



Detection of glutaric acidemia type 1 in infants through tandem mass spectrometry



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ABSTRACT

Glutaric acidemia type 1 (GA1) is a rare inherited metabolic disorder which goes underdiagnosed due to its latency period and subtle presentation. A pilot clinical study was conducted to assess the usefulness, specificity and sensitivity of the tandem mass (MS/MS) spectrometer, specifically the Abbott (AB) Sciex 3200, in the screening for GA1 using dried blood spots. A total of 17,100 specimens, comprising pediatric patients and healthy newborns, were screened from June 2012 to June 2014. A selection criterion was applied to increase the range of samples tested. 14 of the total specimens tested presumptive positive for GA1, of whom all were symptomatic. The diagnosis was confirmed in 4 of the 14 cases and they were started on treatment. 4 cases expired before confirmation. The remaining cases were empirically started on treatment. Most of the patients responded favorably to the dietary management. One important observation was that the older symptomatic children diagnosed with GA1 had poorer outcomes in terms of recovery of delayed milestones and mental deterioration, further emphasizing the need for early diagnosis of organic acidemias along with the other biochemical defects. Tandem mass spectrometry was found to be more than 93.33% sensitive and more than 99.42% specific. The screening test proved to be very simple and economical.

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1. Introduction

Glutaric acidemia type I (GA1) is a rare inherited disorder. The *GCDH* gene that plays a role in this disorder is localized on chromosome 19p13.2 and shows an autosomal-recessive inheritance. THE *GCDH* gene encodes a flavin adenine dinucleotide-dependent mitochondrial matrix protein, the enzyme glutaryl-CoA dehydrogenase (GCDH), which is involved in the degradative metabolism of L-lysine, L-hydroxyl lysine and L-tryptophan [1]. Biochemically, GA1 is characterized by an accumulation of glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA), glutaconic acid (less frequently), and glutarylcarnitine (C5DC). As of 2011, only 500 cases of GA1 have been reported worldwide [1].

The initial progression of clinical symptoms in cases of GA1 is slow; hence GA1 is frequently undiagnosed until an acute metabolic crisis occurs. A child suffering from GA1 usually presents with macrocephaly. Striatal injury induces a variable clinical picture. Dystonia superimposed on axial hypotonia is usually the dominant extrapyramidal symptom [2, 3]. With age, the symptom evolves from mobile to fixed dystonia and

becomes associated with akinetic-rigid Parkinsonism. The other mode of presentation is an infant with delayed motor milestones, hypotonic with impaired voluntary movements. Once the neurological symptoms set in, the recovery is incomplete.

Diagnosis of GA1 starts with newborn screening with tandem mass spectrometry in apparently healthy new born babies; in case of symptomatic children the first would be the history and clinical evaluation and imaging studies as most children present with neurological symptoms. The suspicion of GA1 is from typical risk factors such as family history, consanguineous marriage, and deceased sibling. The other lab findings are severe acidosis, ketosis, hyperammonemia, and abnormal liver function tests. First-line diagnosis in the organic acidemias is urine organic acid analysis using gas chromatography with mass spectrometry (GC/MS). The analytes are elevated and detected in the other body fluids such as CSF and in the tissues by gas chromatography/mass spectrometry (GC/MS) or electrospray-ionization tandem mass spectrometry (MS/MS) [4–6]. Confirmatory testing involves assay of the activity of the deficient enzyme in lymphocytes or cultured fibroblasts and/or molecular genetic testing [7].

Treatment should therefore be initiated early with aggressive symptomatic management and dietary restriction GA1 is included in the panel of diseases that are identified by expanded newborn screening

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in some countries. This study has been taken up to evaluate the sensitivity and specificity of the tandem mass spectrometer (TMS) for detecting this rare organic acid disorder in symptomatic infants and healthy newborns.

2. Objectives of the study

1. To determine the sensitivity and specificity of the TMS to detect cases of GA1 in both apparently healthy newborn and high risk children.
2. To evaluate the effectiveness of TMS in detecting GA1 in children older than 2 months.
3. To emphasize the importance of timely detection and treatment of cases to prevent permanent neurological sequel.

3. Materials and methods

3.1. Study design

A retrospective study in which 17,100 healthy babies and high risk children from all over the country were screened for organic acidemias and amino aciduria by TMS analysis of their dried blood spots. The study was conducted using data of subjects tested between June 2012 and June 2014. Out of the 17,100 cases, 8731 were newborn and 8369 were older children with symptoms. The cases that came as presumptive positive for GA1 were then followed up to obtain information about their outcomes. A written informed consent was obtained from all the participant families.

3.2. Selection criteria

All the subjects were from the Indian subcontinent, both from the north and south regions.

The subjects in this study belong to 2 different groups, one is the low risk group consisting of healthy new born babies up to 7 days after birth and high risk children consisting of pediatric patients between 0 and 14 years of age with symptoms such as macrocephaly, delayed motor skills, metabolic acidosis, dystonia and seizures and presence of risk factors such as deceased sibling and consanguineous marriage. Cases were considered positive for GA1 if they demonstrated a clear elevation in their C5DC values above 0.15 $\mu\text{M/L}$ with a C5DC/C16 ratio of >0.12 , both for routine cases and high risk cases, as per the cutoff values and guidelines followed by the lab (Fig. 1).

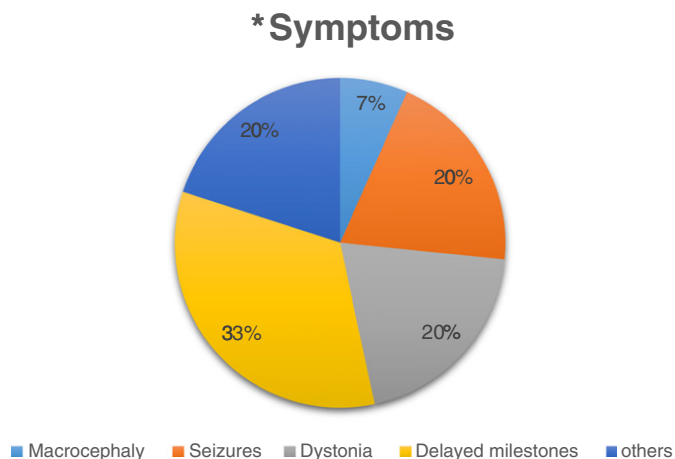


Fig. 1. Distribution of the main clinical features in the positive cases. *Some of the subjects had more than one clinical symptom.

Table 1

Comparison of the baseline characteristics between the negative subjects and presumptive positive cases.

Baseline characteristics of the study subjects	Negative cases N = 17,086	Presumptive positives N = 14
Age (weeks)	52.65	64.24
Gender	Males = 9397 Females = 7689	Males = 7 Females = 7
Term/preterm	3325	3
Maternal complications	Nil	2
Deceased/affected sibling	Nil	2
Geography (%)		
North	7.5	1
South	92.5	13
H/o consanguineous marriage	1392 ^a	2

N = number of subjects.

^a Some of these subjects with positive consanguineous history had other metabolic disorders.

3.3. Exclusion criteria

Cases which could not be followed up to obtain a confirmation of the diagnosis and management. Children older than 14 years as they do not include the pediatric age group.

3.4. Sample collection and processing

Blood samples were collected by heel puncture and blood spots were taken on S903 Whatman filter paper. The acylcarnitine and amino acid profiles of the blood spots were analyzed after their derivatization into butylated esters. The sample with the internal standards was then injected into the liquid column for separation and then into the MS/MS where they were quantified based on their charge/ mass ratio.

Samples were interpreted as normal and GA1 presumptive positive according to the cut off and clinical presentations. Patients who were symptomatic and were presumptive positives for GA1 were followed up for further confirmatory testing such as urine organic acids, enzyme assay or gene sequencing.

The statistical analysis was done by SPSS software version 12. The data was expressed as mean and standard deviation. The sensitivity and specificity of the MS/MS were determined by using receiver operating characteristics curve. The comparison between the study groups was done using chi square and ANOVA Tukey's method. The correlation coefficient was determined by Pearson's correlation analysis.

4. Results

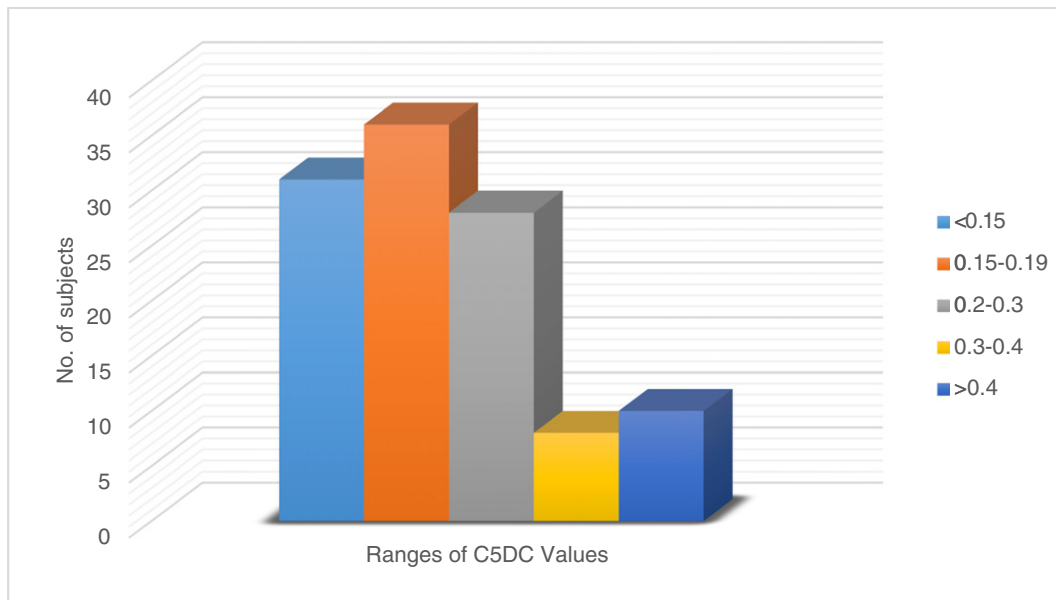
Out of the 17,100 babies that were screened, 8731 were routine screening and 8369 were high risk. Table 1 depicts the demographic distribution of the subjects and the cases were compared with the controls. 4 out of the 14 presumptive positives were apparently healthy babies between 2 and 7 days of age that came for routine screening; the remaining 10 were high risk older children. The 14 positives were then followed up out of which 4 cases were further confirmed with urine organic acids by GC-MS and started on treatment. 4 cases died before

Table 2

The sensitivity, specificity, positive and negative predictive values and prevalence of GA1.

Characteristics	Percentage	95% CI
Sensitivity	93.33%	(67.98–98.89)
Specificity	99.42%	(99.29–99.53)
Positive predictive value	12.28%	(6.88–19.75)
Negative predictive value	99.99%	(99.97–100)

CI = confidence interval.



Comparison of the C5DC values in Micromols/L ($\mu\text{mol/L}$) in the presumptive positive patients. No= number of subjects.

Fig. 2. Cutoff based distribution of positives. Comparison of the C5DC values in micromols/L ($\mu\text{mol/L}$) in the presumptive positive patients. No = number of subjects.

confirmation. 5 cases were started on treatment without further confirmatory testing. 100 cases were found to be false positive as the analytes were elevated but the subjects showed no clinical features of GA1 at the time of testing or on followup. 1 case was not detected as there were no elevated values of the primary and secondary analytes ($\text{C5DC} = 0.05$, $\text{C5DC/C16} = 0.11$) but was diagnosed as GA1 in another lab where the C5DC and C5DC/C16 values were above their cut off (Fig. 2). Remaining patients whose specimens were identified as normal by our method showed no clinical indications of GA1 at the time of testing or on followup. Table 2 shows the sensitivity, specificity and positive and negative predictive values.

Fig. 3 shows the MRI image of a positive case of GA1 with the characteristic “bat wing appearance”. Fig. 4 is the image of a case of GA1 with the spastic limbs and hypertonia. Fig. 5 shows the mass spectrogram of the GA1 positive case, the peak 388.5 corresponds to the primary analyte C5DC which is much higher in this sample when compared to

a normal sample. Fig. 6 shows a chromatogram of a positive case of GA1 where the urine showed elevated levels of 3 hydroxy-glutaric acid and glutaric acid. There was a significant correlation between age at the time of diagnosis and outcome with a coefficient of correlation, $r = 0.332$. There was no significant correlation between gender, birth weight and the outcome of the disorder.

5. Discussion

The routine newborn screening is done using dried blood samples by tandem mass spectrometry. The primary analyte elevated in GA1 is glutaryl carnitine (C5DC) and an elevated C5DC/C16 ratio above the accepted cutoff. The second tier tests include urine for organic acids by GC-MS/MS. The main organic acid elevated in the urine is 3-hydroxyl glutaric acid and glutaconic acid. From the study it has been confirmed that metabolic screening tests help to detect rare inherited disorders

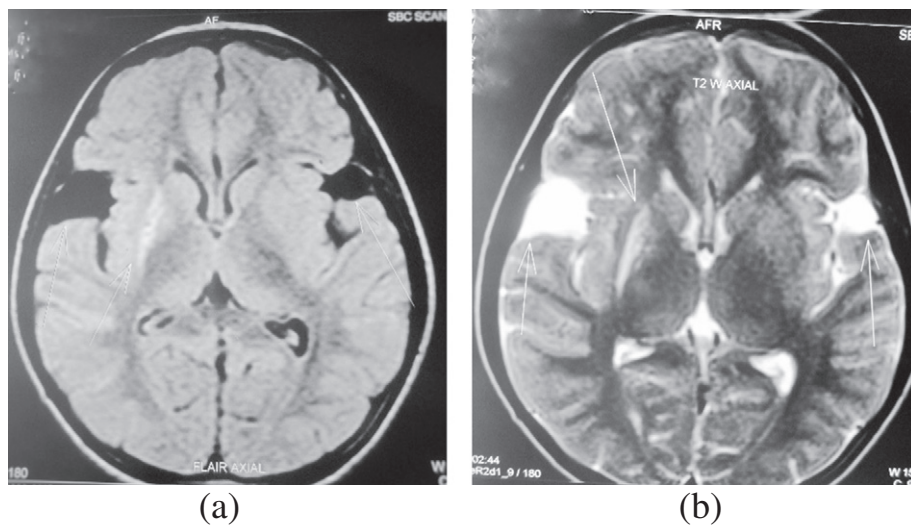


Fig. 3. (a) MRI of brain reveals frontotemporal atrophy, dilated sylvian fissures with open opercula (arrow), diffuse hyperintense lesions in bilateral basal ganglia, and both frontal white matter and bilateral periventricular area. Widening of the sylvian fissure gives the characteristic “bat-wing” appearance. (b) High signal intensity seen in bilateral caudate nuclei and putamen.



Fig. 4. One of the subjects showing the characteristic spastic limbs presented with motor symptoms such as dystonia and athetosis.

such as GA1. An estimated prevalence of 1 in 100,000 newborns has been found. The incidence of GA1 in this study is 14 in 17,100 which makes it higher than the global average as the incidence of consanguinity is much higher in India compared to many nations worldwide and autosomal recessive conditions show an increased prevalence in children of consanguineous marriages. Secondly the population screened by the lab includes high risk population and symptomatic cases. The

positives were more from the high risk group, older children as they were symptomatic cases and they were tested based on suspicion of the disorder.

In the current study the sensitivity and specificity of the screening tests are 93.33% and 99.42% respectively thus establishing it as reliable and sensitive tests. Despite the very rare incidence of this organic acidemia, the TMS was able to pick up as many as 14 positive cases in a 3 year period. Most of these presumptive positive cases were further confirmed by second tier tests and the subjects were started on treatment.

The other means of detecting this disorder is not as effective such as clinical diagnosis is hampered by the lack of characteristic or even pathognomonic signs and symptoms before an encephalopathic crisis. Macrocephaly is found in 75% of patients during infancy [7], but is non-specific. Quantification of the metabolites by urine GC–MS is done in cases which have been confirmed by TMS [8]. In this study also 4 cases were confirmed by urine GC–MS. Though by protocol it is considered as first line of testing there are certain limitations with urine testing, one is the false negative results in low excretor variants [8]. Secondly the urine acylcarnitine profiling is more complex and interpretation can be difficult [6] DNA-based mutation analysis was used for the high-risk screening in one cohort of low excretors. But the DNA mutation studies are better applied once the screening tests show suggestive findings rather than as a first step [9].

Significant differences still exist in the approaches used to diagnose and manage affected patients, and there is a wide variation in the outcome, particularly in patients diagnosed pre-symptomatically [10,11]. The major aim of this study is to re-assess the common practice and to formulate recommendations for diagnosis and management of GA1 based on the best evidence available.

Correlation was found between age at the time of diagnosis and outcome in terms of survival and response to therapy. It was seen that the older the age of detection of the disorder the more adverse the outcome. Older children who were diagnosed had expired. There was no significant correlation between gender, birth weight and the outcome of the disorder [1].

Irreversible neurological symptoms generally occurred between the age of three months and three years [12]. Figs. 3 and 4 shows the changes in the central nervous system and limbs in case of an advanced undiagnosed case of GA1. In the patients identified so far by newborn screening in a study, neurological damage was prevented in 51/65 patients [13]. In this study 1 out of the 14 cases was saved in time to lead a normal life, 5 were already symptomatic at the time of diagnosis yet showed improvement on treatment. 4 out of the remaining expired due to complications. The remaining 4 cases did not improve as they had already progressed to irreversible symptoms. Previous studies

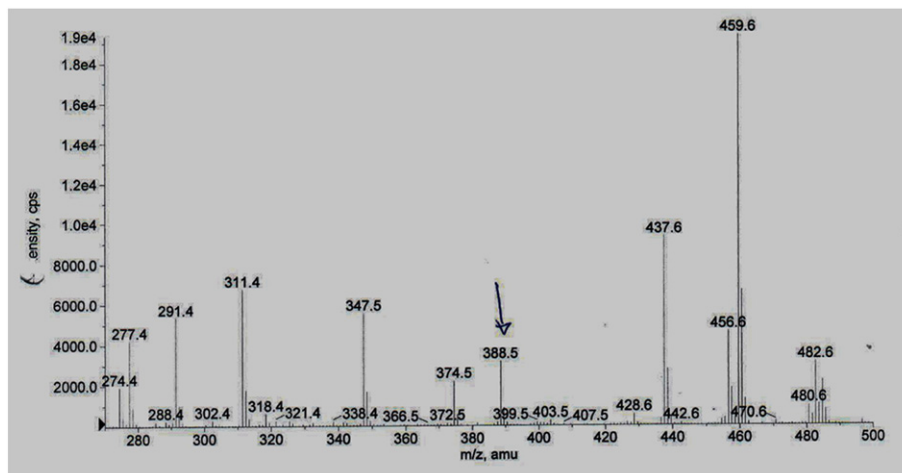


Fig. 5. The mass spectrogram of a presumptive positive case of GA1, the primary analyte is indicated by an arrow.

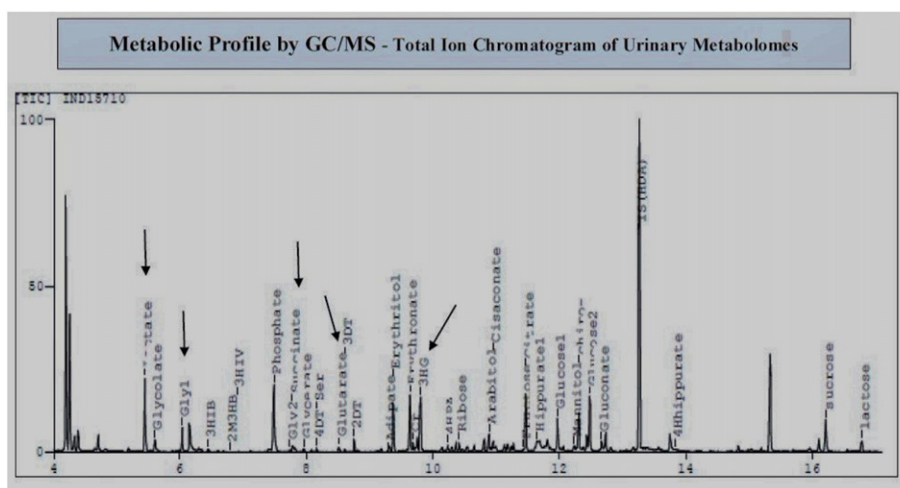


Fig. 6. Image of a positive GA1 GC/MS chromatogram.

have shown that the outcome is mainly determined by a single crisis during infancy or childhood. The diagnosis is done earlier through these screening tests rather than establishing a clinical diagnosis as by the time the child presents with delayed milestones, it is mostly irreversible even with vigorous management.

This emphasizes the importance of newborn screening in early detection of serious debilitating organic acidemias [13–15]. Several different protocols are used in different centers, including low protein diet, diet restricted in lysine/tryptophan, supplementation with carnitine/riboflavin chronic anticonvulsant treatment to reduce risk of neuronal damage post diagnosis [16].

6. Limitations of the study

The information on the presentation and management could be incomplete as most of this information was obtained from the parents with a weak educational background. Also, the geographical distribution of the positive cases is not representative of the nation as most of the laboratory's clientele are from the southern states of the country. The comparison should also be done with the conventional tests used for early detection of this disorder such as imaging studies, clinical evaluation and enzyme assays. But data of these methods are not available to conduct such a comparison.

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