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LETTER TO THE EDITOR

An Audit of Temperature Influence on Urine Specimens with Glutaric Acidemia Type 1 via GC-MS - Is there a Missing Link? Temat

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Glutaric aciduria Type 1 (GA-1) is a progressive neurodegenerative inborn error of metabolism that typically manifests acutely in infants, characterized by glutaryl-CoA dehydrogenase deficiency which is involved in the catabolic pathways of amino acids, such as lysine and tryptophan, impacting approximately 1 in 100,000 births [1,2]. This disorder leads to the accumulation of glutaric acid, 3-hydroxyglutaric acid (3-OH-glutaric acid), and glutaconic acid (occasionally) in bodily fluids, detectable via gas chromatography mass spectrometry (GC-MS). The absence of distinctive signs and symptoms before an encephalopathic crisis complicates early clinical diagnosis, based on clinical presentation or neuroradiology findings, particularly macrocephaly with the typical widening of Sylvian fissures mostly GA-1 is suspected. Owing to the rarity of GA-1 and the difficulties in detecting its presymptomic appearance, prompt diagnosis and treatment plans are essential to reducing the risk of neurological consequences [3]. This emphasizes the significance of raising awareness and conducting screenings among impacted populations. Presymptomatic detection of glutaric aciduria Type 1 (GA-1) typically involves newborn screening programs, where infants are screened for a variety of metabolic disorders shortly after birth.

The section of Clinical Chemistry of the Department of Pathology and Lab Medicine at Aga Khan University (AKU) has been performing Urine Organic acid analy-

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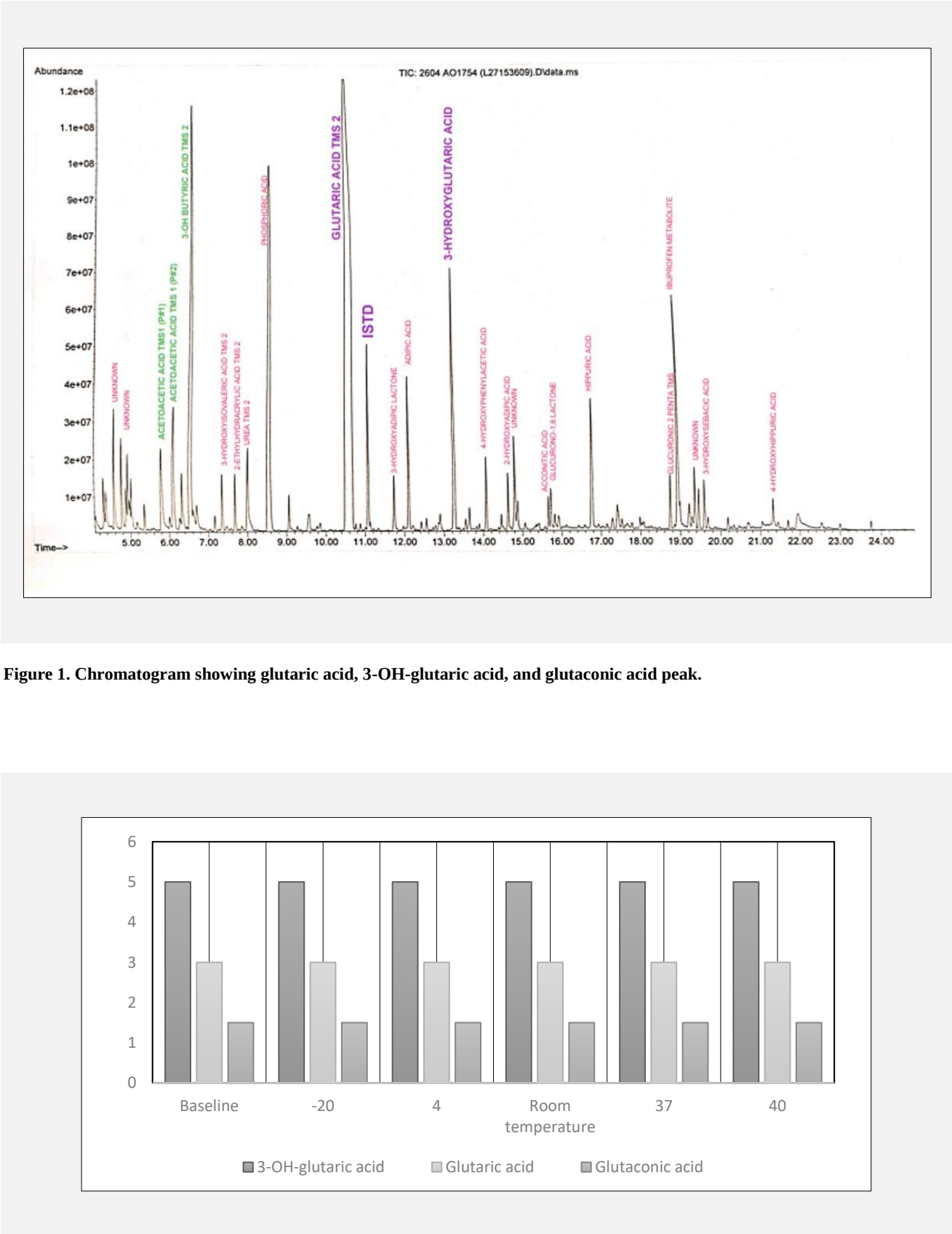

Figure 1. Chromatogram showing glutaric acid, 3-OH-glutaric acid, and glutaconic acid peak.

Figure 2. Peaks of glutaric acid, 3-OH-glutaric acid, and glutaconic acid observed at varying temperature conditions.

sis (UOA) for almost a decade now. Since genomic enzyme analysis is unavailable in Pakistan, presence of characteristic metabolites in UOA is considered essentially diagnostic as they are specific to GA-1.

While reporting, pathologists encountered multiple cases of strong clinical suspicion of GA-1 with only glutaric acid present in urine, creating a diagnostic dilemma. Hence, pre-analytical factors, such as sample exposure to temperature extremities and time delays, were hypothesized to be possible reasons for metabolite instability. This study was designed to test the effect of storage temperature on UOA assay via GC-MS with characteristic presence of these metabolites on baseline analysis.

Urine samples with known GA-1 diagnostic peaks of glutaric acid, 3-OH-glutaric acid, and glutaconic acid were taken. Samples were analyzed using GC-MS (7,890/5,975, Agilent Technologies), urine creatinine (mmol/L) was estimated, and results were analyzed using Chem station software and are shown in Figure 1. Samples were divided into six groups; exposed to six different conditions (temperature), -20℃, 4℃, room temperature, 37℃, exposed to sunlight, and 40℃ for 6 hours with subgroup analysis for glutaric acid, 3-OH-glutaric acid, and glutaconic acid compared to baseline on chromatogram review. Data was analyzed using Microsoft excel software. Results shown in Figure 2 depicted that no significant impact of the temperatures discrepancy was observed on glutaric acid, 3-OH-glutaric acid and glutaconic acid presence in urine. The peaks appeared in their due retention time despite the extreme changes in temperature.

It was concluded that pre-analytical factors, such as extremes of temperatures over time, have no significant effect on the presence of glutaric acid, 3-OH-glutaric acid or glutaconic acid in urine samples. Thus, the phenomenon can probably be linked to other factors such as the inherent variability between individuals. The results coincide with the European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM) that state in its recent findings that external preanalytical factor such as temperature change has no significant adverse impact on the metabolites.

Though according to several studies and guidelines, urine samples are to be stored at specified temperatures 4℃, 22℃, and 40℃, and the samples stored at low temperature were observed to have more stable metabolites [4]. However, our finding suggests that these markers are not affected by the variation in temperature and did not show any thermal sensitivity.

Hence, in cases of discrepant GA-1 diagnoses where no other peaks are detected in samples, plasma glutarylcarnitine levels were also performed but studies have shown this level may not always provide definitive results. While elevated levels suggest GA-1, cases with normal levels have also been observed. Thus, urinary glutarylcarnitine testing emerges as a crucial parameter, offering a comprehensive metabolic profile that can complement plasma analysis [5].

To enhance diagnostic accuracy, molecular genetic analysis of the GCDH gene has been proposed to differentiate patients exhibiting incomplete biochemical phenotypes [6]. We recommend integrating genomic enzyme analysis and testing. This approach provides definitive confirmation of GA-1 by identifying specific genetic mutations. Moreover, genomic testing offers insights into the disorder's underlying genetic mechanisms, paving the way for targeted therapies and personalized management strategies. With no thermal impact on samples, genomic analysis ensures reliable results, advancing our understanding and treatment of GA-1.

Declaration of Interest:
None.

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