Original Article

Canavan Disease: Clinical and Laboratory Profile from Southern Part of India

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Abstract

Background: Canavan disease (CD) is an autosomal recessively inherited leukodystrophy. It affects one in 6,400 to 13,500 people in the Jewish population. However, prevalence and presentation of the disease in India is largely unknown; hence, we are reporting this series. **Methods:** This is a retrospective chart review in a tertiary care hospital from January 2015 to March 2020. CD was confirmed by elevated N- acetyl aspartate (NAA) levels in urinary gas chromatography and mass spectrometry (GCMS)/increased NAA peak in magnetic resonance spectroscopy (MRS) and/or detection of mutations. The data was extracted in a predesigned proforma and analyzed. **Results:** We had 12 children with mean age at presentation being 6.8 months (range 3 months to 10 months.). Males were more commonly affected (83.3%, $n = 10$). Ten children (83.3%) were born out of consanguineous parentage. All of them had visual impairment and pyramidal signs. Seizures were noted in five (42%) children. Normal head size in three (25%) and microcephaly in two (16.66%) cases were noted. Magnetic resonance imaging (MRI) revealed signal changes with bilateral symmetric T2W white matter (WM) hyperintensities in subcortical U fibers in all cases. MRS was done in ten children, all of which showed increased NAA peak. Increased level of NAA in urinary GCMS was noted in six out of eight children. Six cases had homozygous pathogenic variants in *ASPA* gene. Antenatal diagnosis helped in prevention of recurrence in three families. **Conclusion:** Urinary NAA and MRS showing NAA peak are useful in diagnosis of CD. Macrocephaly is not a necessary finding to diagnose CD. Early diagnosis helps in genetic counseling and prevention of subsequent conceptions.

Keywords: Aspartoacylase deficiency, Canavan disease, NAA, spongiform leukodystrophy

Introduction

Canavan disease (CD) is an autosomal recessively inherited spongiform leukodystrophy.[1,2] It is caused due to mutation in *ASPA* gene that encodes the enzyme Aspartoacylase that hydrolyzes N-acetyl-L-aspartic acid (NAA).[2,3] CD usually presents in early infancy with developmental delay or regression of milestones, hypotonia that later progresses on to spasticity, visual impairment, increased head circumference, seizures, and WM dysmyelination.[1,4,5] This disease is more prevalent among the Ashkenazi Jewish population, although non-Jewish cases have also been increasingly reported.[6-8] However, prevalence and presentation of the disease in India is largely unknown; hence, we are reporting this series.

Methods

This is a retrospective chart review of CD from a pediatric tertiary care referral center in southern part of India. The medical records of children attending the pediatric neurology clinic and those who were admitted in wards from January 2016 to March 2020 were analyzed. The disorder was confirmed by elevated N-acetyl aspartate levels in urinary gas chromatography and mass spectrometry (GCMS)/detection of NAA peak in MR spectroscopy and/or detection of mutations in the *ASPA* gene. The data were extracted in a predesigned proforma. Details of history including family history, examination including ocular fundus, investigations like complete hemogram, liver function, renal function, blood glucose, serum electrolytes, serum ammonia, blood lactate, arterial blood gas, urine ketones, neuroimaging, such as computed tomography/magnetic resonance imaging (MRI) including MRS of brain, urine GCMS, and genetic analysis were taken. The voxel placement in MRS was done in the region of abnormal white matter (WM) and compared with an adjacent normal area.

Shimadzu GC-MS2010 machine was used for estimation of urinary NAA level by GCMS. A 20 µl urease was added to urine and incubated at 37°C for 30 min. Three internal standards were added to this mixture at a concentration of

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40 μg per each reaction. The 2-ketoacids were oximized by adding 0.5 ml of 5% hydroxyl ammonium chloride and 0.4 ml of 2.5N sodium hydroxide followed by acidification by adding 0.35 ml of 6N HCl. Liquid phase extraction of organic acids was carried out by using 6 ml ethyl acetate. The organic layer obtained through centrifugation at 3000 RPM for 5 min at 4°C was treated with 5 g of anhydrous sodium sulphate. The supernatant was evaporated in liquid nitrogen at 60°C. The peak identification was based on mass spectral identity using NIST library and quantification was performed in relation to signal intensities with reference to margaric acid. In order to maintain consistency of analysis, the urine volume was adjusted based on the creatinine content (0.2 mg per 2 ml). The reference range was 0–3.7%.

Targeted next generation sequencing for leukodystrophy panel was done in five children and specific ASPA gene analysis was done in one child. Statistical analysis was performed with SPSS version 21. The results were analyzed. Ethical clearance was obtained from institutional ethical committee.

Results

During the study period, twelve (3.1%) children were diagnosed with CD out of 383 leukodystrophies. Clinical findings and laboratory parameters are mentioned in Table 1. The mean age at presentation was 6.8 months (range: 3 months to 10 months). Males were more commonly affected 10/12. Ten patients were born out of consanguineous parentage. Clinical features consisted of developmental delay and visual impairment in all children, seizures in 42%, nystagmus in 50%, optic atrophy in 33.3% and hearing impairment in one child. The mean age of onset of seizures was 9 months. Seizures were generalized tonic–clonic in all cases and one child also had epileptic spasms. All cases had pyramidal signs. Head size was large in seven (58.33%), normal in three (25%), and microcephaly was noted in two children (16.66%). Electroencephalography (EEG) was done in five children with seizures. In one child with epileptic spasms, there was modified hypsarrhythmia, two children had multifocal epileptiform discharges and two children had diffuse slowing and sharp waves. MRI revealed signal changes with bilateral symmetric T2/FLAIR WM hyperintensities in subcortical U fibers in all patients, with hyperintensity in globus pallidi and thalamus structures noted in eight (66.7%) [Figure 1]. MRS was done in ten cases and all of them showed elevated NAA peak. Figure 2 showing MRI of the brain of a child with Canavan disease, diffuse hyperintensities on T2WI in subcortical and periventricular white matter, globus pallidus, midbrain, and anterior limb of the internal capsule and NAA peak on Magnetic Resonance Spectroscopy (MRS). Urine analysis revealed increased level of NAA in urinary GCMS in six out of eight children tested. Six of them showed pathogenic variants in *ASPA* gene. Out of 12 children four children expired, mean age of death was

Table 1: Clinical and laboratory parameters of Canavan disease cases

Case No	1	2	3	4	5	6	7	8	9	10	11	12
Age in Mo	3	10	5	4	8	9	7	7	8	7	7	7
Gender	M	F	F	M	M	M	M	M	M	M	M	M
GDD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dystonia	No	✓	No	✓	No	✓	No	✓	No	✓	✓	✓
Seizures	✓	✓	No	✓	No	No	No	✓	No	No	No	✓
Head size	S	L	L	N	N	L	L	L	S	L	L	N
Vision	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
MRI-WM Signal changes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
MRI-GP/T Changes	✓	✓	No	✓	No	✓	No	✓	No	✓	✓	✓
NAA Peak on MRS	✓	✓	✓	✓	ND	✓	✓	✓	ND	✓	✓	✓
GCMS Increased NAA	ND	✓	ND	✓	Normal	✓	ND	✓	Normal	✓	ND	✓
ASPA Gene HMZ Stat	c. 859G>A/ p.Ala 287Thr	ND	c. 162 C>A/ p.Asn 54Lys	ND	c. 859 G>A/ p.Ala 287Thr	ND	c. 728 T>G/ p.Ile 243Ser	ND	c. 902 T>C/ p.Leu 301Pro	ND	c. 162 C>A/ p.Asn 54Lys	ND

GDD: Global developmental delay, Head size- N-Normal, S-Small, L-Large, PS-Pyramidal signs, HMZ-Homozygous, GP-Globus pallidus, GCMS-Gas chromatography mass spectrometry, NAA- N-acetyl-L-aspartic acid, ND-Not done, M-male, F-female, MRS- Magnetic Resonance Spectroscopy, T-Thalamus, WM-White matter.

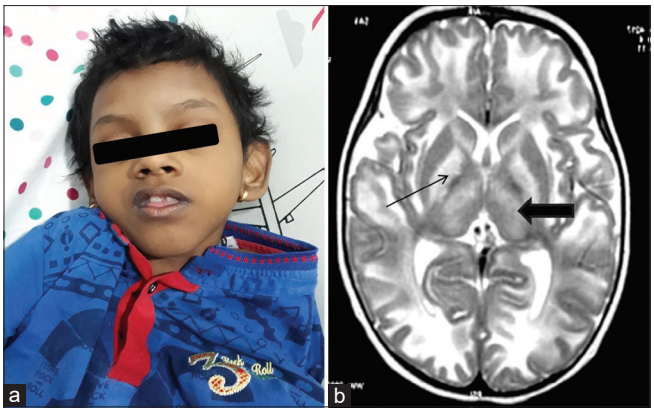

Figure 1: (a) showing clinical photograph of Canavan disease with microcephaly with strabismus in 5-year-old child with head size of 46 cm, (b). MRI of brain of same child showing symmetric involvement of bilateral globus pallidi (thin black arrow), thalami (thick black arrow), internal and external capsules

3 years. The longest surviving child is currently 7 years old, non-ambulatory with complete vision loss, severe spasticity, dystonia, dislocation of hip, and seizures requiring three antiepileptic drugs.

Discussion

In CD, the deficiency of the enzyme aspartoacylase results in pathological accumulation of the substrate N-acetyl-L-aspartic acid in WM, Cerebrospinal fluid (CSF), blood, and massive excretion in the urine. Based on the age of onset, CD could be classified as congenital form presenting at birth, infantile form presenting within 6 months of life, and the juvenile form in which symptoms are evident after the age of 5 years.[9] The classical triad in infantile CD is hypotonia, head lag, and macrocephaly. Macrocephaly is usually not apparent at presentation in infancy but develops with age, with progression of the disease. Most patients succumb in the first decade, but some survive up to the second decade with improved nursing care.[10] Some variations in the course of disease are also known to occur such as normocephaly which is noted in early infancy and in mild juvenile forms.[11]

In this study, high rate of consanguinity, presence of normal, and small head size and MRS showing elevated NAA peak even in the presence of non-contributory urine NAA levels were distinct. Similar findings of normal head size have also been noted by other authors.[6,11-13] We had earlier reported a case of CD with microcephaly.[14] The reason for small head is not clearly known. It may be that the head size of our children may be smaller than the reference specified in WHO growth charts, as we have commonly observed even normally developing children with a relatively smaller head size when plotted on the WHO growth charts and we do not have any geographic and ethnic specific head circumference charts. The classical MRI finding in CD is described as diffuse symmetrical cerebral WM involvement with less marked thalamic involvement with sparing of caudate and putamen.[15]

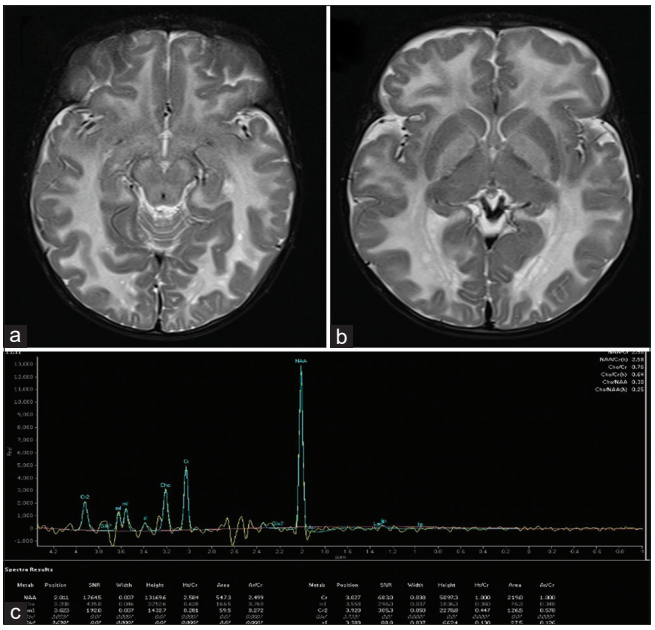

Figure 2: MRI of Canavan disease: axial T2 WI (a and b) showing diffuse hyperintensities of supratentorial WM both subcortical and periventricular WM involvement with, globus pallidus, mid brain, and anterior limb of internal capsule. (c) showing NAA peak on magnetic resonance spectroscopy (MRS)

Two predominant mutations E285A and Y231X are said to account for 98% of chromosomal mutations among Jewish population. A305E is the most prevalent mutation among Western Europe English, Dutch, and German patients.[16] Identification of mutation enabled genetic counselling in three families out of six cases which are genetically confirmed. Novel therapeutic strategies such as use of lithium citrate that decreases central nervous system NAA levels,[17] glyceryl triacetate as an acetate donor,[18] triheptanoin as an energy substrate, lipoic acid as an antioxidant, use of neural stem cell, and gene therapy using recombinant adeno-associated virus have been attempted with variable success.[19] We had not tried any new treatments like lithium in our patients. Bijarnia *et al*.[20] had reported three families with CD from northern India all of which were confirmed by genetic testing. All three cases had large head size compared to 7/12 (58.33%) in our study. Two reported mutations c.162C>A/p.Asn54Lys and c.859GG >A/p. Ala287Thr were identified in different cases of CD. The former mutation is seen in cases 3 and 11. The latter seen in cases 1 and 5. This indicates that the spectrum of mutation could be overlapping in India. This also would aid in screening for these specific mutation in Indian children with suspected CD.

Comparison between microcephalic children with other features revealed that case 1 had small head size, seizures, relatively less NAA peak on MRS compared to other cases with increased head size, and he had normal GCMS. Case 9 also had small head size, no seizures, and normal GCMS. It suggests that CD with small head may be associated with less increase in NAA peak, less NAA level in the urine compared to large head size.

Comparison between normocephalic children with other features: Case 4 had seizures, abnormalities in MRI in WM and basal ganglia (BG), MRS, GCMS. Case 5 had no seizures, no MRI BG changes, normal GCMS. Case 9 had seizures, abnormalities in MRI in WM and BG, MRS, GCMS.

About genotype-phenotype correlation: Cases 1 and 5 had same genotype, case 1 had seizures, MRI GP changes. Case 5 did not have seizures, no MRI GP changes, and normal GCMS. So, to conclude, it is hard to arrive at correlation between variants and clinical features in a small-scale study except that a small head size is associated with lesser NAA peak in MRS and lower NAA level in the urine. A larger scale study or systematic review would be required for the same.

The reason for normal NAA level in the urine despite increased peak of NAA in MRS of brain is due to the accumulation of substrate starts in the brain. Similar findings noted by Karimzadeh *et al*.[6] The limitations of our study were that genetic testing could not be done in all patients. As per our knowledge this is the largest case series from India.

Conclusions

CD is prevalent in communities with high rate of consanguineous marriages. Macrocephaly is not an essential requirement to diagnose CD. MRS depicting NAA peak could be an early diagnostic indicator of CD. Genetic diagnosis is useful for preventing recurrence in the family.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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