 80–140 mg/dl)
- Serum uric acid: 7.9 mg/dl (normal = 2.5–7.2 mg/dl)
- Blood urea: 87 mg/dl (normal = 15–45 mg/dl)
- Serum creatinine: 1.9 mg/dl (normal = 0.6–1.2 mg/dl)
- Blood lactate was high

QUESTIONS

Q.1. What is the most probable diagnosis in this case?

Q.2. What is the biochemical basis of the signs and symptoms?

Q.3. What is the treatment advised?

Q.4. What other disorder is associated with deranged fructose metabolism?

Explanations

Ans.1. This child is suffering with **hereditary fructose intolerance (HFI)** which is due to deficiency of **aldolase B**.

Hereditary fructose intolerance is an autosomal recessive disorder with a frequency of 1 in 20,000. The gene for human aldolase B has been mapped to chromosome 9q22.3.

A single missense "mutation, G to C transversion" is the most common mutation found in this disease.

Ans.2. Biochemical basis of signs and symptoms

Hepatomegaly

Aldolase B is the enzyme which is responsible for splitting **fructose-1-phosphate** into **dihydroxyacetone phosphate and glyceraldehyde.**

Out of three isoenzymes of aldolase in the liver, aldolase A, B and C, aldolase B is predominantly found in the liver.

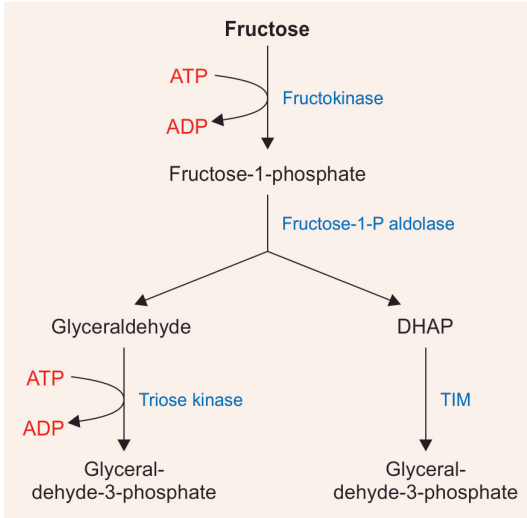

Fig. 1: Fructose metabolism in liver

In deficiency of aldolase B, fructose metabolism is affected and fructose-1-phosphate is not further metabolized. Deposition of fructose-1-phosphate results in hepatomegaly.

In addition, sequestration of Pi in the form of fructose-1-phosphate results in absolute deficiency of Pi in the cell which hampers production of ATP by oxidative phosphorylation. Reduced ATP affects ATP-dependent pumps which otherwise are responsible for maintaining the ionic gradient. This results in osmotic lysis of cells.

Hypoglycemia

Glycogenolysis is affected due to inhibition of glycogen phosphorylase by fructose-1-phosphate, resulting in hypoglycemic episode.

Lactic Acidosis

ATP is utilized in converting fructose to fructose-1-phosphate. This results in acute shortage of ATP which increases glycolysis. Increased glycolysis in limited oxygen supply in liver results in lactic acidosis **(Crabtree effect)**.

Hyperuricemia

ATP is a purine nucleotide and it imparts inhibitory affect on purine nucleotide biosynthesis by inhibiting **PRPP synthetase** enzyme.

Increased purine nucleotide biosynthesis (due to sequestration of ATP in converting fructose to fructose-1-phosphate) and then its degradation results in hyperuricemia. Impaired excretion of uric acid in lactic acidosis is also responsible for hyperuricemia. Many of such babies develop kidney failure.

Ans.3. Treatment of such a baby is complete removal of sucrose, fructose and sorbitol from diet which is certainly very difficult to implement because most fruits, vegetables and medicinal preparation contain fructose. Prognosis is poor in this condition and baby dies because of liver and kidney disorders.

Note: In a few case reports, **bilateral cataract** has been reported in babies having **hereditary fructose intolerance (HFI)**. This is said to be due to inhibitory effect of fructose on sorbitol dehydrogenase which results in excess accumulation of sorbitol

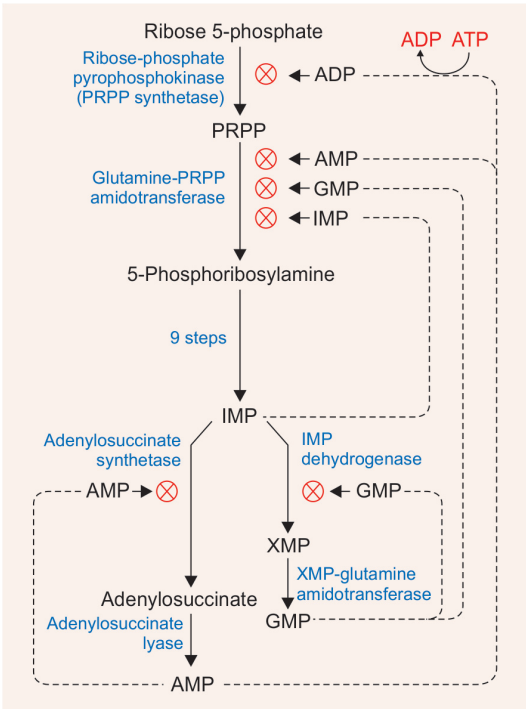

Fig. 2: Regulation of purine nucleotide biosynthesis. ATP is required for supply of ADP which inhibits action of PRPP synthetase

in lens cell. **Sorbitol** being a hygroscopic alcohol absorbs water and results in osmotic lysis of cell and initiation of cataract formation.

Ans.4. Other than hereditary fructose intolerance which is due to deficiency of aldolase B, there occurs deficiency of fructokinase enzyme which results in disorder known as benign fructosuria or essential fructosuria.

As the name itself implies, benign fructosuria is a condition which is a totally benign condition and not harmful. Affected children grow normally. It is detected accidently when urine shows positive Benedict test (due to excretion of fructose which is a reducing sugar) in absence of diabetes.

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Word Meaning

Missense mutation: A subtype of point mutation where change of nucleotide results in occurrence of a new codon which codes for amino acid which is different from original amino acid.

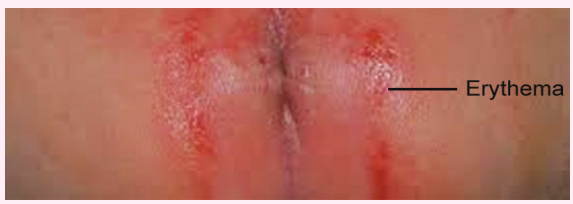
Transversion: A type of point mutation where purine nucleotide is changed for pyrimidine nucleotide and vice versa.

CASE

4

Lactose Intolerance

A 1-year-old boy presents to hospital with complain of swelling abdomen, pain and diarrhea after taking milk and other dairy products. These symptoms were existing for past 4 months and started soon after weaning was introduced. Baby is totally alright when fruit juice is given. On examination, perianal skin showed erythema* which may be due to frequent passage of stool with low pH.



Perianal skin showing erythema due to frequent stool of low pH

QUESTIONS

Q.1. What is the most probable diagnosis in this case?
Q.2. What is the enzyme deficiency in this case?
Q.3. What is the biochemical basis of signs and symptoms in this disease?
Q.4. How to diagnose a case of lactose intolerance in a baby?
Q.5. What is the dietary advice given to mother?
Q.6. What are other clinical variants of this disorder? What is adult hypolactasia?

Explanations

Ans.1. Consumption of dairy products precipitate symptoms while on intake of fruit juice, baby is alright. This history suggests that boy is suffering with **lactose intolerance.**

Ans.2. This is due to deficiency of **lactase** enzyme (which is **beta galactosidase**). The gene of enzyme lactase is located on the long arm of chromosome 2 (region 2q21). Maximum lactase expression in intestinal cell occurs during the first months of life and declines after weaning.

Deficiency of this enzyme is common in preterm infant. This is the last **disaccharidase** which develops during intrauterine development, hence the deficiency of this enzyme is commonly seen in babies born prematurely.

The lactase enzyme is often situated in the microvilli of the small intestine (specially mid-jejunum) and it hydrolyses dietary lactose into its monomer D-glucose and D-galactose which are then being absorbed.

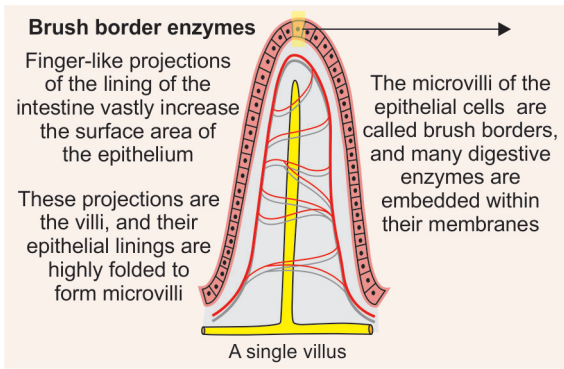


Fig. 1: Lactase is a brush border enzyme

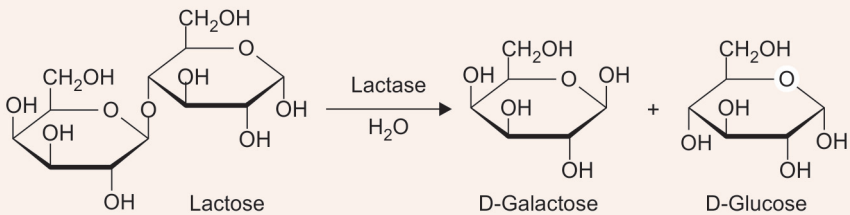


Fig. 2: Action of lactase on lactose

The enzyme spans the apical membrane of mature enterocytes and is homodimer (made up of two identical extracellular 160 kDa polypeptide chains) as well as a short intracytoplasmic part.

In many populations, **lactase levels** decline after weaning. This condition is called **lactase non-persistence (LNP).** LNP affects approximately 70% of the world's population and is the underlying physiological factor for primary lactose intolerance (LI).

While the decline in lactase levels starts soon after weaning, symptoms generally do not manifest before 5 years of age. Presentation in earlier age is most commonly associated with underlying gut condition like viral gastroenteritis, cow milk allergy, giardiasis, coelic disease, etc.

Ans.3.

BIOCHEMICAL BASIS OF SIGNS AND SYMPTOMS IN A CASE OF LACTOSE INTOLERANCE

Abdominal Distension and Bloating

The undigested and unabsorbed lactose in the lumen is osmotically active and tends to absorb fluid, resulting in diarrhea. It also becomes a substrate for action of intestinal bacteria which ferment the lactose in the colon to short-chain fatty acids (SCFA), hydrogen (H_2), carbon dioxide (CO_2) and methane (CH_4).

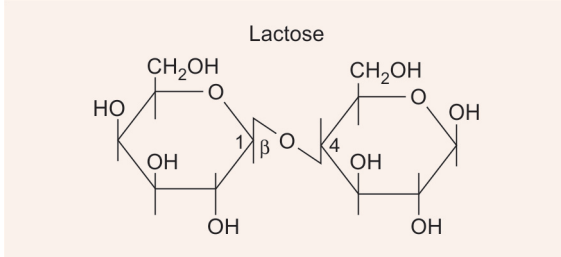


Fig. 3: Lactose is a disaccharide

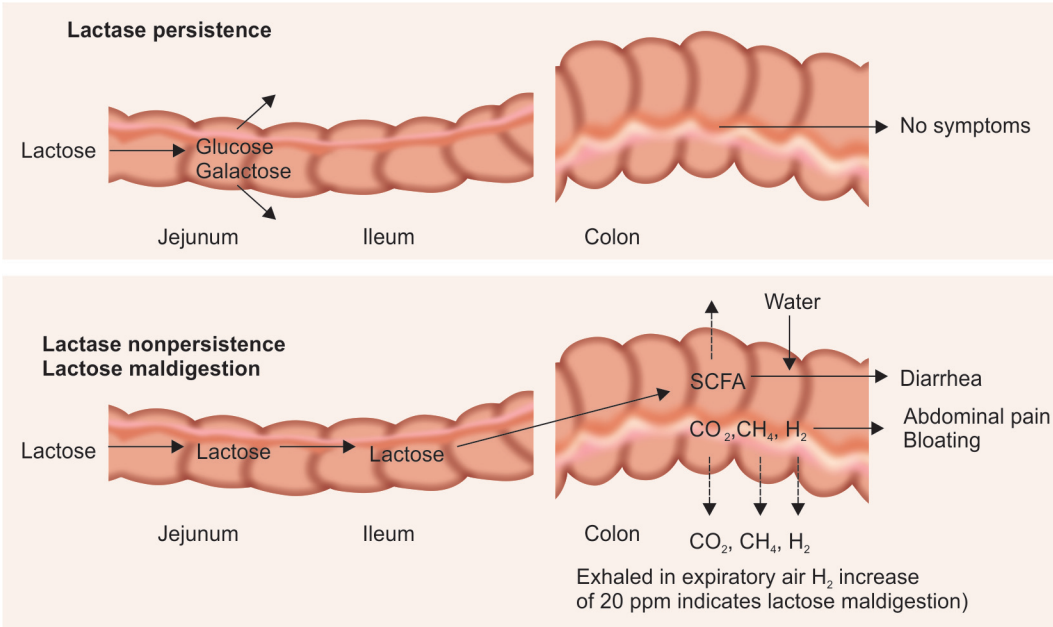


Fig. 4: Mechanism involved in abdominal pain, bloating in lactase nonpersistence (LNP)

These gases are responsible for flatulence, bloating and distension in the abdomen after consumption of milk and dairy product.

Fig. 5: Lactose-containing food items

Ans.4. Diagnosis may be done based on the following findings:

a. The **stool pH** in infants with lactose intolerance is typically **below 5.5 to 6.0**.
b. Measurement of **total and reducing sugars in stool** is an indirect test for lactose malabsorption.
c. **Breath hydrogen test:** In this test, exhaled hydrogen is measured after giving a standard dose of lactose. After an overnight fasting period, baseline hydrogen should be closed to 0 parts per million (ppm). Breath samples are taken every 30 min for 3 hr (from time of the lactose bolus).

   A rise in exhaled hydrogen by 0.20 ppm from baseline is considered diagnostic of lactose intolerance.

Ans.5. Mother is advised to give lactose- and galactose-free diet to this baby till the symptoms subside. Thereafter lactose-containing foods should be reduced but do not need to be eliminated completely.

Breast milk has high content of lactose (7.5 g/100 ml) but it should be continued.

Following are the food items having different amount of lactose:

Food	*Lactose content (g) per 100 g*
Milk (skimmed)	4.8
Milk (full)	4.7
Yoghurt (fresh)	3.0
Buttermilk	3.0
Cheese	3.0
Butter	0.5

Ans.6. Adult-type hypolactasia is characterized by the downregulation of the lactase enzyme activity in the intestinal wall with the advancement of age. Lactose intolerance is generally reversible while adult hypolactasia is an irreversible condition.

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Word Meaning

Erythema: Redness of the skin.

CASE

5

Sucrose Intolerance

A 5-week-old male child is admitted with history of diarrhea for past one week, irritability and refusal to feed for past 4 days. On careful exploring the history, it became evident that baby was exclusively breastfed for 3 weeks after which he was given dried milk powder with added sugar to enhance the taste. He developed diarrhea along with vomiting when dried milk powder was introduced with added sugar.

On examination, baby was dehydrated and irritable and was continuously screaming. Abdomen was full and tender.

Routine blood investigation for complete blood count (CBC), ESR was normal. Blood biochemistry showed normal results for various parameters. Stool culture was negative for bacteria and parasites. Baby was admitted and was treated.

Baby was found to have aversion to sweet food. Symptoms subsided when sucrose was replaced by glucose. He improved and was discharged after 4 days of admission with advice for treatment for dehydration and sugar-free diet.

QUESTIONS

Q.1. What is the diagnosis in this case?

Q.2. Explain the biochemical basis of signs and symptoms in this baby.

Q.3. How to confirm the diagnosis?

Q.4. What is the dietary advice given to mother of this child?

Q.5. What is congenital sucrase isomaltase deficiency (CSID)? What is the gold standard for diagnosis of CSID?

Q.6. How will you detect the presence of sucrose in the urine?

Explanations

Ans.1. This baby is having **sucrose intolerance.** In this condition, there is deficiency of **sucrase enzyme** which is required to cleave sucrose into glucose and fructose.

Ans.2. Deficiency of enzyme sucrase results in no digestion of sucrose which then is acted upon by intestinal bacteria which convert sucrose to various gases. This gas in the intestinal lumen results in bloating, distension and abdominal pain. Baby slowly learns to avoid sweet food and develops aversion.

Ans.3. Noninvasive test is **sucrose tolerance test and ^{13}C sucrose breath hydrogen test.**

Sucrose tolerance test is done by giving 2 g/kg of sucrose as 20% solution and blood glucose estimation is done in 30 min, 60 min, 120 min and 180 min time and is compared with fasting. Flat curve is seen in patients suffering with sucrose intolerance along with watery diarrhea after instituting this test.

This test is considered normal when there is rise of at least 20 mg/dl in glucose level compared to fasting state during the test and absence of diarrhea within 4 hours of test.

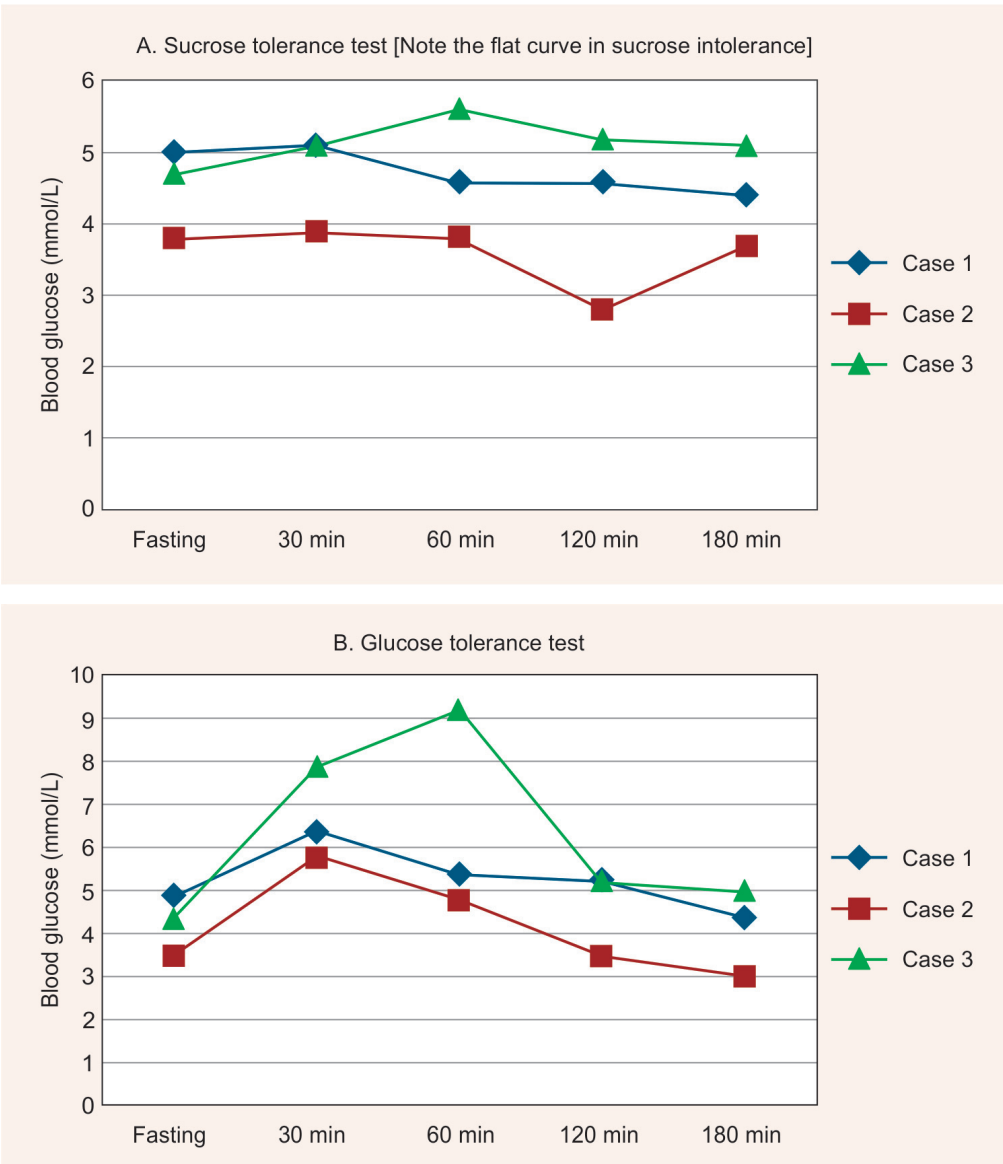

Fig. 1: Sucrose and glucose tolerance tests

Ans.4. **Sucrose (table sugar) free diet** is advised lifelong.

Sucrase (sacrosidase) has been shown to be highly effective, leading to relief of symptoms and improved nutritional status in certain studies.

Baker's yeast consists of lyophilized *Saccharomyces cerevisiae* and has enough sucrase activity, mild isomaltase activity and no lactase activity. It can be advised in sucrose intolerance patient.

Hereditary sucrose intolerance in adult precipitated by gastroenteritis is rarely reported in literatures.

Ans.5. **Congenital sucrase isomaltase deficiency (CSID)** is combined sucrase and isomaltase deficiency. It is an autosomal recessive (AR) disorder. Gold standard for diagnosis of CSID is small intestine biopsy for sucrase-isomaltase **(disaccharidase**) activity.

Ans.6. **Sucrose** can be detected in the urine by sucrose hydrolysis test. In this test first step is to do Benedict's test with the urine containing sucrose. This will result in negative test result as the sucrose is the nonreducing sugar. Thereafter, two drops of dilute HCl is added in the urine and it is boiled. After cooling, Benedict test is performed once again with such urine which now will give positive response as sucrose after acid hydrolysis yields glucose and fructose which are reducing sugars.

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CASE

6

Diabetes Mellitus

A 56-year-old lady comes with complain of increased thirst (polydipsia), increased appetite (polyphagia) and increased frequency of urine (polyuria) for past 6 months.

Following are the results of various laboratory investigations:

- Fasting plasma glucose: 146 mg/dl (normal = 80–110 mg/dl)
- Postprandial blood sugar: 209 mg/dl (normal = <140 mg/dl)
- HbA1c: 7.1% (normal = <6.5%)
- Urine is positive for sugar in dipstick test.

QUESTIONS

Q.1. What is the condition patient is suffering from?

Q.2. What are American Diabetic Association (ADA) and WHO guidelines for diagnosing the diabetes?

Q.3. What are the criteria currently adopted to diagnose diabetes mellitus?

Q.4. In addition to blood sugar and HbA1c, what other investigations would you like to do in this patient?

Q.5. What is the reason behind **polyphagia** and **polyuria** in this condition?

Q.6. What is the line of treatment?

Q.7. What is HbA1c? What is the most common method of HbA1c estimation?

Q.8. Name short-term and long-term complications of diabetes mellitus.

Q.9. What is the conversion factor for converting mg/dl glucose values to mmol/L?

Q.10. What do you understand by the term gestational diabetes mellitus (GDM)?

Explanations

Ans.1. This patient is suffering with **diabetes mellitus**. Diabetes mellitus is the disease which is due to lack of insulin action on various metabolic pathways. This may be either due to absolute lack of insulin or may occur because of insulin resistance.

Ans.2. The ADA and WHO guidelines for diagnosing diabetes are given in Tables 1 and 2.

Table 1: American Diabetes Association (ADA) diagnostic criteria for diabetes

Test	*Threshold*	*Qualifier*
Hemoglobin A1c or	≥6.5%	Lab NGSP-certified, standardized DCCT assay
Fasting glucose or	≥126 mg/dl (7.0 mmol/L)	No caloric intake for at least 8 hours
2-hour glucose or	≥200 mg/dl (11.1 mmol/L)	After 75 g of anhydrous glucose
Random glucose	≥200 mg/dl (11.1 mmol/L)	Plus classic hyperglycemia symptoms or crisis

NGSP, National Glycohemoglobin Standardization Program; DCCT, Diabetes Control and Complications Trial.
*Results must be confirmed by repeated testing.

Table 2: WHO diagnostic criteria for diabetes

Condition unit	*2-hour glucose mmol/L (mg/dl)*	*Fasting glucose mmol/L (mg/dl)*	*HbA1c*	
			mmol/mol	*DCCT%*
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycemia	<7.8 (<140)	≥6.1 (≥110) and <7.0 (<126)	42–46	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42–46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥ 126)	≥48	≥6.5

Ans.3. The international expert committee with the members appointed by American Diabetes Association (ADA), **European Association for the Study of Diabetes,** and the IDF have issued the following **diagnostic criteria for diagnosis of diabetes:**

a. HbA1c ≥6.5%, **or**
b. FPG ≥126 mg/dl, **or**
c. Two-hour postprandial* plasma glucose ≥200 mg/dl after oral glucose tolerance test (OGTT) (75 g of anhydrous glucose dissolved in water), **or**
d. Random blood glucose ≥200 mg/dl along with the symptoms of diabetes mellitus.

Ans.4. In addition to blood sugar and HbA1c, lipid profile, urine **for microalbumin** should be importantly advised.

Patient should be thoroughly worked out to rule out diabetic complication.

Urine should be investigated for microalbuminuria to rule out impending danger of **diabetic nephropathy*.**

Lipid profile should be done, as diabetic patients are prone for **dyslipidemias*.**

Ans.5. In diabetes mellitus, due to lack of insulin, glucose is not adequately metabolized and fails to produce required ATP. Patient thus constantly feels hungry and that is the reason behind **polyphagia*.**

Polyuria* in diabetes is **osmotic diuresis*** and results due to increased excretion of glucose in the urine which drags water along with it.

Ans.6. Patient should be advised **oral hypoglycemic treatment** along with dietary advice and **physical exercise** to enhance **insulin sensitivity.** Frequent monitoring of blood glucose is to be done and antidiabetic medication dose should be adjusted accordingly.

Ans.7. HbA1c is known as glycosylated hemoglobin. It is formed due to nonenzymatic combination of glucose with amino terminal group of β chain of hemoglobin.

Initially the aldehyde group of glucose form a 'Schiff base linkage' with amino group which then is spontaneously changed to more stable 'amino ketone linkage'. This reaction is known as Amadori rearrangement. **Unit of HbA1c is % as it is the percentage of total hemoglobin which is undergoing such glycosylation. Concentration of HbA1c depends upon the status of glucose in the blood and duration of hyperglycemia.**

Normal value of HbA1c = <6.5%

Value of HbA1c ≥ 6.5% is a diagnostic criterion of DM. Assessment of level of HbA1c is important for diagnosis of diabetes mellitus and also to interpret effectiveness and compliance to diabetes treatment (Tables 3 and 4).

Table 3: HbA1c and diabetic status

HbA1c	*Status*
<5.7%	Non-diabetic
5.7–6.4%	Prediabetic
≥6.5%	Diabetes
<7%	Goal of therapy
>8%	Action suggested

Table 4: HbA1c and corresponding mean plasma [*Rule of 8]

HbA1c	*Mean plasma glucose (mg%)*
5	75
6	110
7	145
8	**180 mg%**
9	215

*Rule of 8: According to this, for HbA1c value of 8%, mean plasma value is taken as 180 mg% and for every 1% increase or decrease of HbA1c, 35 mg% of glucose is added or subtracted respectively from 180 mg% value to get the mean plasma glucose value.

Ans.8. DM is a metaolic disease characterized not only by its impact on various metabolic pathways, but also it is prone for developing various complications. Complications of DM can be classified as follows:

- **Short-term complications:** Hypoglycemia, diabetic ketoacidosis, hyperosmolar hyperosmotic coma
- **Long-term complications:** Retinopathy, nephropathy, neuropathy.

Ans.9. mg/dl value of glucose can be converted to mmol/L by dividing it with factor 18:

$$\text{mmol/L value of glucose} = \frac{\text{mg/dl value of glucose}}{18}$$

Similar fashion, the glucose values given in mmol/L can be converted to mg/dl value by multiplying it with factor 18.

$$\text{Value of glucose in mg/dl} = \text{Value of glucose in mmol/L} \times 18$$

Ans.10. In 2013, the World Health Organization (WHO) recommended that hyperglycemia first detected during pregnancy be classified as either 'diabetes mellitus (DM) in pregnancy' or 'GDM'.

Pregnancy is associated with insulin resistance (IR) and hyperinsulinemia that may predispose some women to develop diabetes. Gestational diabetes has been defined as any degree of glucose intolerance with an onset, or first recognition during pregnancy.

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Word Meaning

Diabetic nephropathy: Kidney damage due to diabetes.

Dyslipidemia: Altered lipid parameters.

Polyphagia: Excessive eating

Polyuria: Excessive urination

Osmotic diuresis: Excessive urination due to filtered glucose in urine.

Postprandial: 2 hours after taking meal.

CASE

7

Diabetic Ketoacidosis

A 15-year-old boy is brought to emergency unit in a comatose state. He is a known diabetic since 5 years on irregular insulin treatment. Previous year he had an episode of loss of consciousness while playing for which he was treated with glucose and he recovered. On examination, his palm was cold and clammy and his breath was having fruity/sweet odor. Pulse rate was 120/min and respiration was fast and shallow.

Following are the results of various laboratory investigations:

- Blood glucose (random): 408 mg/dl (normal = random blood glucose = 80–140 mg/dl)
- pH = 7.05 (normal pH = 7.35–7.45)
- *Urine*
  - Protein—nil
  - Ketone bodies ++++
  - Sugar ++++

QUESTIONS

Q.1. What is the probable diagnosis?
Q.2. What is the biochemical basis of signs and symptoms?
Q.3. How is the process of ketogenesis regulated?
Q.4. What test will you do to find out presence of ketone body in the urine? What is the sensitivity and specificity of this test in diagnosing ketoacidosis?
Q.5. How is the ATP derived from ketolysis?

Explanations

Ans.1. This boy is having **diabetic ketoacidosis** (DKA) characterized by ketone body in body fluid (urine) and in breath. DKA is the complication of diabetes mellitus. Lack of insulin results in excessive mobilization of fatty acid and its oxidation results in ketoacidosis.

Ans.2. **Fruity odor of the breath** is due to loss of **acetone** in the breath. **Acetone** is one of the ketone bodies formed due to spontaneous decarboxylation of **acetoacetate.** Body has no mechanism to metabolize the acetone and being volatile this compound is exhaled in breath of the patient giving sweet **fruity odor to the breath.**

Ans.3.

Regulation of Ketogenesis

Ketogenesis is regulated at three important steps:

a. Level of fatty acid in plasma
b. Control at the level of CPT-I
c. Fate of acetyl-CoA

a. *Level of Fatty Acid in Plasma*

Precursor of ketone body is fatty acid. Approximately one-third of total content of fatty acid in the plasma is utilized by liver both in fed and starved states.

In an event of excessive mobilization of fatty acid from adipose cell during starvation, liver uses this fatty acid for ketone body production in addition of its other uses.

b. *Control at the Level of CPT-I (Carnitine Palmitoyl Transferase I)*

The major fraction of fatty acid taken up by the liver from circulation is oxidized in beta oxidation system and remainder is esterified to generate phospholipid and triacylglycerol.

Important step of regulation for diversion of fatty acid for either beta oxidation or esterification is **CPT-I enzyme** which is found at outer mitochondrial membrane.

CPT-I activity is low in well fed state. This is due to inhibitory action of malonyl-CoA (produced during well fed state) on CPT-I. In starvation and diabetes, malonyl-CoA is not synthesized hence the CPT-I is not inhibited.

This explains excess entry of fatty acid in mitochondrial matrix for beta oxidation during starvation and diabetes.

c. *Fate of Acetyl-CoA*

Diversion of acetyl-CoA to either TCA cycle or ketone body production is determined by the fact that ATP generated by acetyl-CoA should remain constant. Surplus acetyl-CoA is diverted to ketone body production.

Lack of oxaloacetate in TCA cycle due to its diversion in gluconeogenesis is also an important factor which determines deviation of acetyl-CoA for ketogenesis.

Ans.4. **Rothera's test** can be done in the urine to find out ketone body (acetone and acetoacetic acid).

This test is done in urine. 5 ml of urine is taken in test tube and is saturated with ammonium sulfate.

Freshly prepared **2% sodium nitroprusside** is mixed in urine. 1 ml of ammonia is added from the side of the test tube and purple ring formed at the junction of two liquids is observed. Appearance of ring suggests presence of ketone body in the urine.

Rothera's test is specific but not a very sensitive test to detect ketone body in the urine. Positive Rothera's test rules in the diagnosis of ketogenesis and ketonuria but negative Rothera's test does not rule out ketogenesis and associated ketonuria.

This is due to the fact that Rothera's test is positive for acetoacetate but not given by beta hydroxybutyrate, which incidently is the major ketone body in plasma and urine.

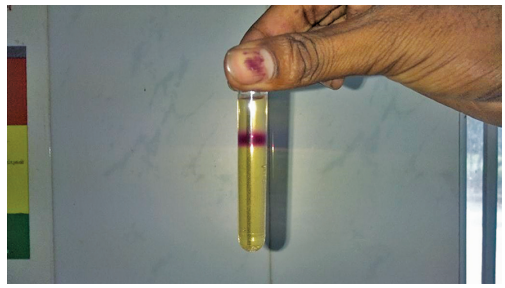

Fig. 1: Rothera's test done in urine showing positive test (note the purple color ring)

Ans.5. Ketolysis takes place in all the cells except in liver and RBC. Liver lacks thiophorase and RBC lacks mitochondria. It generates ATP in the following steps:

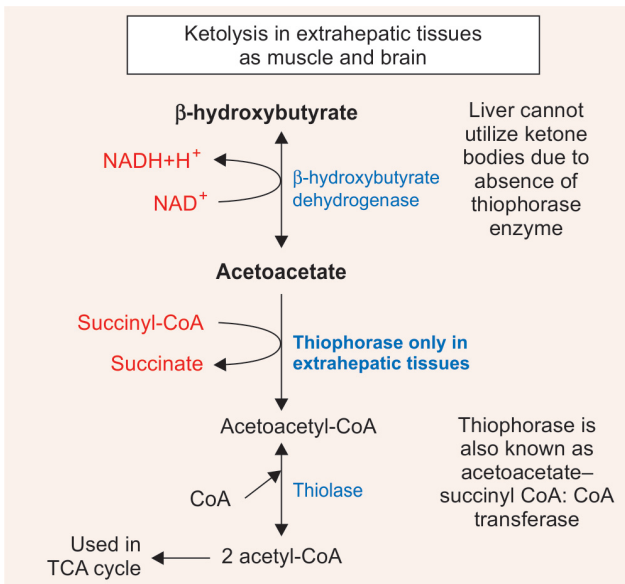

Fig. 2: Steps involved in ketolysis

i. Enzyme **beta hydroxybutyrate dehydrogenase** converts β-hydroxybutyrate to acetoacetate, with simultaneous production of NADH.
ii. Acetoacetate is converted to acetoacetyl-CoA by enzyme **thiophorase** which adds CoA moiety from succinyl-CoA.
iii. Acetoacetyl-CoA is cleaved to acetyl-CoA by enzyme thiolase.
iv. Acetyl-CoA is finally utilized in TCA cycle for production of ATP.

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CASE

8

Correct Vacutainer for Blood Collection for Glucose Estimation

A 45-year-old male is admitted for routine workup. Attending intern collected 5 ml blood in plain vacutainer at 8 am for various organ function tests including blood sugar. Time of sample processing was 11 am and report was received at 12 noon. All laboratory results were normal except blood glucose which was 47 mg/dl.

Consultant biochemistry requested for repeat blood sample in correct vacutainer?

QUESTIONS

Q.1. What is the reason of low blood glucose values in blood collected in plain vacutainer?

Q.2. What is the rate of loss of blood glucose, if blood is collected in plain vacutainer?

Q.3. What all types of vacutainers are being used in various investigations? What vacutainer is recommended for collection of blood for blood glucose estimation and why?

Q.4. What is the preferred sample for blood glucose estimation?

Q.5. Mention various biochemical methods for glucose estimation. Which method is commonly used in routine diagnostic labs?

Q.6. Explain the mechanism by which fluoride inhibits enolase action?

Q.7. As a POCT (point of care testing) glucose is estimated using glucometer. Explain.

Explanations

Ans.1. The reason of low glucose in blood collected in plain vacutainer is spontaneous glycolysis which lowers blood glucose. Blood should be collected in **fluoride**

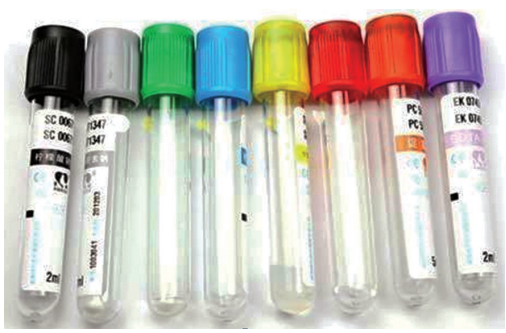

Fig. 1: Various types of vacutainers color coded

vacutainers for estimation of blood glucose as **sodium fluoride** acts as **antiglycolytic agent** and prevents loss of glucose by spontaneous glycolysis in collected blood.

Ans.2. Rate of loss of blood glucose is 10 mg/dl per hour, if blood is collected in plain vacutainer. Three-hour gap since the time of collection and actual analysis makes loss of 30 mg/dl in the above case.

Ans.3. Various types of vacutainers used in laboratory and their purpose are given in Table 1.

Table 1: Various types of vacutainers as coded by color of their cap and their purpose

Type of vacutainer/ color of the cap	*Representation*	*Additive*	*Purpose*	*Mixing instruction*
Red (glass) (clotting time: 60 min)		Nil	Serum biochemistry	Do not invert
Red (plastic) (clotting time: 60 min)		Silicone coating	Serum biochemistry	Invert 4 times
Yellow (clotting time: 30 min)		Clot activator	Serum biochemistry	Invert 4 times
Orange (clotting time: 5 min)		Rapid serum tube (thrombin based clot activator)	Serum biochemistry	Invert 4 times
Light blue		Sodium citrate (1:9)	Coagulation studies	Invert 4 times
Black		Sodium citrate (1:4)	Coagulation study	
Green		Heparin	ABG analysis	Invert 8 times
Lavender/purple		EDTA	Hemogram, ESR, HbA1c	Invert 8 times
Gray		Potassium oxalate and sodium fluoride	Blood glucose, blood alcohol	Invert 8 times

Ans.4. Plasma is the preferred sample compare to serum for blood glucose estimation as all the criteria for blood glucose report interpretation are laid down based on plasma values. Moreover, serum is extracted once the blood is clotted which takes a few minutes. This delay in separating serum from blood alters the value of glucose. Hence, plasma is preferred over serum for blood glucose result.

Ans.5. Various biochemical methods for glucose estimation are:

1. Glucose oxidase-peroxidase (GOD-POD) method.
2. King-Asatoor method.
3. Folin-Wu method.
4. Hexokinase method.

Most common method used in routine diagnostic lab is glucose oxidase-peroxidase (GOD-POD) method.

Ans.6. Enolase, also known as 2-phospho-D-glycerate hydrolase, is the enzyme of glycolysis (Embden-Meyerhof-Parnas) pathway. This enzyme catalyses reversible dehydration of 2-phospho-D-glycerate to produce PEP (phosphoenolpyruvate). This enzyme is dimer having two subunits of enzyme. Each of these two subunits require Mg^{++} for imparting the catalytic activity to this enzyme.

Fluoride interferes with accurate binding of magnesium ion to the enolase enzyme subunit and thus interfere with its catalytic activity.

Ans.7. POCT (Point-of-Care Testing) is also known as near patient testing or bedside testing is the testing system where testing is done near the patient at the point where patient care is being given. POCT is performed outside the laboratory and generally it gives the immediate result which enhances accurate patient care. Limited number of investigations are done in POCT. Glucose assessment by glucometer is one of them.

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CASE

9 von Gierke Disease (Glycogen Storage Disorder Type Ia)

A 3-year-old boy presents with history of yellow discoloration of eyes and passing high colored urine for past 20 days. Mother gives the history of many episodes of loss of consciousness in the past. On examination, baby was small for age with round face and fatty cheeks and thin extremities. He was having swollen and tender right knee joint. On palpation, liver was found to be enlarged 7 cm below the costal margin.

Baby was born by normal vaginal delivery at full term and his growth was normal till age of 1 year.

Following are the results of various laboratory investigations:

- Serum AST: 576 IU/L (normal = 15–45 IU/L)
- Serum ALT: 657 IU/L (normal = 15–45 IU/L)
- Serum ALP: 243 IU/L (normal = 98–278 IU/L)
- Total protein: 7.5 g/dl (normal = 5.5–8.5 g/dl)
- Albumin: 4.1 g/dl (normal = 3.5–5.5 g/dL
- Globulin: 3.4 g/dl (normal = 1.5–3.0 g/dl)

Random blood glucose

- Blood glucose: 47 mg/dl (normal random glucose = 80–140 mg/dl)
- Blood lactate: 6.6 mmol/L (normal lactate = 0.5–1 mmol/L)
- Serum uric acid: 7.9 mg/dl (normal = 2.5–7.0 mg/dl)
- pH: 7.31 (normal = 7.35 to 7.45)
- Ketone bodies in urine: +++

QUESTIONS

Q.1. What is the most probable diagnosis?

Q.2. What enzyme is deficient in this case and how many subtypes of type 1 GSD you know?

Q.3. Explain the biochemical basis of above signs and symptoms.

Q.4. How to approach to diagnosis?

Q.5. What is the treatment advised?

Explanations

Ans. 1 and 2. This baby is suffering with **von Gierke disease (type 1a glycogen storage disorder)** which is due to deficiency of endoplasmic reticulum enzyme **'glucose 6-phosphatase'** enzyme in **liver.** This enzyme is required at terminal step of glycogenolysis and gluconeogenesis and is responsible for conversion of glucose 6-phosphate to free glucose.

Type 1b is due to mutation of glucose 6-phosphate translocase on endoplasmic reticulum membrane. Gene map locus 17q21 is involved in type 1a and gene map locus 11q23 is involved in type 1b. **Type 1 (both 1a and 1b) is inherited as autosomal recessive** disorder.

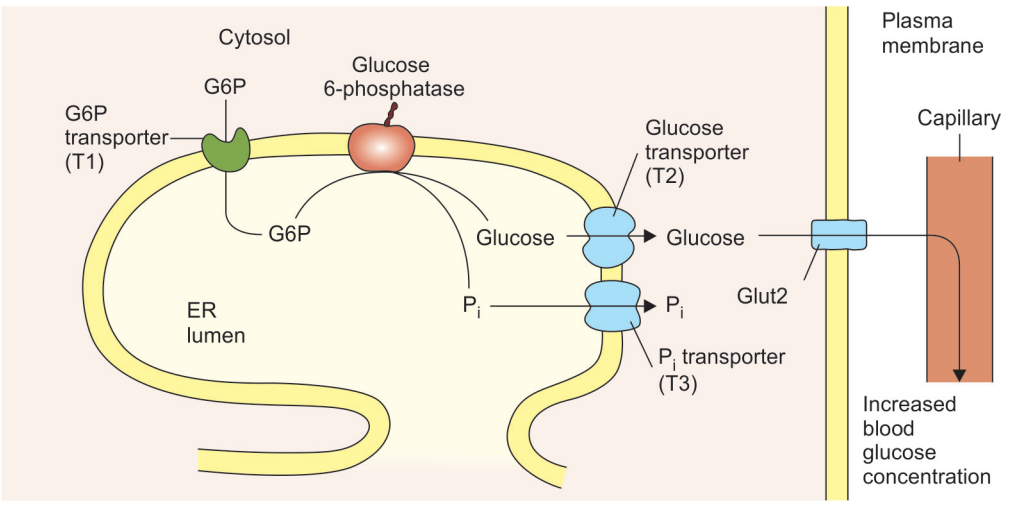

Fig. 1: Schematic plan of conversion of glucose 6-phosphate to free glucose

Type 1a constitutes 80% cases of type 1 and type 1b constitutes 20% of all cases of type 1. Annual worldwide incidence of type 1a GSD is 1 in 1,00,000 live births.

Ans.3.

Biochemical Explanation of Various Signs and Symptoms

Repeated Loss of Consciousness

von Gierke disease results in profound **hypoglycemia** and **ketoacidosis.** Hypoglycemia usually manifests first as tremors, seizures, cyanosis and apnea and, in the long-term, evolves to growth retardation.

High Blood Lactate Level

Lactate is high due to excess utilization of glucose 6-phosphate in hepatic cells for glycolysis in relative oxygen deficiency which results in **anaerobic glycolysis (Crabtree effect).**

Hyperuricemia

Hyperuricemia is due to excess purine nucleotide biosynthesis and then its catabolism resulting in high level of production of uric acid.

Excess synthesis of purine nucleotide is because of the following reasons:

a. **Excess production of ribose-5-phosphate in hexose monophosphate (HMP) shunt** pathway which is the precursor molecule for purine nucleotide biosynthesis.
b. Impaired excretion of uric acid due to **lactic acidosis** also compound to hyperuricemia.

In a nutshell, both overproduction and impaired excretion of uric acid are responsible for **hyperuricemia and gouty arthritis** in such cases.

Hepatomegaly

Liver enlargement is due to excessive deposition of glycogen due to normal synthesis and impaired breakdown.

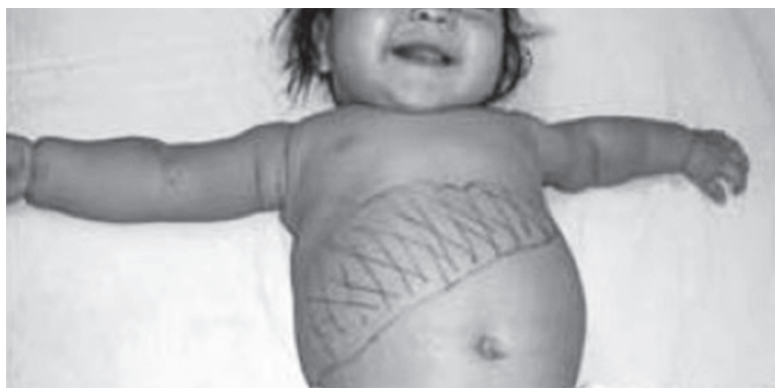

Fig. 2: Hepatomegaly marked

Ans.4. Mutation analysis of the G6Pase (glucose-6-phosphatase) and G6PT (glucose-6-phosphate translocase) genes by PCR-RFLP (restriction fragment length polymorphism), or by direct gene sequencing is done as a method of choice for diagnosis. In some cases, liver biopsy may be done for assessment of enzyme.

Ans.5. Frequent feeding with glucose or corn starch is advisable to **sustain the blood glucose level** so that baby does not get frequent attack of hypoglycemia.

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Word Meaning

Hypoglycemia: This is the condition where blood glucose level falls below 40 mg/dl. If not treated timely, this may prove fatal as neurons does not get adequate glucose and they die.

CASE 10

Cori Disease/Forbes Disease/ Limit Dextrinosis (Glycogen Storage Disorder Type III)

A 3-year-old female child born to consanguineous marriage is admitted with progressive abdominal distension for past 8 months. Mother reported that baby has difficulty in walking and gets easily tired while playing. Mother also gives the history that at times baby gets very restless and sweat in early morning hours. After giving juice or milk, she feels comfortable and sleep.

On examination, child was found to have motor retardation and was small for age. Liver was found to be enlarged (10 cm below the costal margin) with firm consistency.

Following are the results of various laboratory investigations:

AST: 345 IU/L (normal = 15–45 IU/L)

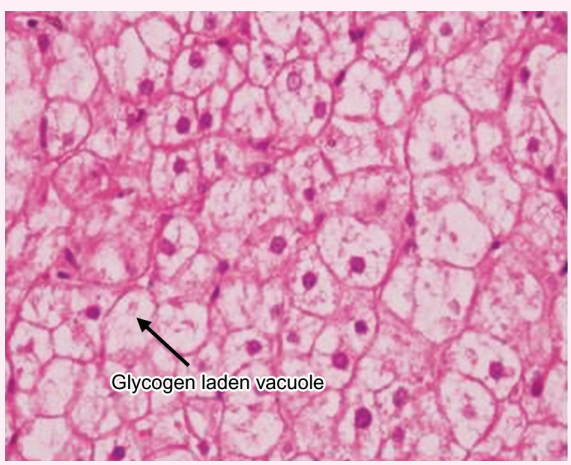
ALT: 234 IU/L (normal = 15–45 IU/L)

ALP: 221 IU/L (normal = 98–279 IU/L)

Total bilirubin: 1.8 mg/dl (normal = 0–1.0 mg/dl)

Fasting blood glucose: 56 mg/dl (normal = 80–110 mg/dl)

Liver histology revealed no fibrosis or inflammatory cell infiltration, but glycogen laden vacuoles were seen.



Glycogen laden vacuoles in Cori disease

Attending pediatrician advised **glucagon challenge test** which mother refused. Baby was diagnosed GSD type III based on liver histology and enzyme analysis.

QUESTIONS

Q.1. What is the causative enzyme deficiency in this disease?
Q.2. Why there is muscle involvement (myopathy)?
Q.3. What type of glycogen is expected in liver cells in biopsy?
Q.4. What is the glucagon challenge test? What is the rationale behind glucagon challenge test?
Q.5. What is the dietary advice given to mother?

Explanations

Ans.1. Enzyme deficient in **type III GSD is alpha-1,6-glucosidase or debranching enzyme** which is responsible for release of last exposed glucose residue from a glycogen chain. In deficiency of this enzyme, glycogen polymer with small branches which structurally resemble limit dextrin tend to accumulate in liver and muscle, hence this disease is called limit dextrinosis.

Ans.2. Muscle is involved due to incomplete breakdown of glycogen in muscle. Glucogenolysis in skeletal muscle provides glucose 6-phosphate which enters glycolytic pathway and generates ATP to sustain exercise. Any metabolic error in muscle glycogen breakdown results in exercise intolerance.

Ans.3. **Limit dextrin** type of molecule tends to accumulate in liver cell. This is due to normal action of **phosphorylase enzyme** and lack of **debranching activity.** This results in formation of limit dextrin which tends to accumulate over time.

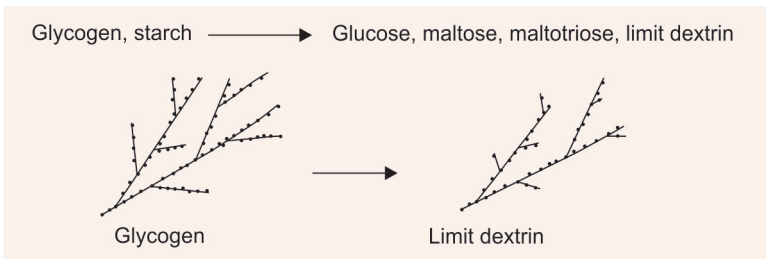


Fig. 1: Formation of limit dextrin after glycogen breakdown

Ans.4. As the symptoms of type I and type III GSD are overlapping (in GSD type III symptoms are similar to GSD I but less severe), the differentiation between type I and type III GSD is made by the **intravenous glucagon test** in the immediate postprandial period when there is usually a rise in blood sugar levels in type III but not in type I patients.

Glucagon Challenge Test

There is rise in blood sugar immediately after giving glucagon in postprandial state but there occurs no increase in blood sugar in giving glucagon in fasting state. This is

due to the fact that glucagon increases the action of phosphorylase enzyme via phosphorylation, but this phosphorylase has action only on alpha 1,4-glycosidic linkages to release the glucose. Well-formed glycogen with intact alpha 1,4-glycosidic linkages are available in postprandial state only, hence infusion of glucagon in well fed state tends to increase glucose in the blood.

In fasting state, the glycogen is already broken by phosphorylase enzyme and so the infusion of glucagon has no effect in increasing the blood glucose.

In other words, in fasting state, the glycogen is already degraded and is present in limit dextrin form and glucagon administration though activates phosphorylase enzyme, it does not get the terminal glucose which can be released.

Ans.5. Dietary advice is given in:

- High protein diet.
- Uncooked starch to maintain blood glucose level.
- Gastric drip at night, if hypoglycemia is frequently reported.
- Frequent small meal with snacks in between.

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Word Meaning

Consanguineous marriage: Marriage between cousins is known as consanguineous marriage. It is a type of interfamilial union defined as marriage between individuals who are second cousins or closer.

Parental consanguinity is associated with increased risk of autosomal recessive disorders and congenital abnormalities in the offsprings.

Limit Dextrin: Limit dextrin is an oligosaccharide which has got 8 to 10 residues of glucose in a slightly branched fashion.

It is the digestive end product of starch and glycogen in the human intestine.

CASE

11 McArdle Disease (Glycogen Storage Disorder Type V)

A 12-year-old boy presents to the OPD with complain of muscle cramps and stiffness in lower limbs soon after beginning of exercise since four years which has worsened in past 6 months. He easily gets tired while playing with friends. On careful history taking, he reveals that he feels better after 10 minutes of continuous exercise.

On examination, low lactate level in the blood was found after exercise. Muscle biopsy shows high content of glycogen (4% *vs* 0.7%).

Following are the results of various laboratory investigations:

- Blood glucose: 100 mg/dl (normal random = 80–140 mg/dl)
- Blood lactate: 0.3 mmol/L (normal level of blood lactate is 0.5–1 mmol/L)
- Creatine phosphokinase (CPK) total: 2289 IU/L (normal = 40–300 IU/L)
- Myoglobin: 556 ng/ml (normal = 25–70 ng/ml).

QUESTIONS

Q.1. What may be the probable diagnosis in this child?

Q.2. What is the biochemical basis of the signs and symptoms? What is second wind phenomenon?

Q.3. What treatment is advised for this patient?

Q.4. Whether this patient can use blood glucose for ATP production or not?

Q.5. What is the reason of dark colour urine in this patient?

Explanations

Ans.1. This child is suffering with **McArdle disease**, a **glycogen storage disorder** type V, which is characterized by deficiency of **muscle phosphorylase enzyme.** It is an **autosomal recessive** disorder which is due to mutation of **myophosphorylase gene** (PYGM) located on **chromosome 11.** This disease is named after **Dr Brian McArdle** who described it in the year 1951. Most of the cases are reported in adolescence or late adulthood.

Ans.2.

Biochemical Basis of Signs and Symptoms

Following are biochemical explanations for underlying signs and symptoms.

a. ***Exercise intolerance:*** Myophosphorylase is the glycogenolytic enzyme in muscle which is responsible for cleaving terminal **alpha-1, 4-glycosidic linkage** in a glycogen

polymer. This releases glucose 1-phosphate units which enter the glycolytic pathway after getting converted to glucose 6-phosphate with the help of enzyme **phosphoglucomutase.**

This disease is characterized by exercise intolerance which is due to lack of proper glycogenolysis in muscle which is not able to supply glucose 1-phosphate. Lack of glucose 1-phosphate results in lack of glucose 6-phosphate which thus is not available for glycolysis.

Pre-exercise consumption of carbohydrate is beneficial as the blood glucose can sustain the exercise for a while in such cases.

b. ***Low lactate in blood even after exercise:*** Lactate is not produced due to lack of glycolysis, resulting in low level of lactate in the blood after the exercise. Muscle shows abnormal high content of **glycogen (4%).** Muscle exercise results in necrosis and high level of **myoglobin.**

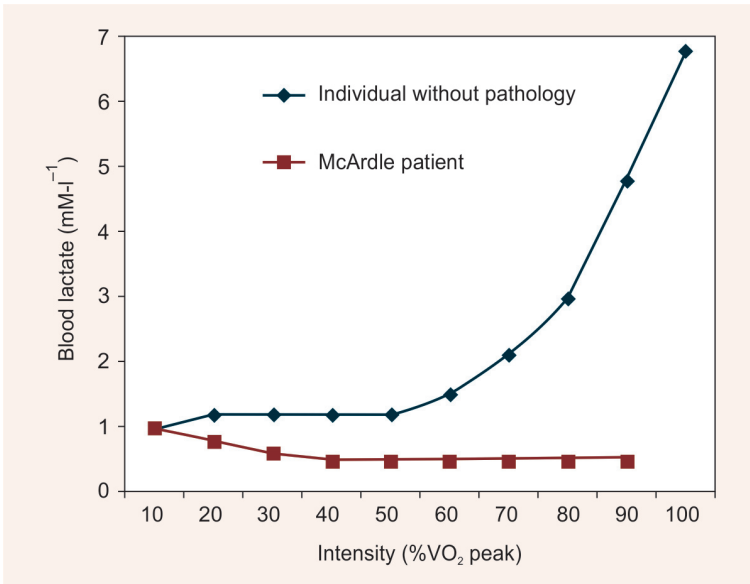

Fig. 1: Graph showing low blood lactate level even after exercise in McArdle disease

c. ***Improvement in exercise tolerance on continuing the exercise:*** To understand this, it is important to understand the second wind phenomenon. There are four important characteristics seen in McArdle disease which help in making a diagnosis:

1. **Exercise intolerance** and low blood lactate level after exercise
2. High total CPK level even at rest
3. High CPK level after exercise
4. **Second wind phenomenon**

Second Wind Phenomenon

Second wind phenomenon was first described by **Pearson** et al and it denotes considerable improvement in exercising capacity after first 10 minutes of exercise. This is explained by increased blood flow in the muscle during early phase of exercise which starts supplying fatty acid for beta oxidation and ATP production which

improves the exercise tolerance. **Second wind phenomenon is pathognomonic of McArdle disease.** Figure 2 explains the second wind phenomenon graphically.

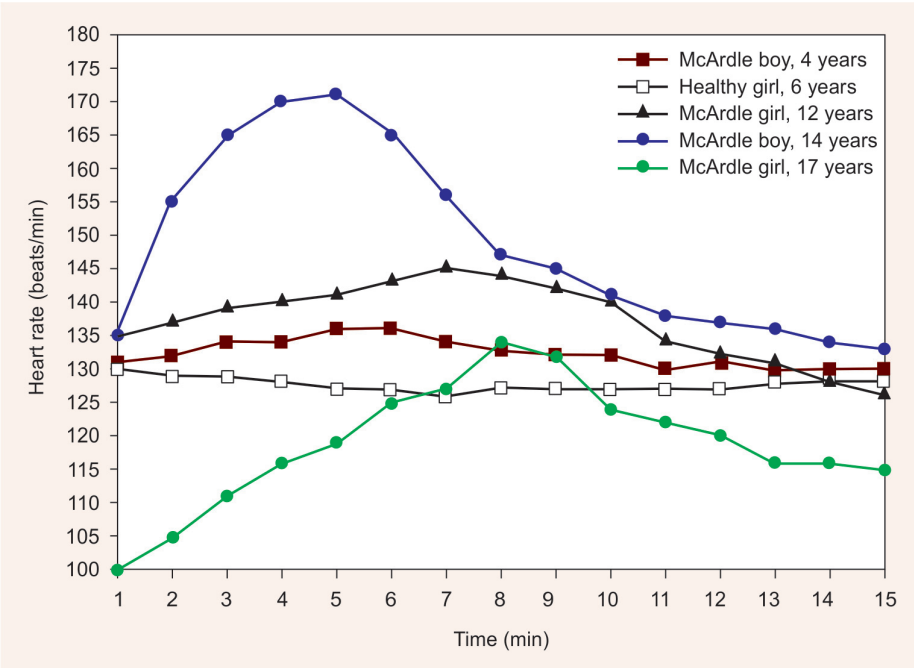

Fig. 2: Graphical representation of second wind phenomenon

d. ***High content of glycogen in muscle on muscle biopsy:*** In McArdle disease, the glycogen synthesis is normal and glycogenolysis is impaired. This results in abnormal accumulation of glycogen in the muscle. Normally there is only 0.7% glycogen in the skeletal muscle, but in **McArdle disease, the glycogen goes up to 4%.**

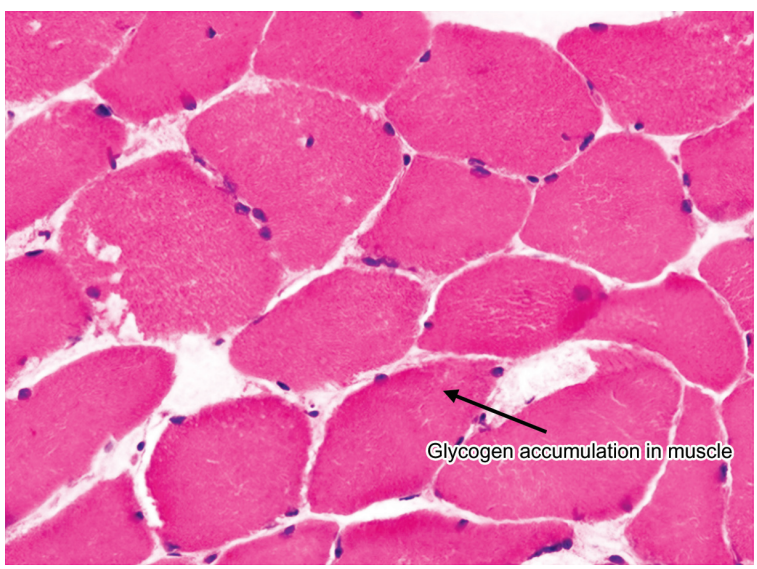

Fig. 3: High content of glycogen in the muscle in McArdle disease

It is important to note that in McArdle disease, liver glycogen metabolism is normal and there is no hypoglycemia or hepatomegaly.

Another glycogen storage disorder where glycogen phosphorylase is deficient in **Hers' disease (type VI glycogen storage disorder)**. In this disease, glycogen phosphorylase is deficient in hepatic cells alone **(Hers' Hepatic)** and muscle glycogen metabolism is normal.

In Hers' disease, presenting symptom and sign is hypoglycemia with hepatomegaly. Muscle is spared and there is no exercise intolerance.

Ans.3. The treatment for this disease is targeted at maintaining the blood glucose level throughout the day and consumption of **20 g of glucose or fructose five minutes before the start of exercise.**

Ans.4. Skeletal muscle of this patient can very well uptake blood glucose and use it in the process of glycolysis as to produce ATP.

Ans.5. Dark color urine is due to passage of myoglobin in the urine. Excessive secretion of myoglobin for a long duration may result in renal failure.

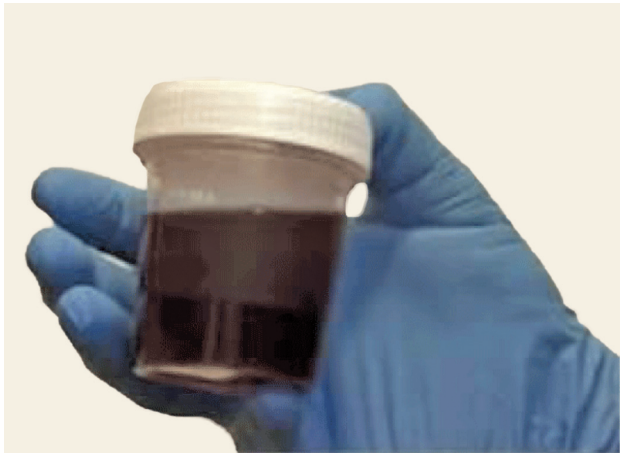

Fig. 4: Dark color urine in a case of myoglobinuria

Cycle Test

It is a physiological test which can be used to diagnose GSD V. This test record the heart rate response seen in second wind phenomenon. Here heart test is found to be notably increasing during first 10 minutes of exercise in addition to muscle cramps and pain.

Word Meaning

Rhabdomyolysis: Muscle breakdown.

Pathognomonic sign and symptoms which is specifically indicative of a disease or condition.

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CASE

12 Pompe Disease (Glycogen Storage Disorder Type II)

A 4-month-old child comes with history of rapid heart beat and restlessness. He was hypotonic and had hepatosplenomegaly.

He was diagnosed to have cardiac failure and was put on diuretics and **ACE inhibitor*.** Chest X-ray showed **left ventricular hypertrophy*.** The assay for **alpha-glucosidase** was done on dried blood spot.

The activity of the alpha-glucosidase in the patient was 0.108 µmol/L/h (normal range: 0.75–7.23 µmol/L/h)

Diagnosis of Pompe disease is made.

QUESTIONS

Q.1. Comment on the disease patient is suffering from. What enzyme is deficient in Pompe disease?

Q.2. How many clinical variants are known for Pompe disease?

Q.3. Which organ gets affected in this disorder?

Q.4. What is the treatment advised?

Explanations

Ans.1. Pompe disease is due to deficiency of lysosomal enzyme **"acid maltase (also known as acid alpha-1,4-glucosidase).** This enzyme plays important role in degradation of old and incompletely formed glycogen in the lysosome. Pompe disease is the only GSD which is included under **lysosomal storage disorder.**

This results in accumulation of glycogen granules in lysosome in virtually every tissue.

Major organ affected is heart where hypertrophic and **dilated cardiomyopathy** is observed.

Ans.2. Variants of Pompe disease are:

- Infantile
- Juvenile
- Adult

Prognosis is poor in infantile onset Pompe disease and death ensues within one year of life because of heart failure.

In adult onset Pompe disease, muscle hypotonia is seen and death is due to **respiratory insufficiency rather than heart failure.**

Ans.3. Key organ affected is **heart and respiratory muscles.** This disease is rapidly progressive and death ensues within first year of life.

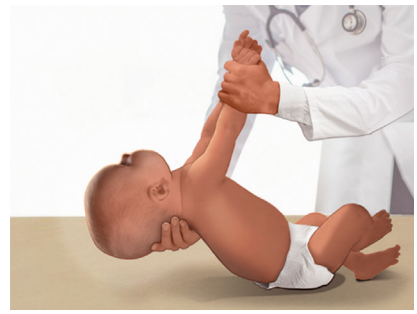
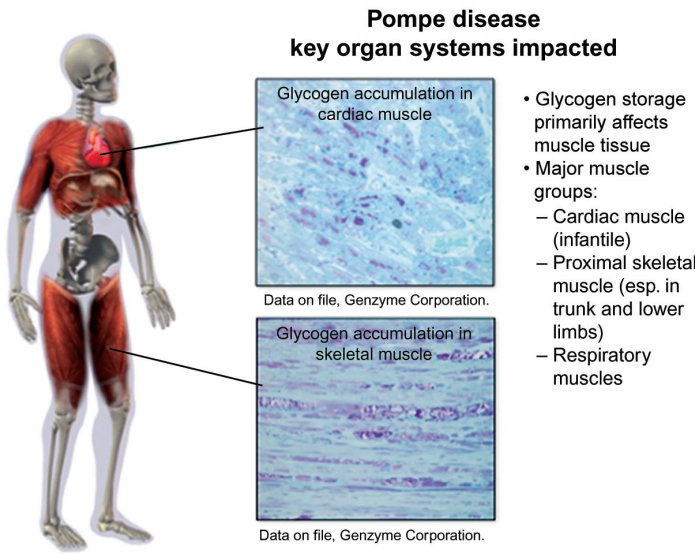


Fig. 1: Hypotonia observed in Pompe disease



Ans.4. **Enzyme replacement therapy (ERT)** is seen to prolong life. ERT can be introduced with standard dose of 20 mg/kg every 2 weeks. Individual response to such therapy varies and largely depends on age of presentation. Literatures have shown considerable improvement in cardiac involvement after such treatment.

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Word Meaning

ACE inhibitor: Angiotensin-converting enzyme inhibitor.

Left ventricular hypertrophy: Thickened and enlarged left ventricle.

CASE

13

Hurler Syndrome (Mucopolysaccharidosis Type I)

A 5-year-old boy is brought by mother with complain of difficulty in walking and joint stiffness for past 6 months. She complains that abdomen is protruded and he has difficulty in seeing objects. On examination, child had short neck, low set ears, depressed nasal bridge and coarse facial features. Abdominal examination revealed hepatosplenomegaly and urine showed presence of heparan sulfate and dermatan sulfate.

Enzyme assay in fibroblast reveals deficiency of L-iduronidase. Attending pediatrician came to a conclusion that child is suffering with mucopolysaccharidosis (MPS).

QUESTIONS

Q.1. What may be the possible type of mucopolysaccharidosis (MPS) in this case?
Q.2. Explain the genetic cause and mode of inheritance of this disease.
Q.3. What are the other MPS (mucopolysaccharidosis)?
Q.4. What is the treatment in such case?

Explanations

Ans.1. This child is suffering with **MPS I (Hurler syndrome)** as diagnosed by deficiency of **L-iduronidase** in the **fibroblast.** Due to deficiency of this enzyme, the **glycosaminoglycans**, which are accumulated in lysosomes, are **heparan sulfate** and **dermatan sulfate** which come out in the urine.

MPS is heterogenous group of disorder where various mucopolysaccharides are accumulated in lysosome in tissues like heart, respiratory system, bones, joints and central nervous system due to deficiency of one or other enzyme involved in degradation of these compounds in lysosome. Such abnormal accumulation of these compounds results in relevant clinical manifestation. **Corneal clouding** and **macroglossia** with **spaced dentition** are **characteristic findings** (Figs 1 and 2).

Definitive diagnosis is usually possible through enzymatic assays of the defective enzyme in cultured fibroblasts or leukocytes.

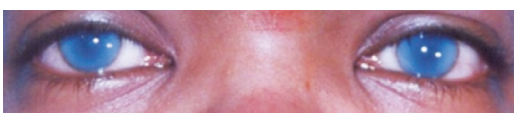

Fig. 1: Corneal clouding in Hurler's disease

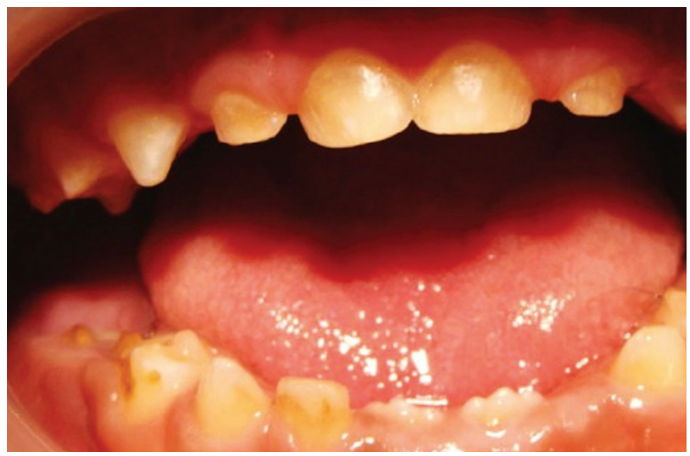

Fig. 2: Macroglossia and spaced dentition in a child with Hurler syndrome

Ans.2. MPS I is **autosomal recessive** disorder which is due to **mutation of gene IDUA** located at short arm of **chromosome 4 (4p16.3).** This results in deficiency of enzyme **alpha-L-iduronidase.**

It was first described by **Gertrude Hurler in 1919** and later the milder form of the disease was described by ophthalmologist **Dr Scheie in 1962.** Depending upon the severity of the disorder, type I MPS has three subtypes:

- **MPS I-H: Most severe**
- **MPS I-H/S: Intermediate form**
- **MPS I-S: Mild form**

Ans.3. There are total 7 types of MPS. They are described in Table 1.

Table 1: Various mucopolysaccharidoses, deficient enzymes and clinical features

Type	*Name of syndrome*	*Incidence*	*Deficient enzyme*	*Accumulated products*	*Main symptoms*
I	Hurler (I-H) (most severe form)	1/100.000	Alpha-L-iduronidase	HS, DS	Mental retardation; corneal clouding, photophobia; organomegaly; heart disease; death in early childhood
	Scheie (I-S) (mildest form)				Corneal clouding; stiff joints; normal intelligence and life-span
	Hurler-Scheie (I-H/S) (intermediate form)				Intermediate phenotype, between MPS I-H and MPS I-S

Contd..

Table 1: Various mucopolysaccharidoses, deficient enzymes and clinical features *(Contd.)*

Type	*Name of syndrome*	*Incidence*	*Deficient enzyme*	*Accumulated products*	*Main symptoms*
II	Hunter	1/250.000	Iduronate sulfatase	HS	Cornea clear, organo-megaly; short stature; death before 15 years
III	Sanfilippo A	1/150.000	Heparan sulphamidase	DS HS	Mild somatic manifes-tations; hyperactivity; profound intellectual deterioration
	Sanfilippo B		N-acetyl-glucosa-minidase		
	Sanfilippo C		Acetyl-CoA: Alpha-glucosa-minide-acetyl transferase		
	Sanfilippo D		N-acetyl-glucosamine 6-sulfatase		
IV	Morquio A	1/75.000	Galactose-6-sulfate sulfatase	KS	Short stature; motor dysfunction
	Morquio B		Beta-galactosidase	CH KS	
V	The MPS I-S was initially described as type V. This is no more being used as separate entity.		Same as type I-S		
VI	Maroteaux-Lamy	<1/250.000	N-acetylgalacto-samine-4-sulfatase	DS	Short stature; motor dysfunction; kyphosis; heart defects; death in early childhood
VII	Sly	<1/250.000	Beta-glucuronidase	HS, DS,CH	Hepatomegaly; dysostosis multiplex; short stature; corneal clouding; develop mental delay

Ans.4. Treatment is **multidisciplinary**. Following plan is adopted:

- Hematopoietic stem cell transplantation (HSCT)
- Enzyme replacement therapy (ERT)
- Supportive therapy

- Physical therapy
- Occupational therapy
- Speech therapy
- Respiratory support

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CASE

14 Hunter Syndrome (Mucopolysaccharidosis Type II)

A 3-year-old male child is brought by mother with history of pain in lower limb and difficulty in walking for past 4 months. History of delayed milestone present.

On examination, child had coarse facial features and low IQ. Liver was found to be enlarged, 4 cm below the costal margin. Cornea was clear and child had stiffness of joints in hand and lower limb.

Urinary examination revealed presence of heparan sulfate and dermatan sulfate and fibroblast showed deficiency of enzyme iduronate-2-sulfatase.

Diagnosis of Hunter syndrome was made.

QUESTIONS

Q.1. Comment on the diagnosis.
Q.2. What is the inheritance of Hunter's disease?
Q.3. What is the treatment plan in such cases?

Explanations

Ans.1. **Hunter disease is MPS II** which is caused by deficiency of enzyme **iduronate-2-sulfatase** resulting in accumulation of **MPS (HS, DS) in various tissues.** This disease is named after **Charles Hunter,** who first described the condition as an **X-linked recessive disease** in the year 1917.

Characteristic finding in this disease is mostly coarse **facial features, macroglossia, short stature, joint contractures, visceromegaly, intellectual disability and dysostosis multiplex*.** In addition, **sleep apnea, cardiomyopathy, cardiac valve dysplasia,** and **dental and dermatological manifestations** may be present.

Presentation is in early childhood as developmental delay and organomegaly, though the milder cases may present in adulthood as joint contracture and hoarseness of voice.

Ans.2. Hunter disease is a rare disorder which is inherited in **X-linked recessive fashion**. Gene is located at **Xq28.** Male child is affected predominantly though reports of this disease being present in female child is also present. Overall incidence of this disease is 1.3/1,00,000 male live births.

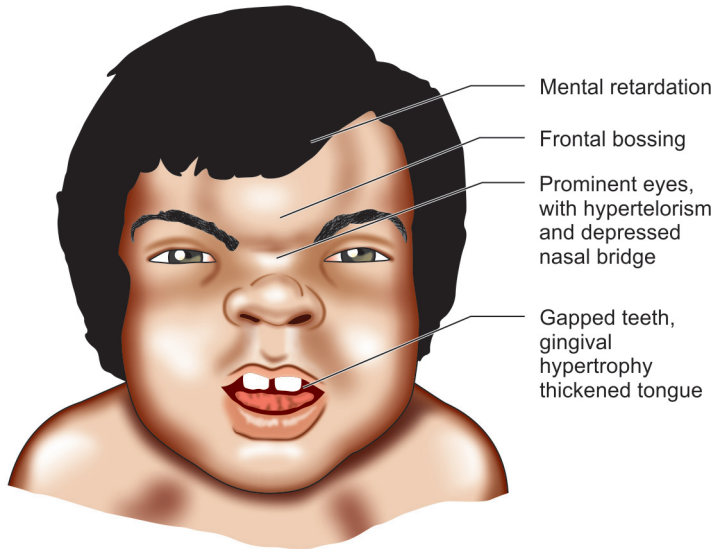

Fig. 1: Facial features in Hunter syndrome

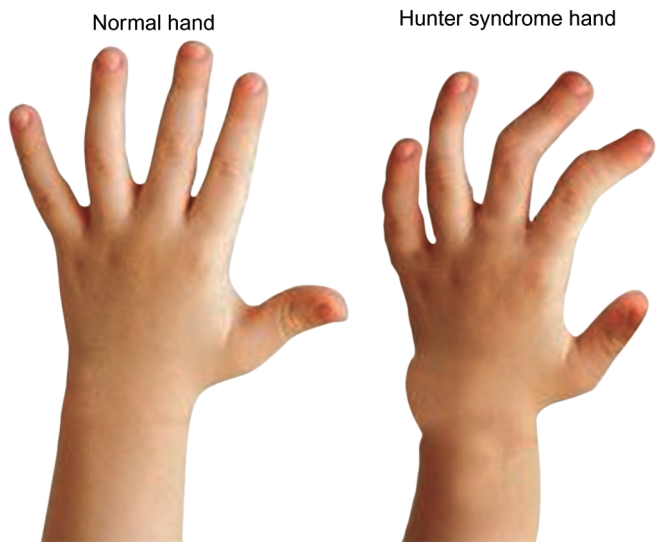

Fig. 2: Joint contracture and claw hand in Hunter syndrome

In fact, Hunter disease is the only MPS which has X-linked recessive inheritance. All other MPS described in Table 1 (Case 13) has autosomal recessive inheritance.

Ans.3. Management is multidisciplinary and a holistic approach to the patient is required, especially for patients with severe neurological involvement. Early diagnosis and initiating early treatment, particularly enzyme replacement therapy (ERT), may reduce possible disease complications and can greatly improve quality of life.

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Word Meaning

Dysostosis multiplex: Skeletal changes due to mucopolysaccharidosis.