Clinico-Biochemical Spectrum of Pakistani Patients with Glutaric Aciduria Type 1 (GA1): Experience from a Specialised Biochemical Genetics Laboratory in Pakistan

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ABSTRACT

Objective: To evaluate the clinical, radiological, and biochemical features of glutaric aciduria Type 1 (GA1) patients identified through urine organic acid testing at a biochemical genetics laboratory (BGL) in Pakistan.

Study Design: Observational study.

Place and Duration of the Study: Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, Pakistan, from January 2013 to December 2022.

Methodology: Medical charts and urine organic acid (UOA) chromatograms of the patients presenting at the BGL from January 2013 to December 2022 were reviewed. Brain imaging was obtained where available. Variables were noted as per the objective and descriptive statistics were obtained.

Results: GA1 was found in 64 (0.4%) patients out of a total of 16,094 UOA requests for high-risk screening cases. The age of diagnosis ranged between one month and three years. The brain MRI findings revealed characteristic abnormalities such as cerebral atrophy, expanded CSF spaces, white matter abnormalities, and a distinct bat wings appearance, in cohesion with the results of biochemical testing.

Conclusion: Sixty-four cases of GA1 from a single centre indicate a high frequency of the disorder in Pakistan. Late diagnosis emphasises the need for increased clinical awareness and preferably newborn screening to ensure optimal outcomes.

Key Words: *Glutaric aciduria Type 1* (*GA1), Brain imaging, UOA analysis, Glutaryl-CoA dehydrogenase* (*GCDH), Pakistan.*

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INTRODUCTION

Glutaric aciduria Type 1 (GA1) (OMIM # 231670) is a rare genetic disorder, inherited in an autosomal recessive model. In individuals with GA1, there is a reduced or absent level of functional glutaryl-CoA dehydrogenase *(GCDH)* enzyme responsible for breaking down amino acid lysine, hydroxylysine, and tryptophan as shown in Figure 1. Without enough functioning of *GCDH*, these amino acids and their by-products can build up to toxic levels in the body, causing damage to the brain and other organs.[1,2]

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The estimated prevalence of GA1 globally is 1/110,000; nevertheless,the prevalence rates tend to be greater in certain populations that share a similar genetic make-up such as the Old-Order Amish of Lancaster County, Pennsylvania (1/300–1/400), and the aboriginal Ojibway-Cree-Indians of Canada (1/300),[3-5] whereas in China, the incidence of GA1 varies from 1/171,411 to 1/52,078.[6] There are no current statistics or estimates on the prevalence of GA1 in Pakistan currently.

Individual studies on GA1 have been done in different hospitals or research institutions in Pakistan. Noor *et al.* recently published a case of a Pakistani male infant with GA1 presenting with fever, seizures, and altered consciousness, and typical radiographic features suggested GA1.[7] The case report emphasises the importance of timely management of metabolic crisis in paediatric patients.[7]

Infants with GA1 deficiency may grow normally during the first one to two years of their lives; macrocephaly is a common observation in these babies, and it occurs before the onset of neurological manifestations.[8,9] The majority of affected infants experience a severe encephalopathic episode, which is brought on by

the catabolic situations like infections or surgery. This can lead to striatal injury and a severe movement disorder that resembles cerebral palsy and can be associated with high morbidity and mortality. Intellectual disabilities may also accompany these symptoms.[10,11] Chronic kidney disease has been increasingly reported in the recent years, alongside other neurological disease manifestations.[12] MRI can be used to detect the characteristics of brain abnormalities that are often seen in patients with GA1 and is an important modality in the diagnosis and management.[13]

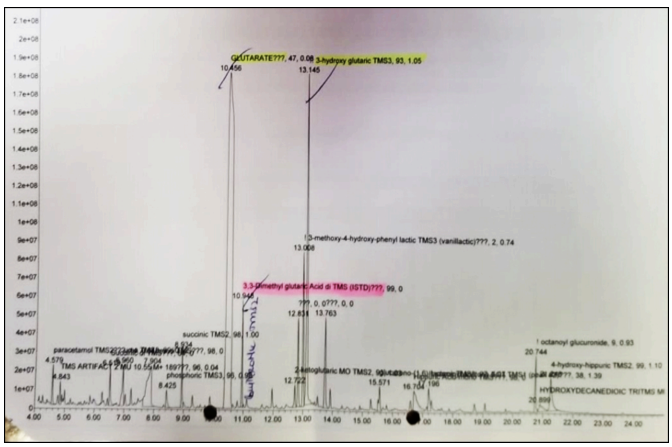

Figure 1: UOA chromatogram depicting representative peaks of GA1.

Newborn Screening (NBS) for GA1 relies on the measurement of glutarylcarnitine (C5DC) in dried blood spots, which has a 96% sensitivity.[3] Positive C5DC levels call for additional biochemical testing *via* UOA to detect glutaric and 3-hydroxyglutaric acid. The diagnosis of GA1 in a proband with suggestive biochemical and / or clinical findings is confirmed by molecular testing based on the detection of biallelic pathogenic variants in *GCDH*.[14,15] In resource-limited countries like Pakistan, where NBS is lacking UOA *via* gas chromatography mass spectrometry (GCMS) is a useful diagnostic modality for timely screening of the patients with suspicion ensuring the prompt management.

The present study's rationale was to obtain insights into the prevalence, diagnostic characteristics, and clinical outcomes of GA1 patients identified through urine organic acid testing in Pakistan, highlighting the importance of early detection and increased clinical awareness for improved management of this disorder. The objective of this study was to analyse the spectrum of clinical, radiological, and biochemical features of the patients identified as GA1 through UOA testing at a biochemical genetics clinical laboratory in Pakistan.

METHODOLOGY

This observational study was conducted at the Biochemical Genetic Lab (BGL), Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital Karachi, Pakistan. A questionnaire containing clinical and biochemical information was completed with each test requisition for UOA. All the patients with diagnosis of GA1 on organic acid analysis were included in the study. Any other diagnosis on UOA and normal UOA profiles were excluded. Samples were received from the entire country *via* a widespread network of 300 phlebotomy centres. The UOA was performed on GCMS using ChemStation software (http://www.agilent.com/en-us/-support/software-informatics/multiinstrumentsoftwarerev) with the help of multiple libraries.

This retrospective study involved a review of UOA chromatograms suggestive of GA1 from 2013 to 2022 by two consultant chemical pathologists after the approval by the institutional Ethical Review Committee (ERC#8393). The cases with diagnosed GA1 on urine organic acid analysis were included and disorders other than GA1 were excluded from the final analysis. The clinical, biochemical, and MRI findings were retrieved from the database. A pre-structured questionnaire was used for the data collection. Frequencies and percentages were calculated for gender, consanguinity, geographical distribution of the cases, MRI findings, and clinical presentations.

RESULTS

A total of 16,094 UOA tests were performed over the period of nine years, for high-risk screening cases. A total of 64 (0.4%) (39 males and 25 females), with ages ranging from one month to three years, had GA1 as suggestive diagnoses. The patients were representative of the geographical distribution of the country, as shown in Figure 2.

The most common clinical features noted was developmental delay followed by lethargy and fever. Detailed clinical features are presented in Table I. The characteristic metabolites on UOA are glutaric acid, 3-hydroxyglutaric acid, and glutaconic acid as depicted in Figure 1. The brain MRI findings available for 26 patients, revealed cerebral atrophy [n = 4 (15.3%)], expanded CSF spaces [n = 5 (19.2%)], and white matter abnormalities [n = 6 (23.06%)], indicating progressive degeneration and impaired connectivity in the brain's white matter pathways. A distinguishing characteristic feature i.e., widening of the silvian fissure giving bat wings appearance.

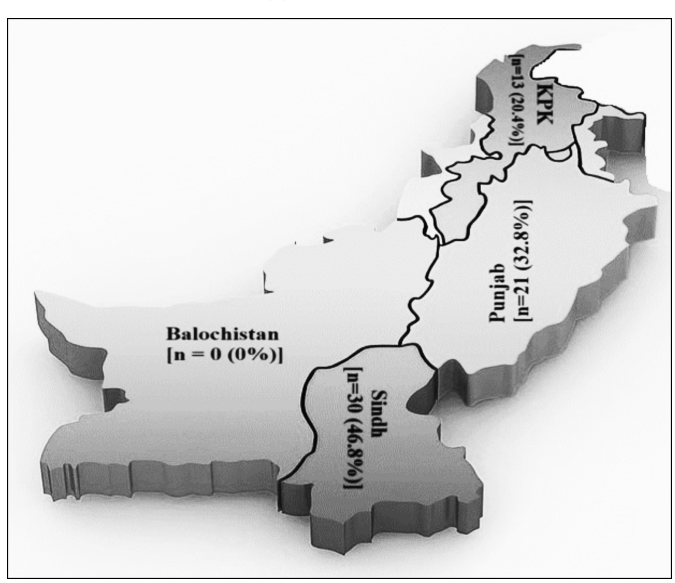

Figure 2: Geographical distribution of the cases.

Table I: Clinical features of the patients with GA1 (n = 64).

Clinical features	Total number of patients (n = 64)	Percentage
Developmental delay	36	56.2%
Lethargy	26	40.6%
Fever	24	37.5%
Seizures	21	32.8%
Failure to thrive	19	29.6%
Hypotonia	18	28.1%
Involuntary movement	18	28.1%
Poor sucking	15	23.4%
Septicaemia	10	15.6%
Vomiting	10	15.6%
Mental retardation	10	15.6%
Dysmorphic	8	12.5%
Smell in urine	7	10.9%
Skin lesions	6	9.3%
Coma	6	9.3%
Coloured urine	4	6.2%
Jaundice	3	4.6%
Eye lesions	2	3.1%

DISCUSSION

GA1 is frequently accompanied by a variety of neurological signs and complications, including irreparable brain damage.[11] To stop the emergence of acute encephalopathic crises and lessen the long-term effects on the patients' health and well-being, it is essential to diagnose GA1 early and treat it promptly. This single centric experience with 64 cases from the high-risk screening masks the fact that this rare disorder has a considerable frequency in the country which may rise further in the presence of national NBS.

To properly diagnose and treat GA1, the MRI of the brain is essential. Twenty-six of the 64 GA1 patients in this study underwent brain imaging tests, which allowed the authors to assess the typical radiological abnormalities connected to this condition. The examination of these radiographs exhibited several noteworthy abnormalities that were frequently observed in severely affected individuals with GA1. Previously, Larson *et al*. reported MRI findings in 18 Dutch individuals with GA1, whereas the current study exhibited 26 individuals with GA1 using brain imaging.[15] Larson *et al.*'s brain imaging findings included various abnormalities such as open opercula, widening of cerebrospinal fluid (CSF) spaces or ventriculomegaly, white matter abnormalities and subdural haemorrhage, typically associated with frontotemporal hypoplasia. The present study also found very much similar radiographic results (cerebral atrophy, widening of CSF spaces, batwings appearance, and white matter abnormalities) as of Larson *et al.*'s findings. Both the studies highlight the presence of structural brain abnormalities in the patients with GA1.

Gelenar *et al*. exhibited a patient with unspecific mild neurological symptoms who was diagnosed with GA1 based on the detection of typical neuroimaging GA1 features.[16] These features included multiple subependymal nodules, frontotemporal hypoplasia, mild leukodystrophy, and consistent biochemical abnormalities. Conversely, this study focused on GA1 patients who underwent brain imaging tests to assess the typical radiological abnormalities associated with the condition. The examination revealed several noteworthy abnormalities commonly observed in severely affected individuals with GA1. While both studies examined GA1 patients and used neuroimaging techniques. Gelenar *et al*. emphasised the diagnosis of GA1 in a patient presenting with mild neurological symptoms, while this study aimed to characterise radiological abnormalities in GA1 patients, particularly those severely affected. Funk *et al*. and Kimura *et al*. also reported that brains of the children affected with GA1 exhibit wide sylvian fissures, basal ganglia atrophy, white matter lesions, and enlarged frontal ventricles.[17,18] These symptoms were similar to those found in the current study.

These findings emphasise the importance of conducting MRI scans to accurately diagnose and treat GA1. By understanding the radiological abnormalities associated with this condition, healthcare professionals can better assess the severity of the disease and develop appropriate treatment strategies for affected individuals.

For the purpose of identifying GA1, this study showed that there was 100% agreement between the brain imaging results and UOA analysis. The precise diagnosis of GA1 is supported by the findings of distinctive brain abnormalities, such as cerebral atrophy, increased CSF gaps, aberrant white matter, and the batwings appearance, in addition to the elevated levels of metabolites in the urine.

This study strengthens the argument for setting up local diagnostic facilities and NBS for GA1. It stands as the pioneering study that comprehensively evaluates the clinical, biochemical, and radiological aspects of GA1 in Pakistan, presenting findings from a substantial group of symptomatic patients within the native population. NBS plays a significant role in identifying infants with GA1 before they develop symptoms or experience metabolic crises.[10] As there is no national screening centre in Pakistan, this may limit the early detection of GA1. In such instances, the healthcare personnel must maintain a high index of suspicion for GA1 and consider diagnostic testing if a neonate or child exhibits symptoms or clinical signs. When there is no national screening programme, it is even more important for healthcare professionals to be aware of the signs and symptoms and available limited testing facilities of GA1. Through UOA testing, GA1 can be detected early, allowing for prompt counselling of the metabolic emergencies in those individuals impacted. Furthermore, the association between these biochemical indicators and the results of brain imaging enhances the diagnostic precision of GA1. Consequently, in the diagnostic process and continuing monitoring of those suspected of having GA1, the incorporation of both UOA analysis and brain imaging methods, such as MRI, can be considered.

However, this study had certain limitations. Firstly, since there is no mandated GA1 screening in Pakistan, most of the patients were referred to the authors' centre for a diagnostic evaluation due to a clinical suspicion of an inherited metabolic disorder. As a result, the findings cannot be generalised to the public or individuals who were not referred to the centre. Secondly, because the test was not available in Pakistan, a conclusive diagnosis utilising molecular analysis was not possible. The primary reason for this was the financial consequences of the additional expense of

outsourcing the specimen, which the families had to pay. Moreover, as this was a laboratory-based study, details pertaining to the follow-up and further investigations were not included.

CONCLUSION

A single centre's reporting of 64 instances of GA1 during a 10-year period suggests that the disease is present in Pakistan and should be anticipated in the relevant context. The inclusion of GA1 in NBS programmes is necessary to enable the early management and better patient-centred outcomes because most patients with GA1 in Pakistan are only diagnosed after they are already exhibiting symptoms.

ETHICAL APPROVAL:
The study was approved by the institutional Ethical Review Committee (Ref# 2023-8393-24002).

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
MB: Literature review, data compilation, and write-up.
LJ: Data collection, write-up, and reviewing of the final manuscript.
HM: Write-up and data cleaning.
AHK: Write-up of the first draft and reviewing of the final version for critical analysis.
SA: Conceived the idea and write-up.
All authors approved the final version of the manuscript to be published.

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