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A COMPREHENSIVE REVIEW ON A RARE GENETIC MITOCHONDRIALDISEASE: ALPER'S DISEASE

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ABSTRACT

Alper's disease known as Alper's Hutttenlocher syndrome is a rare genetic disorder results from mutation of POLG (Polymerase gamma) gene. This gene is required for Mitochondrial DNA replication and repair. This disease has mode of recessive inheritance. More than 145 mutations are identified which is responsible for pathogenicity of the disease. Mutation in POLG is not specific for Alper's disease as it can be responsible for other mitochondrial disease, juvenile onset and involvement of liver differentiate the disease from other disorder like Myoclonus epilepsy with ataxia and ataxia neuropathy syndrome. Alper's disease generallyaffects brain, liver and muscle as these organs requires large amount of energy. Onset of symptoms usually occurs at the age of 1-3 year or 17-21 year of life followed by rapid progression of disease. Main characteristic features of the disease are epilepsy, psychomotorretardation and liver failure. Symptoms may be varied from headache and visual loss, ataxia, hallucination, cognitive impairment to complete ophthalmoplegia and liver failure dep ending upon stage and severity of the disease. Electroencephalogram (EEG), magnetic resonance spectroscopy (MRI), Genetic testing, Mitochondrial DNA content can be used for diagnosis purpose. Currently complete cure for disease is not available however symptoms are managed by supportive treatment.

KEYWORDS: Alper's Hutttenlocher syndrome, Mitochondrial disease, Polymerase Gamma gene, Epilepsy, Hepatic failure, Neuro-degeneration.

INTRODUCTION

Alper's disease is a rare genetic disorder which falls in the category of disease having mode

of recessive inheritance. Alper's disease is also known as Alpers syndrome, Alpers-Huttenlocher syndrome, Alpers hepatopathy polio dystrophy, and hepeatocerebral degeneration of early childhood. Originally diagnosis of Alpers syndrome shown as a result of brain injury, post encephalitic syndrome, neurometabolic and epileptic encephalitis. Later on, after number of research it was noted that Alpers disease refer to the entity of infantile diffuse cerebral degeneration and hepatic cirrhosis.[1] Human mitochondrial DNA polymerase gamma (POLG) are nuclearly encoded and responsible for replication of mitochondrial genome. Mutation in this POLG is responsible cause for Alpers disease.[2] As mitochondrial DNA is largely needed for liver, brain and muscles, these organs are largely and primarily affected during the disease. Mutation in this gene leads to depletion of mitochondrial DNA, so symptoms can be presented in the form of intractable seizures, psychomotor retardation and liver failure. Rapidly progressive cerebral grey matter degeneration result in change in MRI and EEG findings which can be used as a diagnostic tool for the disease.[3] Usually, Alpers disease is fatal and no treatment can completely cure this disease, however symptoms can be managed by drugs, therapy and nutrition depending upon severity of disease.

History

The neuropathology and clinical features of Alpers' syndrome were described by Bernard Alpers in 1931 in which they give description of four-month girl with normal development whohave developed refractory seizures within the context of a one-month illness.[4] Although disease was described earlier, the information given by Alpers supported to outline the disease.[4] Huttenlocher et. al represented the association of hepatic features and cerebrospinal fluid findings in Alpers disease.[6] It also confirmed earlier suggestions of autosomal recessive inheritance associated with the disease.[7] Blackwood at al. supported the eponym of Alpers disease based on the review of five case reports in which two siblings also had livers cirrhosis with episodes of epilepsyia partialis continua (EPC).[8] Blackwood named the collection of findings as Alpers disease. After 25 years, Harding gave description of thirty-two patient with a definite liver and brain pathology that defines the typical clinical course of the disorder.[8] clinically the initial development was normal, though seizures often herald the disorder. In all, with the onset of seizure of the disease progress rapidly. The onset of liver involvement was varied, with some preceding seizure onset whereas in others, it had been only seen at the end of the clinical course and few had no significant liver finding. The biological research and characterization of the gene sequence of polymerase gamma, by Roop and Copeland ushered within the molecular era of polymerase gamma, induced disorders

specifically Alpers Huttenlocher syndrome.[10] Eventually polymerase gamma induced disorders facilate to define a class of mitochondria nuclear communication disorders. The enzymatic relevance of polymerase gamma, to Alpers disease was established by Navyaux et al. with their description of mitochondrial DNA depletion and decreased polymerase-g enzyme activity.[11] In 2001 the first patient was reported with pathogenic mutation in polymerase gamma having progressive external ophthalmoplegia syndrome.[12] Link of genotype to phenotype for Alpers Huttenlocher syndrome was established in 2004 by Navyaux and Nguyen who described mutation in polymerase gamma is accountable for the clinical entity of Alpers disease.[13] Huttenlocher et al described case repots of eight patients having distinct kind of hepeatocerebral degeneration and neuropathological finding demonstrating the degeneration of cerebral grey matter of Alpers Huttenlocher syndrome. The liver findings were described as cirrhosis or subacute hepatitis with superimposed fatty infiltration of hepatocytes and also increased cerebral protein level. Huttenlocher et al. was the first to propose that the progressive degeneration of the cerebral cortex with distinct hepatic findings representing a specific hepeatocerebral degeneration syndrome.[6] Sandbags and Lerman provided the first information of a possible mitochondrial abnormality.[7] They were the first who described that Alpers disease is inherited mitochondrial disorder. The above-mentioned data gives us information about the pathophysiology of the clinical phenotype, how to identify the genetic etiology, and the physiologic changes of decreased mitochondrial DNA content which leads to the mitochondrial DNA depletion syndrome known as Alpers Huttenlocher syndrome. Within the succeeding 3 years, a summaryof the full spectrum and clinical descriptions of polymerase-g disorders was nearly completed, with both dominant and recessive mutations known to cause a wide spectrum of clinical disease.[14]

Structure of POLG (Polymerase gamma)

Mitochondria have their own small 16.5 kb circular double stranded DNA which are liable for coding 22 tRNA, 2 rRNA and 13 polypeptides needed for process of electron transport and oxidative phosphorylation. Remaining 1000-1500 proteins needed for mitochondrial biogenesis are encoded by nuclear genome and incorporated to mitochondria.[15] These proteins are concerned for mitochondrial DNA (Mt-DNA) replication. Therefore, defects in these proteins turn out depletion of mitochondrial DNA, which ultimately result in mitochondrial dysfunction and cellular failure.[16] Among the different 16 DNA polymerases present within the cell, alone DNA polymerase gamma

(POLG) is believed to be responsible for DNA replication and repair.[10,17,18] POLG is the sole DNA polymerase gamma gene functional to mitochondrial DNA replication and repair.[19,20] Protein is a part of mitochondrial trimer replicase consist of catalytic(α) subunit of the Mt-DNA POLG accessory (β) subunits (POLG2). It is synthesized in cell nucleus and transported across mitochondrial inner and outer wall and reach inside the mitochondria where it associates with other protein for process of DNA replication.[21] This POLG is more prone to oxidative damage by presence of free oxygen species. The human enzyme, heterotrimer containing catalytic subunit, POLG A, and dimer of an accessory subunit,POLG B.[22] POLG A belongs to the family of polymerase enzyme A. This DNA POLG created of three domains: an N-terminal domain containing 3'-5' exonuclease (exo) activity, a spacer domain and a C-terminal domain, containing 5'-3' DNA polymerase (POL) activity. C terminal end activity which is known as pol activity distributed in three pars known as palm, finger and thumb. Additionally, POLG consist of a 50-deoxyribose phosphate lyase activity however the location of its site which is responsible for this activity is not known yet.[22] The accessory subunit, POLG B serves as a processivity factor, as it is liable for enhancing the DNA -bindingaffinity and catalytic activities of POLG A.[24,25]

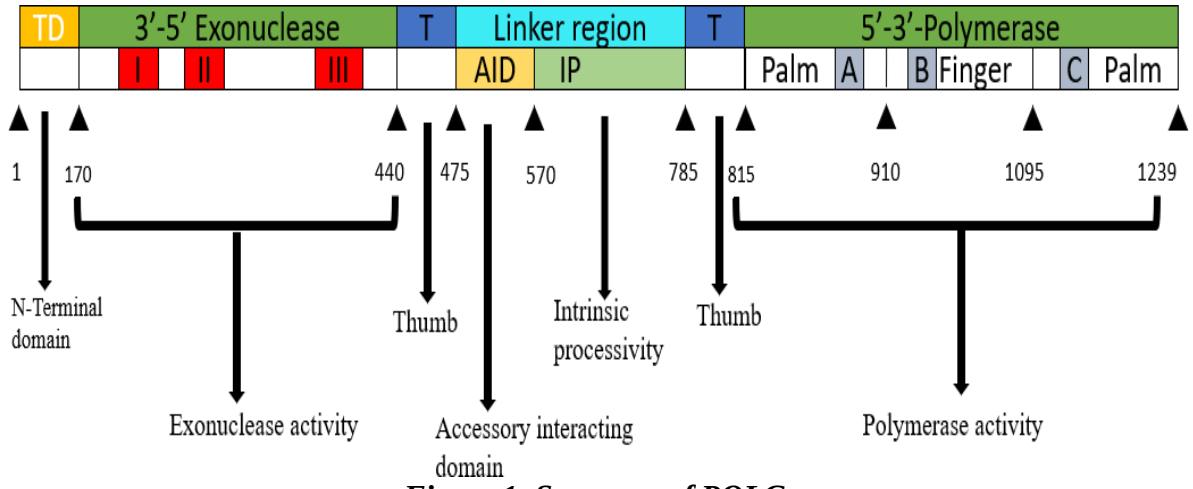

Figure 1: Structure of POLG.

Structure of Polymerase gamma is described as below.

POLG is a protein containing a 3-domain system.

1. **N-terminal (Amino terminal) domain:** This part contains 3'-5' proofreading exonuclease. It also functions to provide targeting sequence for amino acids. This p art contains highly conserved sequence motifs known as subpart 1, 2 and 3.
2. **Linker region domain:** contains 2 thumb part (T) which separates exonuclease part and

polymerase domain. Linker domain also provide binding site for POLG 2 protein. It contains 2 motifs namely as Acessory interacting determinant (AID) which is required for binding with POLG2 and Intrinsic processivity (IP). Both are required for full processivity. The average number of nucleotides added by the enzyme per association/disassociation with the template DNA is termed as processivity.

3. **Carboxy terminal domain:** This part contains 5'-3' polymerase activity and 5' deoxyribose phosphatelyase activity. The polymerase region contains one thumb region (T) and the palm (two motifs) and finger which are represented as region A, B and C domain.

There are three highly conserved sequence motifs within the exonuclease region (1,2&3) and three within the polymerase region (A, B, & C), which are essential for full enzyme activity.

Pathophysiology of alpers disease

Mitochondria are main source for formation of ATP (Adenosine tri phosphate). ATP synthesized by oxidative phosphorylation which is carried out by mitochondrial respiratory chain (MRC) components. MRC consist of 90 protein subunit which are distributed as five complexes (complex1-5). From this 13 are encoded by mitochondrial DNA (Mt-DNA) while other are required for mitochondrial structure and function and are encoded by nuclear DNA (n - DNA).[26] The DNA polymerase responsible for Mt-DNA replication and rep air is polymerase gamma.[27] POLG is a DNA polymerase which is responsible for Mt-DNA replication and repair. Mutation in gene encoding POLG will cause damage to Mt-DNA in form of depletion, and multiple deletions. Increase in load of mutation is a generally results in inherited mitochondrial disease.[28,30]

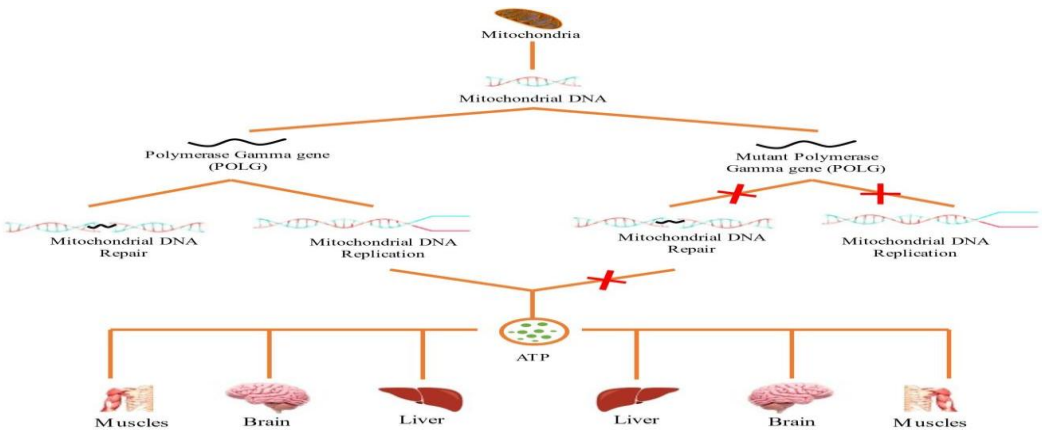

Figure 2: Pathophysiology of alper's disease.

Mutation of POLG is a major cause for mitochondrial disease specially those affecting children and adult. More than 200 mutations found which are responsible for mitochondrial disease that vary in age of onset, inheritance pattern, and range of organ involvement.[31,32] Mutation responsible for this type of disease have mode of either dominant mutation or recessive mutation. In case of dominant mutation, it does not require presence of similar gene for expression of disease, therefore this mutation is more likely to be inherited and more prone to produce disease. While for recessive mutation, expression of disease requires similar type of gene which decrease chance of inheritance and less prone to produce disease. Dominant mutation of POLG results adult-onset myopathies and encephalopathies.[33,34] and recessive mutation of POLG can cause adult/juvenile onset ataxia epilepsy syndrome or Alpers syndrome. Autosomal dominant mutation always seen in adult while autosomal recessive mutation present throughout the lifespan.[35] The phenotypic expression and timing of this autosomal disorders are quite variable at onset and progression of disease is unpredictable. Thus, depending upon these criteria recessive mutation of POLG falls in different syndrome as described in Table 1.

Table 1: Disorders caused by mutation in POLG.

Age of onset	Syndrome	Reference
Neonatal/infancy	Myocerebrohepatopathy spectrum (MCHS)	[36,37]
Infancy/childhood/juvenile	Alpers-Huttenlocher syndrome (AHS)	[38,39]
Adolescent	Myoclonus epilepsy with ataxia	[39]
Adolescent/young adult/elderly	Ataxia neuropathy spectrum (ANS)	[38]
Adult	Autosomal recessive progressive external opthalmoparesis	[35]
Adult	Autosomal dominant progressive external opthalmoparesis	[35]

Early onset POLG syndrome MCHS and AHs have overlapping symptoms but with distinct difference. Features that distinguish MCHS from AHS are earlier onset of symptoms, liver findings, severe myopathy and having mild seizure. Progression of AHS is more prolonged and in stepwise manner.[31,36,40] Alper's syndrome is accompanied by either mitochondrial DNA depletion, deletion or combination of both.[27,41] Till date more than 145 mutation are identified as responsible cause for Alpers syndrome, in which more than half belongs to recessive mode of inheritance. Dominant mutation primarily affects catalytic residues in POL domain which can be identified by effective competition for the DNA

subtract by mutant with enzyme.[42,44] In most cases recessive mutation linked to most severe form of POLG syndrome known as Alper's syndrome. Manifestation of syndrome requires presence of minimum two recessive compound having heterozygous mutation.[41,45] Consideration of newly identified amino acid substitution as pathogenic mutation are generally based upon absence of variant in normal gene sequence. However, this task is challenging due to presence of large variation in normal gene sequence of POLG. In Alpers syndrome these mutations are uniformly distributed among three structures of catalytic domain. It is not known yet which property of enzyme is affected and how it leads to mitochondrial depletion and deletion. One study was performed to assess the structure–functionrelationships for recessive disease mutations, by reviewing existing biochemical data on site - directed mutagenesis of the human, Drosophila and yeast POLG, and their homologs from the family A DNA polymerase group. In this study they have modelled primer-template DNA on the structure of POLG and given evidence of recessive mutations cluster within five distinct functional modules in the catalytic core of POLG that designate as 'Alpers Clusters 1–5'. From study it was concluded that cluster prediction can be used as a diagnosis-supporting tool to evaluate the pathogenic role of new POLG variant.[46] AHS is autosomal recessive disorder caused by mutation in Mt-DNA POLG. No homozygous dominant mutation and simple heterozygous compound containing one dominant and one recessive mutation inheritance was reported for AHS. Mutations in AHS can be found in the form of homozygous recessive or as compound heterozygotes in trans. Homozygous recessive mutations ideally produce the milder and latter onset phenotype, while patients with compound heterozygote mutations present earlier and have severe and more progressive disease.[20,47] Although some exceptions are there for these mutations resulting in variation of onset and progression of disease. There are also some mutation combinations induce worse disease with earlier onset of symptoms.[46] Homozygous and compound heterozygous mutations have been reported, including E873X, A467Y, G848S, and W748S from which heterogenicity is due to A467Y and G848S mutations.[39,48] The most common finding of mutation is compound mutation of linker region p.A467T/pW748S and mutation in polymerase region. Mutation of latter region are associated with increased incidence of Liver failure compare to homozygous mutation.[45,49,50] Surprisingly, some compound mutation in linker region causes milder juvenile onset of AHS while homozygous mutations in the linker region can be found in the more severe childhood onset of AHS. Therefore, although the combinations of mutations within distinct regions of POLG likely responsible for the phenotypic expression but also other mechanism are involved for progression of

disease. In more than 70% patient of AHS, at the onset of disease copies of Mt-DNA in liver and muscle is in the normal range. Which decrease <35% of normal range upon disease progression due to Mt-DNA depletion in liver, brain eventually muscle.[51] Enzymatic activity of POLG is less than 10% of the control value throughout the lifespan of patient. During the course of disease respiratory chain involvement is not specific, even later on respiratory chain complex may having normal activity or variable deficiency ranging from single complex to multiple complexes.[37,47,48,52] Mutation in POLG results in Mt-DNA depletion and deletion in neurons which results in cellular dysfunction and disables energy metabolism. This delicately balanced neuronal energy metabolism has two consequences: 1. Chronic and continual neurodegeneration, and 2. The neuron's inability to deal with greater demand, which can result in acute localized/focal necrosis. (Figure 3) This damage may result in onset of epilepsy.

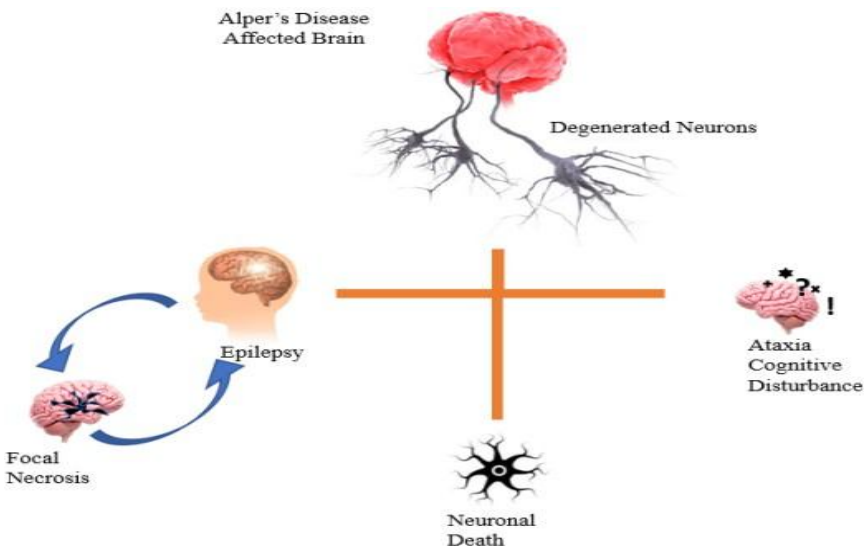

Figure 3: Neurodegeneration in Alper's Disease.

The neuronal depletion up to 40% resulted from recessive mutation is a threshold for neurons for maintaining survival, if it goes below that level, it impairs MRC component and affect complex1 which may lead to progressive respiratory chain activity. Gradual loss of this neurons leading neurodegeneration is associated with ataxia, encephalopathy, and cerebral atrophy. Upon progression of disease focal lesion may be develops which can be identified by MRI. This focal damage triggers for developing seizure.[30,53] Focal seizures are most commonly seen in patient of Alper's disease having epileptiform discharges occurring in occipital region of cortex.[54,55] In juvenile case seizures are accompanied by headache, vomiting which resembling presence of migraine with aura. Occipital lobe function alteration

may result in visual hallucination, hamianopia, nystagmus and ocular clonus.[49,54] Focal clonic, myoclonic seizure present in almost all patient and may result in generalized status epilepticus/Epilepsia partialis continua (EPC).[56,57] Some cases also reported having refractory status epilepticus which they might never recover,[31,54,55,57] EPC is closely related to stroke like episode which may be characterized by acute/subacute neurological dysfunction results in migraine like headache, visual dysfunction and mental changes. EPC associated with high morbidity and mortality.[58] Epilepsy is a prognostic factor for Alper's disease. It has been noted that patient not suffering from epilepsy having slightly long survival as compare to patient with epilepsy.[53] Prolonged seizure activity and status epilepticus can be linked with development of cortical focal lesion which can be seen in MRI as high T2 signal changes. Although any part of neocortex is affected, changes predominantly affecting occipital lobe are observed during seizure activity.[56,58] Magnetic resonance spectroscopy finding in acute cortical focal lesion shows lactate peak and reduced amount of N-acetyl aspartate, suggesting mitochondrial dysfunction and neuronal loss respectively.[53] Positron emission tomography shows high level of glucose uptake in cortical focal lesion.[59]

Organ involvement

Organs those require large amount of energy and are more prone to oxidative damage are majorly involved in AHS. This involves organ like brain, peripheral nervous system, liver and gastrointestinal system.

1. Central nervous system

Seizure is a most common feature of a disease as it is seen in about 50% of a people with Alper's disease. Once appearance of seizure in a patient, disease become rap idly progressive and usually results in death after 4 years of onset.[60,61] While in other patient progression of disease is slower and etiology for variation of progression of disease still remains unknown.[31,49] Valproic acid which is used for treatment of seizure also induce liver failure. The first case of valproic acid associated rapid onset of liver disease in patient having Alper's disease was reported in 1992.[62] So, for treatment of Alper's disease it is necessary to analyze seizure frequency, duration and medication without treatment with valproic acid to understand natural history for progression of disease. EEG finding and seizure semiology vary from patient to patient and changes upon progression of disease. Some patient of disease has Febrile convulsion while others have afebrile convulsion, but for most cases occipital lobe findings are common. Focal, asymmetric, occipital predominant

convulsion which p resent as visual and tactile hallucinations, nausea and vomiting, dysautonomia, headache and eye nystagmus auras, or present often with focal or generalized motor seizures.

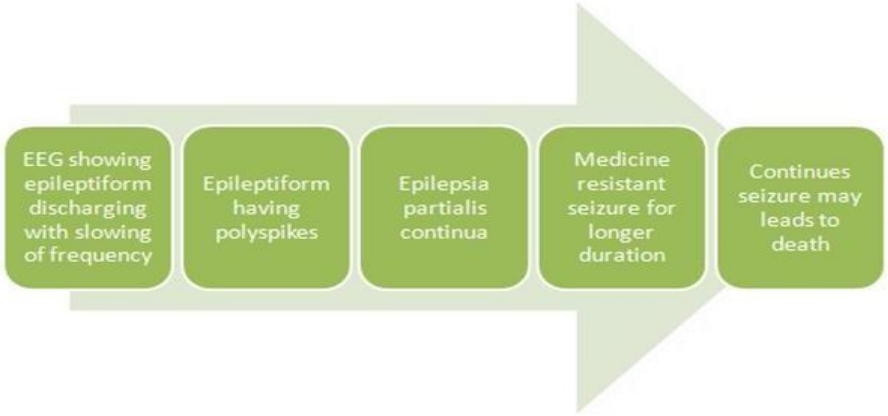

Figure 4: Progression of Alper's Disease in form of seizure.

The progression of disease in the form of seizure illustrated in above Figure 4. During initial period of progression of disease, EEG shows occipital predominant epileptiform discharges, with focal slowing of frequency. Morphologically epileptiform are having Poly spikes which may be unilateral or bilateral over posterior head region.[63,66] Later on, patient manifest repeated episodes of seizure which indicate status partials continua. As the disease progress, Seizure becomes resistant to medical treatment having longer duration of action. Almost continues myoclonus and myoclonic seizure become prominent and continues seizure may usually result in death,[39, 49, 63, 67-70] Seizures and lack of medical control over treatment of seizure are major attributable reason for comorbidities in patient having Alper's disease. Cortical blindness may be result from neuronal loss in the calcarine and striate cortices. Initially visual loss may be transistory which may become permanent upon progression of disease. Spongiosis, neuronal loss and astrocytosis are the changes observed during progression and it also extend into the depth of the cortex.[71] Part of cerebellum is involved variably in the disease progression which can be observed often as Purkinje cell dropout and Bergman gliosis with a preserved granular cell layer. This loss of purkinje cell is directly related to loss of ataxia.[67] Some patient having migraine complaint associated with visual hallucination auras which can be observed due to involvement of occipital lobe in disease progression.[49,69] Delayed maturation of cranial nerve and abnormal eye movement network within cortex and cerebellum leads to central nervous and peripheral nervous dysfunction. Sensory neuropathy evaluation is difficult in children which escapes identification of disease until reflex of muscle stretch is lost. Late finding may result in loss

of sensation of pain, temperature, vibration, light and touch. While for older patient is seen that sensory neuropathy attributable to loss of peripheral axonal branch of dorsal root ganglianeurons which is secondary to neuronal death.

Liver

Environmental factors may accelerate liver dysfunction and valproic acid induce liver damage is a characteristic feature of Alper's disease.[39, 62 – 64] According to the description given by Huttenlocher et al. and by Harding,[41, 42] liver involvement without exposure to valproic acid has been comprised as one of the most defining features of the disease. Pathophysiological changes for liver dysfunction in the Alper's disease are different from chemically induced toxic liver disorders. Liver dysfunction vary from patient to patient although valproic acid act as a catalyst for liver damage regardless patients' inheritance and early or late liver dysfunction. Almost all patient having Alper's disease who live long enough having history of liver involvement results in failure of liver during progression of disease. When patient is on valproic acid, liver dysfunction results within 2-3 month which may delayed up to 6 months depending upon progression of disease and condition. Hypoglycaemia, reduced synthesis of albumin and coagulation factors and mild elevation of transaminase level indicate liver dysfunction. Early finding of liver dysfunction may stop progression of disease to liver failure. In one study it was observed that use of IV levocarnitine may reverse course of valproic acid induced liver failure. Hypoglycaemia can represent as early sign of Alper's disease specially in 1 -2 years of age.[73] The precise etiology still remains unclear, but this may result in secondary impairment of mitochondrial fatty acid oxidation due to dysfunction of electron transport chain. Coagulopathies may be occurred with or without presence of cirrhosis in Alper's disease. Thesecoagulopathies result from the hepatic biosynthetic failure of vitamin K dependent clotting factors II, VII, IX, and X. Prothrombotic episodes of deep venous (e.g., subclavian or femoral) thrombosis at the site of venous line placement are commonly observed in patients with Alper's disease. This thrombosis can be result from a relative deficiency in the hepatic synthesis of anticoagulant proteins C and S or anti-thrombin III.

Gastrointestinal involvement

Difficulty in swallowing, delayed gastric emptying and intestinal immobility can be seen as a GIT dysfunction and these symptoms worsen as the disease progress. Some children having difficulty in swallowing may require placement of gastric tube for nutrition purpose.

The continuous gastric tube feeding is often required as normal bolus feeding is not tolerated because of gastric immobility. Disease progression results in complete dysmotility, as result ofthis patient may require jejunal tube feeding leads to total parenteral nutrition. A complete loss of the longitudinal muscularis propria, external muscle layer in the gastrointestinal tract was observed in two patients having Alpers-Hutttenlocher syndrome. Mt-DNA depletion within this layer is present in an infant who died at age 20 days with polymerase-g mutations.[74] Pancreatitismay also present as the disease progressed although mechanism of pancreatitis in Alper's diseaseremains unknown.

Cardiac involvement

Although involvement of cardiac in disease is not common, cardiomyopathy and congestive cardiac failure present in 10% children suffering with Alper's disease.

Signs and Symptoms

Alper's disease an autosomal recessive disorder which is characterised by refractory seizures, episodic psychomotor regression, cortical blindness and liver disease.[13] Symptoms usually appear between the ages of one and three years, with the development of intractable seizures (generalised, focal, and myoclonic), which can lead to death in a matter of month to year. Initially stroke like episode may be occur which may develop to a state of Epilepsia partialis continua. Hypotonia, hemiparesis, ataxia, cortical blindness, and indications of hepatic failure with ascites and jaundice like symptoms may be present depending upon the stage and severityof disease. In juvenile cases central peripheral axonal neuropathy can be occur.[72,75] The acute liver failure after exposure to valproic acid is a defining feature of Alper's disease. [1] Patient may suffer from symptoms like headache or migraine, vision loss, movement disorder like dystonia, anxiety, depression, gastrointestinal dysmotility, muscle weakness. Severe disease progression may result in ophthalmoplegia, to liver failure and nutritional failure.[76]

Valproic acid induce liver damage

Alper's disease includes liver degeneration of which mechanism is still unknown. Valproic acidis an antiepileptic drug having property to induce idiosyncratic liver injury. Valproic acid is used for treatment of epilepsy which is a characteristic feature of disease. In one study, experiment on model of Alper's disease induced pluripotent stem cell was performed to explorerelationship behind the high risk of Valproic acid induced liver damage in Alper's

disease. In this study it is observed that AHS induced pluripotent stem cells hepatocytes are more sensitive to Valproic acid than normal cells. Also, it is noted that carnitine and N-Acetylcysteine used fortreatment purpose can rescue Valproic acid induced sensitivity.[77] In study of two children having confirmed Alper's disease history, during diagnosis drug resistant seizure and liver damage induced by sodium valproate is reported. Other cases are reported where during treatment of disease, Valproic acid is used to control seizure will induce liver disease.[78,79]

Diagnosis of alper's disease

1. Genetic testing

As POLG gene mutation is responsible factor for pathophysiology of Alper's disease, sequencing of POLG should be done whenever disease is suspected. In most cases experts recommended full sequencing of POLG for diagnosis. Greater than 60 mutations in POLG are reported which are related to Alper's disease. As a result of this number and combination of mutation make method of full sequencing of the gene to be most sensitive and specific for confirmation of diagnosis. This method has more importance as there is no specific biomarker is available for POLG mutation related disorder.

2. Electron transport chain enzymology

Reduction of electron transport chain enzymatic activities in muscle and liver is not specific for Alper's disease, which shows inspecificity of method for diagnosis of Alper's disease. Reports of patients shows a variable pattern of electron transport chain defects.[81] This variability of electron transport chain complex activity findings is also dependent on the stage of disease and the tissue tested for diagnosis. As the disease become more progressive, catalytic subunits of the various complexes encoded by mitochondrial DNA limit enzyme activity, due to which abnormal electron transport chain activities become more pronounced. Based on this it is seen that electron transport chain enzymology should not be used to confirm the diagnosis of Alper's disease.

3. Mitochondrial DNA content

Generally, it is observed that number of Mt-DNA is 3-40% of normal Mt-DNA present in patient having Alper's disease.[13,36,81] Although this can helpful as a diagnostic tool, confirmation of diagnosis is not solely depended upon Mt-DNA number due to following two reasons.

a. Mt-DNA number can be normal at the initial stage of disease.

b. Lack of specificity and sensitivity due to depletion of Mt-DNA may be reason for other disease involving liver and muscle which makes diagnosis misleading.

In some cases, mutation may alter transcript without affecting Mt-DNA number, in such cases this diagnosis become useless.[82] Since POLG mutation is not always leads to DNA depletion in blood cell, for diagnosis by Mt-DNA tissue of muscle and liver should be considered for diagnosis of disease by Mt-DNA analysis.[83] Almost all patient shows DNA depletion upon progression of disease with some exceptions.[84] Therefore, it is concluded that Mt -DNA can be helpful as a diagnostic tool but absence of depletion cannot exclude possibility of disease.

4. Electroencephalogram (EEG) Findings and Seizures

Specific EEG finding and pattern of seizure during initial stage of disease may helpful as a diagnostic tool for Alper's disease. Explosive seizure with an asymmetric occipital lobe predominant epileptiform discharge that observed during stage of epilepsia partialis continua with presence of psychomotor delay is generally observed in Alper's disease.[54, 55, 63] Combination of epileptiform location and seizure semiology also signal the clinician to confirm that POLG gene is normal before initiating valproic acid. In one study EEG of nine patient having proven Alper's Huttenlocher syndrome and fifty-five patients with status epilepticus were examined by clinical expert and it is proven that RHADS (rhythmic high-amplitude delta with superimposed (Poly spikes) provide a specific tool for AHS diagnosis.[85]

5. Neuroimaging

MRI and CT can be used for diagnosis of disease, although it is also nonspecific for Alper's disease. Initially CT and MRI result may be normal but upon progression MRI shows pathologic changes which may be acute or chronic in nature. Normal MRI may not detect lesions produced during disease, for this reason diffusion-weighted imaging acquisition should be used for diagnosis purpose.[86] Following are the types of changes which are observed during disease progression.

1. Electroencephalogram anomalies and neuronal loss/gliosis in the occipital regions were seen as hyperintensities on T2/fluid attenuated inversion recovery sequences, imp lying mitochondrial malfunction.[87]

2. In the thalami and basal ganglia, T2/fluid attenuated inversion recovery hyperintensities are common when seizures are uncontrolled.[55]

3. With normalising of the signal alterations, a brief resolution of magnetic resonance variations may also occur.[88]

4. However, as the disease advances, MRI shows atrophy, which indicates Alper's disease-induced pathologic alterations in the basal ganglia and brainstem.[72,84]

5. Some patients have cerebral involvement, which is linked to the significant Purkinje cell loss seen during autopsy.[71]

Diagnostic criteria

Nguyen et al has described criteria for liver involvement in progression of disease that is independent to the exposure of valproic acid. These criteria and neuropathological investigation at autopsy should be to considered for the diagnosis when there is no suggestion of liver involvement.

Specific histological features of Alper's disease

Minimum 3 feature from the following criteria must present for confirmation of liver involvement during diagnosis.

1. Bile duct proliferation
2. Micro vesicular steatosis
3. Liver-cell plates collapse
4. Localized necrosis hepatocyte dropout with or without portal inflammation
5. Bridging fibrosis or cirrhosis
6. Typical lobular architecture parenchymal disorder or disorganization
7. Regenerative nodules
8. In dispersed hepatocytes not impacted by steatosis, oncocytic alteration (mitochondrial growth with strongly eosinophilic cytoplasm) was seen.

As signs and symptoms of disease varies in timing, intensity and severity, clinical suspicion is necessary during early stage of diagnosis. Generally clinical symptoms eventually expressed, POLG mutation will finally confirm the diagnosis.

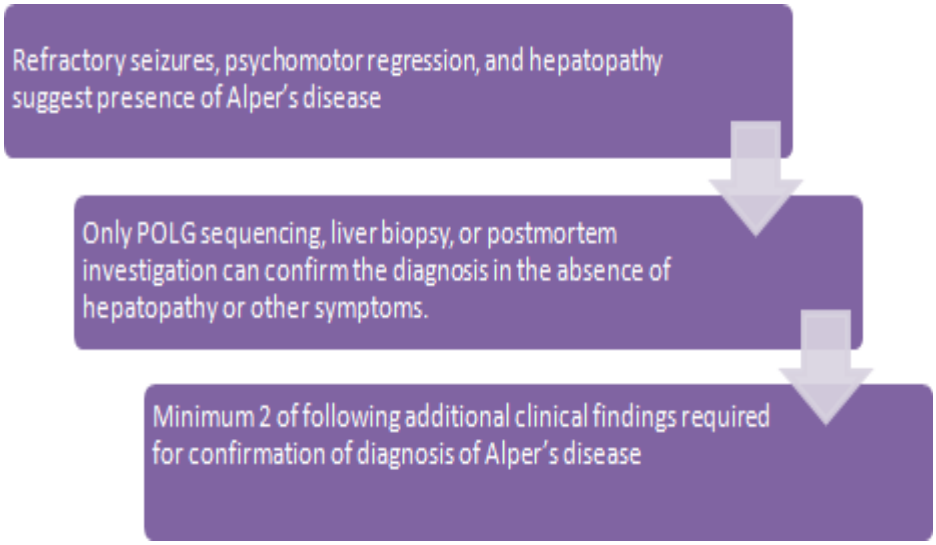

Figure 5: Diagnosis of Alper's Disease.

a. Reduced N-acetyl aspartate, normal creatine, and increased lactate as determined by cranial proton magnetic resonance spectroscopy.
b. A high level of protein in the cerebrospinal fluid (>100 mg/dL).
c. Repeated magnetic resonance imaging or computed tomography shows cerebral volume reduction (central more than cortical, with ventriculomegaly).
d. Multifocal paroxysmal activity with high-amplitude slowing (200-1000 V) and spikes/Poly spikes (10-100 V, 12-25 Hz) on at least one electroencephalogram.
e. Optic atrophy or cortical blindness.
f. Visual-evoked potentials that are abnormal yet electroretinogram findings are normal
g. Quantitative loss of mitochondrial DNA in skeletal muscle or liver (35 percent of the mean). A 10% reduction in polymerase-enzymatic activity in skeletal muscle or liver
h. In the absence of severe liver failure, elevated blood or cerebrospinal fluid lactate (3 mM) on at least one occasion
i. Electron transport abnormalities in the liver respiratory chain (complex IV or a mix of complex I, III, and IV) that are less than 20% of normal

Treatment of alper's disease

Although disease having clinical pathological genetic characteristic features there is no treatment available to correct disorder. Logically it is possible that correction of deficit created by mutation in POLG1 would help in increasing mitochondrial DNA content. At initial stage ofdisease level of POLG1 Is normal although symptoms are present and in other hand it is also noted that enzyme activity is significantly altered in all cells although the disease resides in thebrain liver and muscle. This both things make the mechanism of disease

more complex. Complete enzyme activity of POLG is required for Mt-DNA replication and repair. Mutation inthis POLG1 alter all 3 processes. For expression of POLG1, both copies are needed for full enzymatic activity due to its billing nature. Both p. Ala467Thr and p. Trp748Ser contribute to 5% residual activity of POLG 1 gene.[89,90] The heterozygous mutation in one of these sites in normal individuals leads to deterioration of disease owing to 50% residual activity exerted bythem.

Mutation in p. Glu848Ser significantly lowering processing. This may not induce disease ifmined with wild type allele but in combination with other variant may result in death.91 This study suggests that depending on the Location of mutation and nature of variant on either allele Single nucleotide editing may not abrogate disease. Multiple copies of mitochondrial DNA in each mitochondrion are there and multiple mitochondria are present in each cell, so incompletegene transfer in only subset of mitochondria may not fully correct the needed 50% activity. If genetic alteration is performed multiple gene transcripts to majority of mitochondria would be needed.

1. Exosomes

Exosomes are membrane bound vesicles released by a variety of cell type and involved in intercellular communication. Engineered extra cellular exosomes may allow a delivery of drugs and genetic materials as they have ability to cross biological barrier such as blood brain barrier. Use of exosomes derived from wild type POLG progenitor Sales may help in correction of abnormal POLG function.[92]

2. Exercise

Studies performed on mouse model shown that endurance exercise induced systematic mitochondrial biogenesis and prevent mitochondria DNA depletion and thereby results in complete phenotype protection.[93] Exercise may help it early stage of disease because as disease progressed, ability of exercise for protection is severely compromised.

3. Dietary supplements

Most mitochondrial experts use variety of vitamins and supplements for increasing ATP production.[94] High oxidative stress can be responsible for respiratory chain disease.[95] Combination of various antioxidants, N-acetylcysteine, vitamin E and vitamin C, coenzyme Q10 can be used in combination. Use of N-acetylcysteine and Vitamin E together help in reduction of oxidative stress an increasing life span of zebra fish having complex 1

disease.[96,97] Treatment of the disease have limitation to provide only symptom management and supportive care. Disease having range from minor illness to fatal encephalopathy/liver failure with varying levels of treatment are available for symptomatic relief. A gastronomy feeding tube will be placed for medicine, hydration and nutrition as a part of supportive care and treatment. Surgical ventilation can range from less invasive treatment like continues positive airway pressure to more invasive procedure like tracheostomy installation and mechanical ventilation. When neurologic functions began to deteriorate occupational, physical, search therapy is used to maintain function as long as possible while also ensuring comfort. In early stage of disease consultation with gastrointestinal expert is necessary to manage liver function and pathology. Ventilation problems are common in Alper's disease and can exist long also. Assessment of ventilation function Can be performed using polysomnography by measurement of carbon dioxide partial pressure and by measuring pulse oximetry. Tracheostomy and artificial ventilation may be performed for treatment purposes. Seizure should be controlled as soon as possible by therapy. The stage of epilepsia partialis continua (EPC) may not be possible to control with any treatment. There is no evidence is there that's suggesting better therapeutic advantage of lamotrigine, topiramate, oxcarbamazepine and levetiracetam over older medicine (phenytoin, phenobarbital, carbamazepine. New medicines required less processing by liver having less drug interaction any less sedative in nature. Other anticonvulsants may deteriorate liver function so it is required to monitor liver function continuously during treatment.

I. Treatment of liver failure includes small meals taken frequently to encounter impaired gluconeogenesis. Low dietary protein and non-absorbable sugar may also help as supportive treatment.
II. Levocarnitine benefits for liver failure and due to its low toxicity, it may use for treatmentpurpose.
III. In Alper's disease liver transplantation is not recommended for treatment purpose.
IV. Recent study shows that CSF folate deficiency can occur in Alper's disease.[98] Lumber puncture is required for testing of CSF folate deficiency to detect 5 Methyl tetrahydrofolate concentration. This can be treated by calcium leucovorin which have ability to cross blood brain barrier and increase level of cerebral folate.
V. There are no any guidelines at present to suggest frequency at which test are performed tomonitor stage of disease. It is required to monitor blood counts, electrolytes and liver enzyme level and liver function reasonably at every few months. For treatment of live r during course of therapy it is essential to monitor level of levocarnitine in blood.

Other primary test is performed to monitor disease state in conjunction with liver ultrasound, EEG.[99]

CONCLUSION

Alper's disease is a mitochondrial disorder results from autosomal recessive inheritance of Polymerase gamma (POLG) gene solely responsible for Mitochondrial DNA (Mt-DNA) replication and repair. Mutation in POLG ultimately results in Mt-DNA depletion and deletion leading Mitochondria of cell work insufficiently to produce required energy. As liver, brain andmuscle are the organ which require more energy disease usually affect these organs. As a result of this neurodegeneration occurs which ultimately leads to phenotype expression of the disease. Alper's disease can occur due to from homozygous or compound heterozygous mutation of POLG. Location of mutation have significant role in phenotype expression of disease. Usually, death results within 4 years of onset of symptoms. Generally patient with Alper's disease havenormal development initially, although onset and type of symptoms or organ involvement depends upon severity and progression of disease. Clinical feature involving brain, liver and muscle are a defining feature of a disease. Rapid progression of disease after onset of symptoms and non-uniform mutation makes disease more complex and challenging to treat. Seizure is most common and first symptom observed in patient. Progression of disease at the end may result in liver cirrhosis or liver failure. Valproic acid which is used for treatment of epilepsy may induce liver damage in patient having Alper's disease although mechanism behind this stillnot clear. If disease diagnosed at an early stage of disease, exercise may help to significantly delay the manifestation of disease. Currently complete cure for disease is not available howeversymptoms are managed by supportive treatment.

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