METABOLIC MYOPATHIES: CLINICAL, BIOCHEMICAL, GENETIC AND HISTOPATHOLOGICAL BASIS OF DIAGNOSIS

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Abstract

Metabolic myopathies are inborn diseases of carbohydrate and lipid metabolism that takes place in cytoplasm and mitochondria. These characteristically result from lack of activity of one or more specific enzymes or their failure in the transportation of proteins. In this article, we present a systematic review of the clinical and biochemical basis of metabolic myopathy as most of this work has come up in last twenty years or so. Metabolic myopathies are frequently misdiagnosed because of lack of general awareness particularly among primary and secondary caregivers at neonatal, intensive care and pediatric out-patient units. This review includes inheritance patterns and clinical and laboratory findings of the more common metabolic myopathies within a clinical classification that give a general idea about these disorders. A summary of possible and hopeful treatment types for these diseases is given.

Key Words: Metabolic Myopathy, GSD, Carnitine, CPT, Lipid metabolism, Genetics, Histopathology, mitochondrial myopathy.

Introduction

Metabolic myopathies are the muscle diseases that occur due to alteration in the cellular physiological reactions as a result of deficiency or absence of enzymes catalyzing the specific reaction. The basic defect may be genetic or acquired, the genetic one being point mutation, deletion or re-arrangement in nuclear or mitochondria encoded genes. It appears that the general spectrum of myopathies described in the world literature is also seen in India, although the prevalence is difficult to ascertain, as there is very little published epidemiological data. Much information has accumulated globally in last two decades on metabolic muscle disorders through advanced laboratory techniques especially biochemical, genetic and histopathological analysis. The descriptions of muscle diseases in India can be traced back to ancient Indian medical treatise "Charak Samhita", (3000B.C.-500 BC) (1). Gouri-Devi and Venkataram analyzed these writings in the light of current clinical observations to fathom what was the concept of muscle diseases in the mind of those ancient physicians when they described them (2). The ancient Indian physicians were aware of diseases with proximal muscle weakness in spite of muscle enlargement, the finding that is commonly seen in various metabolic myopathies and muscular dystrophies. They however, attributed this phenomenon to excessive accumulation of morbid humour (with its interaction with fat) that melts from upper part of the body and gets deposited in the lower limbs particularly in hips and calves. Mc Ardle was the first person to describe metabolic myopathy in 1951(3). Metabolic myopathies are a group of muscle disorders caused by a biomedical defect of skeletal muscle energy system that results in inefficient muscle performance. The biochemical defect primarily affects ATP production. The major sources of ATP in skeletal muscle are phospho-creatine, glycogen and free fatty acids (4). For minimal routine muscle activity, high-energy phosphate bonds derived from phospho-creatine are utilized. Free fatty acids are the major source of energy at rest or during a few minutes activity; during the exercise of more than 5-10 minutes, glycogen is used for ATP production and during prolonged exercise (of more than 40 min) fatty acids derived from distant sources (adiposities) again form the major substrate for energy production.

The presentation of metabolic myopathy is variable. It usually presents with episodic cramps, weakness or myoglobinuria after exertion or prolonged exercise. However, some patients of metabolic myopathies present with fixed or slowly progressive weakness.

Metabolic myopathies by classification (Table-1) can be primary or secondary (5, 6). But, here we described it in most generalized forms i.e., myopathies due to disturbance in carbohydrate and lipid metabolism & mitochondrial myopathies.

Table-1. Classification of metabolic myopathies

Primary metabolic myopathies	Secondary metabolic myopathies
Disorder of Carbohydrate Metabolism Disorder of Lipid Metabolism Mitochondrial Myopathy Myoadenylate Deaminase Deficiency	Endocrine Myopathies Drug Induced Myopathies Periodic Paralysis Myoglobinuria Hypermetabolic state: AIDS, CHF, COPD Secondary myoadenylate deaminase deficiency

Myopathies due to disturbance in carbohydrate metabolism

The glycogen storage diseases (GSD) are a group of inborn error of metabolism characterized by glycogen accumulation in tissues due to enzyme defects in the glycogenolytic or glycolytic pathways. Among the glycogen storage diseases some present as pure myopathy, whereas others involve other tissues like liver or RBCs. When an enzyme defect affects mainly glycogen storage in liver,

a common symptom is hypoglycemia. When the defect is in the muscle tissue, weakness and exercise intolerance result from defect in the glycolytic pathway. These glycolytic pathway enzymes are found in cytoplasm, endoplasmic reticulum and lysosomes. Some enzymes are specific to individual organs. All the GSDs are inherited as autosomal recessive trait except phosphoglycerokinase deficiency, which is inherited as X-linked recessive disorder. The most important GSDs from clinical standpoint are acid maltase deficiency and myophosphorylase deficiency (7). Fourteen types of GSDs have been described so far designated as GSD-0 to GSD-XIII. Out of them GSD-0, GSD-I and GSD-VI are pure hepatic disorders and will not be discussed here. Rarely however, may these have lactic acidosis and related muscle cramps (7, 8).

GSD Type II (Pompe's Disease / Acid Maltase Deficiency - AMD)

Type II Glycogen Storage Disease is an inborn error of metabolism that belongs to a group called lysosomal storage disorders. Acid maltase deficiency is a clinically heterogeneous group of diseases that has relatively distinct age of onset variants (consisting of three forms viz. infantile, childhood and adult ones) with specific phenotypic characteristics (9). The infantile form presents in first few weeks to years of life with diffuse hypotonia and weakness of the muscles. It mimics SMA I (Werdnig Hoffman's Disease) in presentation. Hepatomegaly, macroglossia, cardiomegaly and early respiratory muscle involvement are common in infantile and childhood varieties. Infantile form is fatal before 2 years of age. The childhood and adult forms of acid maltase disease are primarily the disorders of skeletal muscle. They present as slowly progressive muscle weakness associated with calf hypertrophy, which bears close resemblance with Duchenne's muscular dystrophy (9, 10). The frequency of GSD II is low occurring in less than 1 in 100,000 (11).

GSD II primarily involves skeletal and cardiac muscles. The deficient enzyme is "acid alpha-1, 4-glucosidase" (glucan 1, 4 - alpha-glucosidase or acid maltase). This enzyme is present in lysosomes and catalyzes the hydrolysis of the glycogen into glucose. Glycogen is a polymer of glucose (Figure -1) and glucose molecules are linked together by two types of bonds -

α' (1 $\rightarrow$ 4) glycosidic bonds (mainly), and

α' (1$\rightarrow$ 6) glycosidic bonds (at branch points)

Without the proper function of this enzyme, the glycogen fails to degrade to glucose and gets accumulated in the lysosomes. These inclusions disrupt the normal functions of the muscle cell and eventually the cell dies. When muscle cells are injured, their contents spill into the blood (10, 12).

Biochemical findings

Deficiency of alpha-glucosidase activity can be demonstrated in muscle fibers, fibroblasts, leukocytes, lymphocytes and urine. Severe deficiency of alpha-glucosidase activity occurs in infantile form but residual activity is observed in the less severe adult onset form. Serum creatine kinase (CK) levels are elevated in all forms of the disease but to variable degree. The infantile form is associated with the highest CK levels, but these levels are often less than 10 times the upper limit of normal. In adults, the CK level can be normal. Thus serum CK levels may be useful in differentiating this disorder from closely mimicking Duchenne muscular dystrophy (9, 11 and 12).

Histopathology

On light microscopy, vacuoles are seen within type -I and II fibers. The vacuoles are most prominent and more uniformly seen in nearly all the muscle fibers in the infantile form. In the childhood and adult forms, vacuoles are apparent in only 25% to 75 % of fibers in clinically affected muscles and may be absent in clinically unaffected muscle groups (13). These vacuoles react strongly with periodic acid-Schiff stain, which is sensitive to diastase. The vacuoles also stain intensely to acid phosphates, confirming that the vacuoles filled with glycogen are secondary lysosomes. Electron microscopic study shows abnormal accumulation of glycogen in lysosomes as well as cytoplasm. Glycogen accumulation can also occur in anterior horn cells, bulbar nuclei and Schwann cells, accounting for the superimposed findings in some patients (12, 14 and 15). Fiber size variation is common as is fiber splitting. In severe cases, muscle fiber atrophy and connective tissue proliferation in the endomysial regions may be present (16).

Gene Involved

Alpha-1, 4-glucosidase gene is coded by chromosome 17q25.2-25.3, (17) which is of 20 kb and consists of 20 exons. Forty-three different deleterious mutations have been identified in this gene ranging from missense, nonsense and splice site mutations to single base pair deletions, larger deletions and insertions. In addition, thirty-one apparently harmless, polymorphisms have been identified in this gene (13, 18 and 19).

Symptoms

Infantile-onset form

Patients with this form usually present during early infancy with weakness and floppiness. They are unable to hold their heads up and cannot do other motor tasks common for their age. The muscles do not appear wasted. Limb girdle muscles and the muscles involved in respiration are affected. The heart muscle thickens and progressively fails in its pumping function. The patients usually die before 12 months of age due to heart failure and respiratory weakness (12, 16 and 20).Childhood-onset form

Patients with this form of disease have a later onset, in infancy or early childhood, and progresses more slowly than the infantile form. Organ involvement varies among the individual patients but muscle weakness is generally seen. The life expectancy is better than for the infantile form (10, 16).

Adult-onset form

Patients with this form of Type II GSD do not usually show signs

of organ enlargement, but are marked by muscular weakness mimicking other chronic muscle diseases. Problems with walking are seen, due to weakness of the hip muscles. Some patients present with pulmonary insufficiency due to muscle weakness, and nighttime breathing must be evaluated. The involvement of the muscle weakness progresses slowly over the years. Heart involvement does not appear to be a significant feature (21).

GSD Type V

Type V GSD is an autosomal recessive disorder with variable ages of onset characterized primarily by exercise intolerance leading to Myoglobinuria in severe cases. According to clinical heterogeneity, different forms are reported such as severe infantile form, a late-onset form and a mild form with symptoms of excessive fatigue and poor stamina. Existence of manifesting carriers has also been reported. Primary organ involved in this disorder is skeletal muscle and the deficient enzyme is Muscle Glycogen Phosphorylase, also called Myophosphorylase (9, 10 and 12). This is responsible for the degradation of glycogen in the muscle (22):

Glycogen (n) + Phosphorus (inorganic) → Glycogen (n-1) + Glucose-1-Phosphate.

Biochemical finding

Phosphorylase activity is normal in erythrocytes, leucocytes, platelets, skin biopsy specimens and cultured skin fibroblasts from patient with Myophosphorylase deficiency. Thus, definitive diagnosis of the disease relies on the measurement of Phosphorylase activity in a muscle biopsy specimen. There are two major path physiological mechanisms involved with this enzymatic deficiency: (1) block of anaerobic glycolysis deprives muscles of the energy needed for the isometric exercise, and (2) block of aerobic cycle due to consequent shortage of pyruvate and acetyl-Co A, impairs dynamic exercise above a certain intensity (~50 % VO_2 max). In agreement with the concept that oxidative phosphorylation is curtailed by decreased substrate availability, it has been shown that oxygen extraction and maximal oxygen uptake are decreased in Myophosphorylase deficiency but can be at least partially restored by intravenous glucose infusion (12, 23). Haller et al. have shown that concentration of Na^+-K^+ ATPase pumps are lower in muscle biopsy specimens of patients with this disease. This finding is also supported by an increase in the concentration of potassium ions in the serum after exercise (24). The Phosphorylase enzyme plays a vital role in the breakdown of glycogen into glucose. In the absence of Phosphorylase in muscles, glucose cannot be released from the glycogen stored in skeletal muscles to create energy. The patients experience problems performing and completing most exercises, especially anaerobic exercises. Because they lack the enzyme to metabolize glycogen, which is the main source of energy for anaerobic activity, their body struggles to find other sources of energy to complete a given activity or exercise. Under these circumstances, the body breaks down muscle when trying to attain energy (20, 23and 24).

Histopathology

Light microscopic analysis of muscle biopsy specimen shows subsarcolemmal deposits of glycogen as bulges or blebs at the periphery of fibers. Accumulation of glycogen between myofibrils generally is less marked but may be sufficient to give the fibers a vacuolar appearance. In milder case, there may be no vacuole, and even the PAS stain may fail to show any focal accumulation of glycogen (25).The histochemical reaction for Phosphorylase is a valuable diagnostic tool because in most patients muscle fibers have no enzyme activity. In two conditions, however, the reaction can be positive in muscle biopsy specimens from patients with Myophosphorylase deficiency: (1) when there is some residual enzyme activity and (2) when there are regenerating fibers. The reaction, which provides false positive result due to expression of different isozymes in immature muscle cells and regenerating fibers may be misleading in biopsy specimens taken too soon after an episode of Myoglobinuria when regeneration is active (21, 23,25).

Gene Involved

The gene for Myophosphorylase (PGYM) is approximately 14 kb and has been cloned, sequenced and assigned to chromosome 11 q13. Two other tissue-specific iso-forms exist that are encoded by two different genes (Liver: 14q21-q22, Brain: 20q11). Autosomal dominant transmission has been documented in a few families (26). Two possible mechanisms may explain this pseudo-dominant inheritance: (1) heterozygote may be the manifesting carriers with 20% to 35% of normal enzyme activity, although the mechanism is unknown or (2) homozygous or compound heterozygous patients in two successive generations occurring by chance or because of frequent mutations. For the Myophosphorylase, at least 16 different mutations have been reported, but the most common mutation is R49X (27).

Symptoms

In this disease, the patient shows the symptoms such as muscle pain, cramps, fatigue, and muscle tenderness. With the breakdown of muscle (rhabdomyolysis) and the release of the red protein Myoglobin, Myoglobinuria may develop, as evidenced by dark-red or red-brown urine. Serum creatine kinase levels are greatly elevated. The physical exam of patients with Type V GSD is normal. They complain of painful muscle cramps after exercise. These persons are commonly muscular; they do not have large livers, and are normal in height. Their liver Phosphorylase activity is normal, and they do not have hypoglycemia (28, 29 and 30).

Type VII (Tarui Disease)

GSD VII in its typical form is similar to McArdle's disease. Clinical heterogeneity however has been observed in patients who have only hemolytic anemia or who have fixed weakness of early or late onset. In this case, serum CK (creatine kinase) is variably increased and Myoglobinuria occur infrequently. There is no increase in lactate. Primary organ involved in this disease is skeletal muscle and the deficient or defective enzyme is phosphofructokinase (PFK). This enzyme is needed to facilitate the breakdown of glycogen into energy in muscle. With this

deficiency, effective glycogen breakdown (glycolysis) during muscle stress cannot be accomplished, resulting in pain, weakness, and cramping in the exercising muscle (12, 31).

Biochemical findings:

PFK is the rate-limiting enzyme of glycolysis and its deficiency blocks glycolysis, thus explaining the flat venous lactate response to forearm ischemic exercise. The phosphomonoesters accumulate in muscle examined by 31P-NMR spectroscopy in the case of PFK-deficiency but not in Myophosphorylase deficiency, as there are different sites of two metabolic blocks. The negative effect of high carbohydrate meals on exercise tolerance has been attributed to the fact that glucose lowers the blood concentration of free fatty acids and ketones, which are alternative fuels used for the second wind in patients with PFK deficiency. The pathogenesis of contracture and Myoglobinuria remains unknown; abnormal accumulation of metabolites such as ADP may be an important trigger of premature fatigue (30, 31, 32).

Histopathology:

Due to PFK-deficiency, the glycogen accumulates in muscles and reaches a concentration upto two to three times greater than normal. Morphologically, the stored glycogen is seen mostly at the periphery of the fibers. PAS histochemical staining by preincubation with diastase can reveal this abnormality. A peculiarity of PFK deficiency is the finding in some muscle fibers, usually in older patients, of abnormal polysaccharides that stain with PAS but are not digested by diastase. Ultra-structurally, the abnormal glycogen is composed of finely granular and filamentous material, similar to the polysaccharide that accumulates in branching enzyme deficiency. The presence of this material has been attributed to the accumulation in muscle of G-6-phosphate, an activator of the enzyme glycogen synthase, which might alter the ratio of the synthase and branching enzyme (31, 33).

Genetics:

The phosphofructokinase, which is found in muscle, is coded by 12q13.3. The other two forms coded by 10p and 21q22.3 are found in platelets and liver, respectively. The enzyme present in muscle is a homotetramer of the "M" form. This is an autosomal recessive trait. In a family in which the disease was seen in two generations, compound heterozygosis in two successive generations explained the pseudo dominant inheritance. Two common mutations for this disease are IVS5-1G->A and 2079delC (34, 35, 36).

GSD IV (Andersen's disease/ Amylopectinosis)

Contrary to other forms of GSD, in type IV GSD the amount of glycogen is not increased in the tissues. Instead, the glycogen that does accumulate in small amounts has very long outer branches, as there is a genetic deficiency of the branching enzyme. The organs primarily involved include muscle, heart, liver and brain. A separate branching enzyme attaches the C1 point of a glucose residue of one glycogen chain to the C6 hydroxyl group of the glucose residue of another glycogen chain to yield a branch with an a 1-6 linkage. Three isoforms exist in the muscle but some more are expected to be discovered in future (37, 38).

Biochemical finding:

In this disease serum CK is inconsistently increased. There is an abnormal deposition of basophils in the muscles. Abnormal glycogen (i.e. without branching) accumulates in the muscle tissues as it fails to degrade when required.(12, 15,38).

Histopathology:

In muscle biopsy specimens there are deposits of a basophilic, intensely PAS-positive material, which is only partially digested by diastase. In the electeron microscopic examination, the storage material consists of filamentous and finely granular material often associated with normal-looking glycogen beta particles. This abnormal polysaccharide is found in skin, liver, muscle, heart and the central nervous system but the amount varies markedly in different tissues. This structural abnormality of the glycogen is thought to trigger the body's immune system, causing the body to actually attack the glycogen and the tissues in which it is stored. The result is remarkable scarring (cirrhosis) of the liver as well as other organs, such as muscle. The typical symptomatology of this disease is the result of this scarring process (39-40).

Symptoms:

A baby with the typical Type IV GSD appears to be normal at birth. The first indication of a problem is a failure to thrive. The rate of growth and mental progress of the baby stops at a certain point and does not continue normally. The liver and spleen enlarge. There is little weight gain and muscles develop with poor tone. The course of the disease is one of progressive cirrhosis and associated problems. Death typically occurs by five years of age. There have been a few older patients seen with severe muscle problems, who are found to have abnormal glycogen (40,41,42).

Genetics:

The gene for the branching enzyme is GBE1 and this is located on chromosome 3. The gene is mutated in this disease and there are several mutations reported with the classic phenotype. The base size is 2106-bp and respective protein contains 702 amino acids. A 210-bp deletion caused by a splicing junction mutation was associated with fatal neonatal neuromuscular variant. Another mutation, Y329S, initially associated with the non-progressive hepatic form was observed later in Ashkenazi Jewish patients with adult polyglucosan body disease. The same mutation in a ubiquitously expressed single gene can cause two completely different phenotypes, a hepatic disease in childhood and a neurological disease in adulthood (40, 42).

GSD III (Cori Disease/Forbes Disease/Limit Dextrinosis)

With this type of glycogen storage disease, the enzyme deficiency

causes the body to form glycogen molecules that have an abnormal structure. This abnormal structure also prevents the glycogen from being broken down into free glucose. A variety of subtypes have been observed of this disorder and there appears to be considerable variation in the tissues affected by the defect (white blood cells, muscle and liver). In GSD Type IIIa, the disease involves both liver and muscle tissues. The less commonly seen Type IIIb appears to involve only the liver. The deficient enzyme is Debrancher enzyme. Debrancher enzyme has two independent active sites, consisting of residues in different segments of a single polypeptide chain, that catalyzes a (1→6) glucosidase and transferase (transglycosylase) reactions. The transferase of the debranching enzyme transfers three glucose residues from a 4-residue limit branch to the end of another branch, reducing the limit branch to a single glucose residue. The a (1→6) glucosidase moiety of the debranching enzyme further catalyzes hydrolysis of the a (1→6) linkage, yielding free glucose (10, 12 and 43).

Biochemical finding:

The Debrancher enzyme is a monomeric 160-kDa polypeptide endowed with two distinct catalytic functions, oligo-1, 4-1, 4-glucantransferase and amylo-1, 6- glucosidase. After Phosphorylase has shortened the peripheral chains of glycogen to about four glucosyl units, the debranching enzyme in two steps removes these residual stubs. In patients with myopathy, muscle glycogen concentration is usually increased markedly, (ranging from 3.02 to 6.1 g/100 g in one study on eight patients). As expected, the stored polysaccharide has abnormally short peripheral chains characteristic of PLD (44)(Phosphorylase limit dextran). Studies on anaerobic glycolysis of muscle extracts in vitro show that lactate production is virtually absent with endogenous substrate (PLD) but is detectable with added glycogen and is normal with hexose-phosphate glycolytic intermediate. Chemical analysis of the blood usually shows low blood sugar, elevated glycogen content in red blood cells, and elevated levels of fat. Uric acid and lactic acid levels are usually normal (45, 46).

Histopathology:

Specimens of muscle biopsy show severe vacuolar myopathy. Numerous sub-sarcolemmal and inter-myofibrillar vacuoles contain PAS (periodic acid Schiff) positive material that is digested by diastase. By electron microscopy, the vacuoles correspond to large pools of free and apparently normal glycogen particles. Glycogen-laden lysosomes often are seen but are not as frequent as in acid maltase deficiency. Excessive glycogen accumulation has also been observed in biopsy specimen from skin, endomyocardium and cultured muscle. Glycogen accumulates in intramuscular nerves, Schwann cells and axons of nerve specimens (46, 47).

Genetics:

The Debrancher enzyme is a single protein encoded by a single gene (AGL gene) that expresses in all the tissues. The gene is located on chromosome 1p21. Several mutations have been identified in patients with Debrancher enzyme deficiency in muscle and liver (type IIIa). Two nonsense mutations, R864X and R1228X, are common in white people, whereas a one-base deletion (4455 delT) is common in North African Jewish patients. In type IIIb patients, two mutations in exon3 are observed and one of these, a 2bp deletion is at 17delAG(48, 49).

Symptoms:

Children with GSD III are often first diagnosed because of a swollen abdomen due to a very large liver. Some children have problems due to low blood sugars on fasting but this is not as common as in GSD I. Growth may be delayed during childhood but most reach a normal adult height. Muscle weakness is commonly present in childhood and can, at times, be severe. Often the liver returns to a normal size at puberty, although the enzyme defect persists (50, 51). Cardiomyopathy also can complicate debrancher enzyme deficiency, again, usually in adulthood.(45).

GSDXII

Deficiency of aldolase was first reported in 1996. Primary organ involved is Muscle and the deficient enzyme is aldolase. Aldolase carries out the breakdown of fructose-1, 6 bis-phosphates into glyceraldehyde-3-phosphate and dihydroxy acetone phosphate.(6,10)

Biochemical finding:

Aldolase is an enzyme, which belongs to the "Lyase" class. There are three isozymic forms of this enzyme - one of them predominates in muscle and erythrocytes, other in liver, kidney and small intestine and the third one in neural tissue. This is a rare disorder and in one case report serum CK levels were found to be very high (6480 U/L). (6, 10, 12).

Histopathology:

In one case report, the muscle biopsy specimen showed nonspecific myopathic changes and PAS staining was not abnormal (46).

Genetics

Aldolase enzyme, which is present in muscle, is encoded by gene located at 16q22-q24, whereas the enzyme present in liver, kidney and intestine is encoded by gene located at 9q21.3-q22.2. 17cen-q21 encodes the brain's enzyme. A missense mutation E206K, has been identified in a patient who had myopathy. One other missense mutation had been reported in a patient with hemolytic anemia, but the reason why mutations in the identical gene lead to different phenotypes is not known.(52, 53).

Symptoms:

The clinical picture in the 4.5-year-old boy with myopathy consisted of episodic exercise intolerance and weakness following febrile illness and no pigmentation was observed. Ischemic exercise test results were not described.(12).

GSD X

Hereditary deficiency of muscle Phosphoglycerate mutase is referred to as glycogenosis type X. GSD X was first described in 1981. The disease primarily involves muscle. This enzyme belong to the enzyme class "Isomerase" and it catalyze the interconversion of 2-Phosphoglycerate into 3-Phosphoglycerate (53)

Biochemical finding:

According to compositional point of view, the Phosphoglycerate mutase exist in three isozymic forms. There are two subunits of mammalian Phosphoglycerate mutase, a muscle specific subunit (PGAM-M) and a non-muscle specific subunit (PGAM-B). Thus, three types of PGAM dimers may exist in mammalian tissues. Mature muscle contains mainly the homodimers MM forms; liver, kidney and brains contain mainly the BB form, and the heart contains all the three dimer forms: MM, BB & MB. Erythrocytes contain a releated enzyme, diphosphoglycerate mutase, which can be considered also as one of the isozymic form. The serum creatine Kinase level is increased. Forearm ischemic exercise test increases venous lactate less than two-fold above the pre-exercise level. (12,14,54).

Histopathology:

Histologic examination of muscle biopsy specimens shows variable PAS staining. Tubular aggregates were present under the sarcolemma of type IIB fibers in one patient. Glycogen concentration in muscle is normal or only moderately increased. The residual PGAM activity (5 %) in muscle biopsy specimen corresponds to the BB isozyme expressed in normal muscle (55).

Genetics

The PGAM-M is coded by 759bp gene that contains two introns and is located at chromosome 7p12-7p13. Some three or four types of mutations are reported. The patients were homozygous for a nonsense mutation W78X and one was a compound heterozygote for W78X and a missense mutation, E89A.There was also a different homozygous missense mutation R90W. Recently, a new mutation G97D was identified in a Japanese patient with partial muscle PGAM deficiency, who presented with exercise intolerance and cramps.(56).

Symptoms:

Exercise intolerance, cramps and recurrent myoglobinuria characterize GSD X disease (57).

GSD XI

The condition occurs due to deficiency of enzyme Lactate dehydrogenase and the organ primarily affected is muscle. This enzyme carries out the reaction in both directions and converts lactate into pyruvate (58).

Biochemical finding:

LDH is a tetrameric enzyme composed of two subunits M and H, resulting in five isozymes, the two homotetramer M4 and H4, and three hybrid forms. M-subunit containing isozymes predominate in skeletal muscle whereas H-subunit containing isozymes predominate in heart and other tissues. During an attack of Myoglobinuria, serum LDH level does not increase as much as that of creatine kinase. Forearm ischemic exercise shows an abnormally low rise of venous lactate, contrasting with excessive increase of pyruvate (58, 59).

Histopathology:

Histologic finding in muscle biopsy specimens were not well documented in most cases, but in one patient, non-specific myopathic changes have been described. Glycogen concentration was normal in muscle fibers (55, 58).

Genetics:

The gene encoding the M-subunit of LDH consists of 7 exons and was assigned to chromosome 11. At least seven different mutations in the gene causing muscle LDH deficiency have been reported. No common mutation has been described except for a 20-bp deletion that seems to be common in Japanese patients (58).

Symptoms:

The patients showed exercise intolerance and recurrent Myoglobinuria. Some asymptomatic individuals can be identified through blood screening. All reported patients so far had been Japanese except the two unrelated white individuals. Eczematous rash was described in some patients (55, 58).

GSD VIII

Glycogen storage disease VIII is a group of hereditary disorders caused by the lack of one or more enzymes involved in glycogen synthesis or breakdown and are characterized by deposition of abnormal amounts or types of glycogen in tissues. Primary organs involved are liver, leucocytes and muscle. The deficient enzyme is Phosphorylase b kinase (60)

Biochemical findings:

Phosphorylase b kinase is a multimeric enzyme composed of four different subunits alpha, beta, gamma and delta. The molecular weight of the enzyme is 1.28 x 106 kDa. The gamma subunit is catalytic and its activity is regulated by the degree of phosphorylation of alpha and beta subunits. The delta subunit is a calmodulin protein and this is regulated by calcium sensitivity. There is episodic Myoglobinuria and variably increased serum CK (10, 12, 60).

Histopathology:

Analysis of muscle biopsy specimens shows sub-sarcolemmal accumulation of glycogen predominantly in type II B fibers. Ultra structurally there are pools of free normal looking glycogen particles (55,60).

Genetics:

Different genes code the different enzyme subunits in different tissues. The alpha subunit of the muscle is encoded by Xq12-q13 whereas the alpha subunit of the liver is encoded by Xp22.2-22.1. 16q12-q13 encodes the beta subunit of the muscle, liver and brain. The delta subunit is encoded by three different calmodulin genes i.e., 14q24-q31, 2p21 and 19q13. In patients with liver and muscle disease, mutations have been reported in the beta subunit. In X-linked liver disease, several mutations have been reported in the alpha subunit and in autosomal recessive disease of the liver, gamma subunit is mutated. In muscle specific disease, a missense mutation E1112X and a splice junction mutation have been reported in the alpha subunit of the muscle. (61, 62)

Symptoms:

GSD VIII with pure muscle involvement is characterized by exercise intolerance with myalgia, cramps, weakness of exercising muscles and myoglobinuria. Infants may have hypotonia and middle-aged patients may have progressive muscle weakness. GSD VIII with involvement of both liver and muscle (enzyme is defective in both liver and muscle) has an autosomal recessive trait and is characterized by hepatomegaly and non-progressive myopathy of childhood. Fatal infantile cardiomyopathy is apparently inherited as an autosomal recessive trait. The enzyme defect is only in the heart and not in muscle or liver. GSD VIII with pure liver involvement has both X-linked recessive and autosomal recessive inheritance and is characterized by hepatomegaly, growth retardation, delayed motor development, hyperlipidemia and fasting hypoglycemia. (60-62).

GSD IX

Phosphoglycerate kinase is a complex enzyme. Based on tissue involved and the type of inheritance, several subtypes of Phosphoglycerate kinase deficiency syndromes have been identified. The inheritance of GSD IX can be autosomal recessive or X-linked recessive, the latter accounting for nearly 75% of all cases. Muscle is the primarily involved organ and the deficient enzyme is Phosphoglycerate Kinase. This enzyme catalyzes the transfer of the acylphosphate group of 1, 3-bisphosphoglycerate to adenosine diphosphate and forms 3-phosphoglycerate and ATP in the terminal stage of the glycolytic pathway. The Phosphoglycerate kinase enzyme is a regulatory enzyme in the breakdown of glycogen, thus the deficiency of this enzyme results in glycogen accumulation (10, 12, 63).

Genetics:

Two distinct isoforms of the Phosphoglycerate kinase genes are located at Xq13.3 and 19p13.3 for muscle and testis, respectively. This is an X-linked trait. A single gene encodes the enzyme. PGK1 enzyme is found in muscle and PGK2 enzyme in the testis. The complete amino acid sequence and cDNA sequence have been determined and the coding region is 1254bp. The gene spans 23 kbp and contains 10 introns. The mutations found so far were all missense, except the two splice junction mutations and a single-codon deletion. The single gene for PGK expresses ubiquitously and therefore any mutation in the gene would affect all tissues except sperms (64, 65, 66).

Biochemical findings:

Residual PGK activity varies in different tissues and in different cases. The resting serum creatine kinase level is increased inconsistently in patients with myopathy. Blood tests show no signs of hemolysis. Forearm ischemic exercise causes no or only slight rise of venous lactate. A variable myoglobinurea occurs. (65).

Histopathology:

In muscle biopsy specimens, there is a mild increase in the glycogen level and this is seen with the help of histochemical staining (55, 65).

Symptoms:

PGK deficiency was reported in 1981 in 14 years old boy with exercise intolerance, cramps and recurrent Myoglobinuria.Untill recently, it was believed that hemolytic anemia and brain dysfunction were common, whereas myopathy was rare (10,12,65).

Myopathies due to disturbance in Lipid Metabolism

Lipid metabolism in muscle is a complex process that is closely associated with mitochondria. The fundamental steps in lipid metabolism are - (1) uptake and activation of fatty acids into skeletal muscle; (2) transport of fatty acids across the mitochondrial membrane by means of carnitine cycle and (3) beta oxidation. In the resting state, the prime source of energy that sub-serves 70% of the energy requirement is fatty acid.

Disorder of lipid metabolism in muscles is sometimes referred to as lipid storage myopathy, implying excessive lipid deposition in muscle. Excessive lipid storage dose occur in carnitine deficiency and in the acyl-CoA dehydrogenase deficiency; however, for other disorders of lipid metabolism, such as CPT (carnitine palmitoyltransferase), lipid storage myopathy is a misnomer because excessive lipid deposition dose not occur and this term should be avoided (67). So, the disorders of lipid metabolism in muscle are used interchangeably with the terms disorders of fatty acid oxidation and lipid myopathy.

L-carnitine deficiency syndromes

L-carnitine is chemically a beta-hydroxy-gamma-trimethylbutyric acid. This is a small, water-soluble compound that is essential for the normal oxidation of fatty acids by mitochondria in mammalian biological systems.

The cause of the carnitine deficiency was unknown but the following mechanisms were hypothesized: (1) a defects in carnitine biosynthesis, (2) excessive carnitine catabolism, (3) failure of

carnitine transport in muscle, or (4) failure of carnitine storage in muscle and other tissues (68).

On the basis of clinical features the carnitine deficiency syndrome are divided in following way:

Primary Myopathic Carnitine deficiency syndrome

In view of the possibility that many patients with presumed Myopathic carnitine deficiency may have other underlying disorders, there is uncertainty about the existence of this entity as a clinical disorder. Existence of purely myopathic form of carnitine deficiency may be resolved by the molecular genetic study. (67, 68).

Biochemical finding:

The serum creatine kinase level is often normal or elevated up to about 15 times normal. In plasma there is less than 10% of normal total carnitine.1-2% of the total carnitine is present in muscle, liver and other tissues and lipid storage occurs in these tissue (69).

Histopathology:

Light microscopic study showes vacuolar myopathy that affects primarily type I fibers; the vacuoles are filled with neutral lipids. Lipid vacuoles are not membrane bound and are generally located in rows between myofibrils, distorting but generally not disrupting, the cellular architecture. There are aggregates of mitochondria, which concentrate at the periphery of the cytoplasm. Lipids filled vacuoles are also seen in muscle, nerve and leucocytes (68,69).

Genetics :

Exact cause of myopathic carnitine deficiency remains unknown. However, the organic cation transporter (OCTN2) encoded by 5q31-q32 is supposed to be defective in this disease. In most of the cases, the inheritance of this disease is autosomal recessive type. (70).

Symptoms

Symptoms begin in middle adulthood. Proximal limb weakness is common to all patients and is often progressive; facial and respiratory muscle weakness may occur. Muscle cramps and exercise intolerance have been described but are not particularly major features of this disorder. Myoglobinuria has been reported rarely (67).

Primary Systemic Carnitine deficiency syndrome

Primary systemic carnitine deficiency is a widespread disorder of impaired fatty acid oxidation that is not caused by another disease that might deplete tissue carnitine stores. The criteria for the diagnosis include: (1) severe reduction of carnitine levels in plasma or tissues, (2) prejudice fatty acid oxidation due to low or reduced level of carnitine, (3) disorder correction by the replacement of normal carnitine and (4) absence of other defects of fatty acid oxidation.(67, 70).

Biochemical finding-

Serum CK levels are mildly or moderately elevated in approximately 50% patients. During attack of encephalopathy, liver transaminase and lactate dehydrogenase levels are usually elevated. Condition of hyper-ammonia also occurs. In contrast to myopathic carnitine deficiency, systemic carnitine deficiency is associated with severely reduced plasma and tissue carnitine levels, including skeletal muscle, cardiac muscle and liver. In addition, there is impaired renal absorption of carnitine; therefore, even when plasma carnitine levels are low, urine levels remains high. (53, 67)

Histopathology-

There is no specific Histopathological finding for this disease.

Genetics -

A gene responsible for primary systemic carnitine deficiency has been identified recently. This gene assigned to chromosome 5q31, encodes for a sodium ion-dependent carnitine transporter. The gene product, a novel transporter protein termed OCTN2 is expressed in kidney, skeletal and cardiac muscle and placenta. Mutations in the gene have been identified in affected individuals. Autosomal recessive inheritance is suspected in some cases (67).

Symptoms

The clinical features of patients with systemic carnitine deficiency are heterogeneous. In its full-blown state, systemic carnitine deficiency is characterized by proximal myopathy, hypoglycemic encephalopathy resembling Reye's syndrome, hepatomegaly and cardiomyopathy. This disorder can be fatal. Patients may present with one or any combinations of signs and symptoms. Disease onset is usually in infancy or early childhood, but generally before age 10 years (67, 70,71).

Secondary Carnitine deficiency

Carnitine deficiency should be considered as secondary until proved otherwise. This occurs in a wide variety of clinical situations. This is occasionally the result of severe dietary deprivation or impaired hepatic and renal function. However, in most cases, there are defects of beta-oxidation of fatty acids. Rarely do these defects affect muscle alone or predominantly. Practically always other features of disordered fatty acid metabolism- liver disease, hypoketotic hypoglycemia, Reye-like syndrome, SIDS and so on - are present in some combination. All of the beta oxidative defects are characterized by dicarboxylic aciduria. The abnormal organic acid in each case is determined by analysis of blood and urine; identification of the specific enzyme deficiency requires tissue analysis (71,72).

Carnitine Palmitoyltransferase (CPT) deficiency

The two CPT enzymes, CPT I and CPT II , and carnitine assist in the transfer of long-chain fatty acids across the mitochondrial membrane for their subsequent oxidation. CPT CPT II and I are distinct proteins encoded by different genes. CPT I exist in at least two isoforms, designated liver CPT I (L-CPT I) and muscle-CPT I (M-CPT I) for the tissues originally identified as expressing these isoforms; the two isoforms arise from separate genes. In contrast, only a single isoform of CPT II has been identified and it seems to be the product of a single genes. CPT II is distributed uniform ally in all tissues. CPT II and I are positioned in different places in the mitochondrial membrane. In 1987, it was commonly accepted that CPT II and I were located on the outer and inner faces, respectively (68, 71).

CPT I deficiency

CPT I deficiency is rare, and only deficiencies of L-CPT has been described. It is not known if M-CPT I deficiency exists because no case of isolated M-CPT I deficiency has been reported yet. Perhaps one reason for the lack of reports of M-CPT I deficiency is that this mutation may be incompatible with life because of the importance of the activity of this enzyme for heart function; more than 90% of cardiac CPT I is the M-isoform.

L-CPT I deficiency usually presents in infancy with hypoketotic hypoglycemia and is potentially lethal. Hepatomegaly and hepatic encephalopathy are common in this rare disorder. Muscle weakness generally is not a feature of this disorder, because L-CPT I is not found in the skeletal muscle. In cardiac muscle, L-CPT I contributes less than 10% of total CPT; cardiomyopathy would not be predicted in L-CPT I deficiency, but has been reported. The gene encoding for the L-CPT M-CPT and I I are localized on chromosomes 11q13 and 22q13.3, respectively (10, 12, 71).

CPT II deficiency

There are three forms of CPT II deficiency:

(1) Lethal Neonatal CPT II deficiency-In newborns, CPT II deficiency is a rare fatal disorder associated with its profound deficiency in multiple organs. Symptom onset occurs within the first few days of life, with encephalopathy, cardiomyopathy and hepatomegaly; deaths occur within the first week of life. Hypoglycemia metabolic acidosis, seizures, hypotonia and dysmorphism have been reported. Common finding include diffuse lipid accumulation in heart, liver, kidney and adrenal glands; cystic renal dysphasia and cerebral dysgenesis have also been reported. There is widespread profound reduction in CPT II activity in liver, heart, muscle, brain and skin fibroblasts.

(2) Severe infantile or childhood CPT II deficiency-Children with the severe infantile and childhood form of CPT II deficiency are normal at birth. Symptom onset occurs after the first month of life, and usually is precipitated by the fasting state or an infection. This is a systemic disorder. Hypoketotic hypoglycemia, hepatomegaly, cardiomegaly and hypotonia are characteristics clinical features. This form of CPT II deficiency is not necessarily fatal, but sudden death may occur with the first or any subsequent episode.

(3) Adult Myopathic CPT II deficiency-In 1973, the first case of CPT deficiency found in a 29 years old man with recurrent muscle cramps and myoglobinuria. Adult CPT II deficiency is now probably the most common inherited disorder of lipid metabolism in skeletal muscle and is the most common cause of recurrent myoglobinuria. The clinical hallmarks of myopathic CPT II deficiency are recurrent episodes of exercise induced myalgias or muscle stiffness, weakness, rhabdomyolysis and myoglobinuria. Although muscle fatigue or overt weakness occurs with attacks, between attacks, most patients are entirely normal (68,70,71).

Biochemical findings-

Between attacks often there is no apparent abnormality in urine and serum. Some patients may have elevated triglyceride and cholesterol levels. Serum CK elevation up to 10 to 30 times normal also occurs. Forearm exercise testing demonstrates a normal rise in serum lactate to vigorous or ischemic exercise (68).

Genetics-

All disorders of fatty acid oxidation are inherited in an autosomal recessive manner, CPT II being probably encoded by a single gene, which has been localized to chromosome 1p32. At least 15 mutations have been reported. (70, 71).

Carnitine -Acyl carnitine translocase deficiency

Carnitine -Acyl carnitine translocase assists in the transfer of acyl carnitine across the inner mitochondrial membrane in exchange for carnitine.There have been only rare reports of this carnitine-acyl carnitine translocase deficiency. This condition causes muscular weakness, cardiomyopathy, hypoketotic and hyper ammonia, which develop in early infancy with death in the first month of life (71,72).

Acyl-CoA dehydrogenase - The group of acyl-CoA dehydrogenase catalyze the first reaction in the mitochondrial ß-oxidation of straight chain fatty acids of various lengths. There are specific enzymes for short-(4 to 6 carbons), medium-(6 to 12 carbons) and long chain (12 to 18 carbons) fatty acids. Very long-chain acyl-CoA dehydrogenase (VLCAD) also is active on long chain fatty acids.(72).

Short-chain Acyl-CoA dehydrogenase deficiency

This deficiency is very rare. In this type of myopathy a limb-girdle distribution may appear initially in older children and adults, or it may follow episodic metabolic disorders in infancy. The neonates sometimes lethal form usually presents in the first few days of life with poor feeding, vomiting and metabolic acidosis.

Survival beyond 1 year of age has been reported, with progressive muscle weakness and hypotonia becoming outstanding within the first 6 months of life. In adult form, there are proximal weakness with predominate myalgias. In the neonatal and adult onset forms, the muscle biopsy specimens exhibit excess lipid deposition in type I fibers along with reduced carnitine content (67,70,71).

Medium-chain Acyl-CoA dehydrogenase deficiency (MCAD)

In early 1980s, MCAD was first described. This is now believed to be the most common form of metabolic defect of fatty acid oxidation. Symptoms occurs within first two years of life with Reye's-like episodes of lethargy, vomiting and coma precipitated by fasting state and associated with hypoketotic hypoglycemia. There is occurrence of low level of plasma and tissue carnitine levels. This may produce sudden death. (71, 72).

Long-chain Acyl-CoA dehydrogenase deficiency (LCAD)

The symptoms of LCAD are similar to the MCAD, but these occur at a very early age and the clinical phenotypes may be more severe. Infantile hypoketotic hypoglycemia is associated with cardiomegaly and hypotonia. Two of three patients have been reported by Hale et al (73).

Very Long-chain Acyl-CoA dehydrogenase deficiency (VLCAD)

Very long chain acyl-CoA dehydrogenase is a rate-limiting enzyme in the long chain fatty acid oxidation pathway. When we compare it with other ACD, it has unique size, structure and mitochondrial distribution. Most patients are under 2-years of age at the onset of symptoms and there are two main phenotypes: (1) without cardiomyopathy, a mild form consisting of hypoketotic hypoglycemia and (2) a severe early-onset form with hypertrophy cardiomyopathy and a high incidence of death. Sudden death of infants occurred in this disease. There is a very rare report of adult onset of this disease (69,71, 72).

Mitochondrial myopathies

Mitochondrial myopathies are a heterogonous group of disorders associated with metabolic abnormalities in various tissues particularly myopathies (74):

(1) **Total cytochrome-c-oxidase deficiency:** A distinctive clinical picture has been reported in three infants, who showed lack of cytochrome -c-oxidase deficiency in their muscles. The infants were noted to have a weak cry and to suck poorly in the neonatal period, but their condition deteriorated so much that they had to be admitted in hospital with muscle weakness and failure to thrive in between 4 and 8 weeks of age. At this time the infants were pale, lethargic, floppy and areflexic, with severe metabolic acidosis. Two infants died after 13 and 15 weeks of age (75)

(2) **Mitochondrial myopathy with lack of cytochrome b and decreased cytochrome a:** Spiro et al (1970) described an unusual family where a 46-year-old man and his 16 years old son had a disorder of muscle and nervous system, which bore some resemblances to the Kearns Sayre syndrome. Muscle biopsy showed group atrophy, central nuclei and increased numbers of mitochondria; in both patients there was reduction of cytochrome a and b, particularly of b (76).

(3) **Syndrome of growth retardation, lactic acidosis, cerebral disease and myopathy :** Shapira et al and Hart et al have each described pairs of sibs with a distinctive condition consisting of onset during the first decade of diminution of growth rate, episodes of vomiting, headache and acidosis, a myopathy and the later development of epilepsy, dementia and sensor neural deafness. This is a type of autosomal recessive inheritance (77).

Treatment

There is no permanent treatment of all these diseases. But, some possible treatment is available. Muscle glycogen storage diseases (GSDs) are disorders of inborn error of metabolism, in which gene therapy restoring the deficient enzymes may ultimately cure the diseases. However, considering the physiological basis of GSDs other treatments such as substrate supplementation, activation of the residual enzyme and enzyme replacement, are also important. Therapeutic trials in progress include the combined use of vitamin B6 and cornstarch for GSD type V, enzyme replacement therapy using rh-alpha-glucosidase for GSD type II, and ketogenic diet for GSD type IX. Recombinant human acid alpha glycosidase (rhGAA) enzyme replacement therapy (ERT) for the Pompe disease is available but to a little extent (78-80). In future, hopefully the gene therapy would be available for the cure of most of these diseases.

Conclusion

Metabolic myopathies are currently underestimated by the doctors in neonatal and intensive care units of national health clinics or in private clinics. Intensity in the rate of identification of these disorders is directly related to clinical decision and the custom of thinking of these diseases not as scarcity but as possibility, in the subset of cases that cannot be explained by more familiar physiopathology. From this stride forward, advances in information and biochemical techniques will in actual fact be able to increase the rate of diagnosis.

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