Screening for X-ALD by Tandem Mass Spectrometry Using Dried Blood Spots

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In May 2017, two distressed families approached our hospital to get their respective sons, aged 7 and 12 years examined. These young boys, hailing from small suburban villages of Andhra Pradesh and Odisha, were requested by their school heads to consult a city doctor. Reportedly, there was a sudden decline in their scholastic performance and attention span in school. From being bright and intelligent children without any medical issues post birth, they had suddenly become inattentive and dull in just a matter of 6 months. The worried parents tried to counsel their children but every attempt failed. Unable to meet the expectations of the parents and teachers these boys became socially withdrawn and eventually refused to go to school. Soon, one of them developed a problem with walking and had multiple falls. His speech was unclear so he stopped talking and going out to play with his friends. His mother observed that his skin colour was darkening day by day. The other boy could not see the blackboard as his vision had started blurring. He repeatedly visited the washroom at home and in school. Parents scolded him for being careless and considered it as a pretext to escape from studies but did not realise something was wrong with him until one day he could not recall recent or past events. Both the families had an income which was below the poverty line so they could only consult a doctor in their town who gave them some vitamin syrups and left them in this condition for another one month. As things worsened, they were referred to a neurologist in the city which was closest to their hometown. Magnetic

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* Ms. Archana Natarajan, Ph.D. Scholar from National Institute of Mental Health & Neurosciences, Bengaluru, is pursuing her research on "Mass Spectrometry-Based Targeted Metabolomics for the Diagnosis of X-Linked Adrenoleukodystrophy." Her popular science story entitled "Screening for X-Ald by Tandem Mass Spectrometry using Dried Blood Spots" has been selected for AWSAR Award.

Resonance Imaging (MRI) and other neurological evaluations were carried out where doctors suspected X-Linked Adrenoleukodystrophy (X-ALD). With a referral letter, these patients were sent to NIMHANS, Bengaluru for a check-up. Detailed evaluation was done at the neurology, neuroradiology and neurochemistry departments to investigate into the issue these boys were independently facing with some overlapping symptoms.

MRI revealed changes in the brain associated with a genetic disorder, X-ALD where very long chain fatty acids (VLCFA) accumulated in the body leading to symptoms coinciding with some of those described above. It could affect the nervous system, create hormonal imbalance, weaken their muscles and cause many other complications. It can affect all age groups but mainly affects young boys/adult men and women above the age of 50, who inherit the faulty gene. The symptoms are not visible after birth but can appear at any time in the later stages of life and if left undiagnosed at the right time, it can even lead to permanent disability and death in some cases. Apart from suspecting X-ALD based on MRI and neurological evaluations, clinicians suggest for plasma VLCFA estimation by a technique called gas chromatography mass spectrometry (GC-MS), which is expensive and requires more blood volume to be collected from the patient.

We could understand the mother, Binodini's, from West Bengal, distress when she found out that her son was suspected to have X-ALD. Seeing her 21-year-old son (A: earlier it says 12?) run away from home after aggressive outbursts on multiple occasions made her visit different doctors and get him checked. Post his MRI testing in Kolkata, he was sent to our hospital (A: the name?) where the clinicians suggested a plasma VLCFA after which they would get the diagnosis confirmed by genetic testing. Unable to pay INR18,000 for the test, she requested the doctors to discontinue the evaluation and left for her hometown. We requested the patient to make a visit to the hospital again but being illiterate and from a poor financial background they could not understand the importance of the test.

Meanwhile, we had received the funding from the Department of Biotechnology to establish a low-cost test method for screening of X-ALD which could be quick and a procedure that would cause less pain to the patients during blood sample collection. Our method included the testing of the by-products of VLCFA, called VLCFA-LPCs (lysophosphatidylcholines) in dried blood spots (DBS), where a finger prick could get us close to 0.5-1 ml of blood. Collecting spots on the filter paper, drying them and measuring multiple analytes in just a small 3 mm spot of blood from it is a well-known method for performing newborn screening of children for many inherited metabolic disorders. For a decade, our laboratory has been offering the testing facility for the screening of inborn errors of metabolism by tandem mass spectrometry, not just for patients consulting in NIMHANS but also from other parts of India at a very low cost compared to other private laboratories. We wanted to establish another diagnostic test with a similar concept to screen patients for X-ALD. So, we coupled DBS technology with liquid chromatography tandem mass spectrometry (LC-MS/MS) technique and used it to our advantage where multiple samples can be tested quickly at a lower cost when compared to the conventional plasma VLCFA testing by GC-MS. When the patient has been pricked multiple times sample collection from veins becomes extremely challenging. In such conditions, collecting blood as dried blood spots by a finger prick

can help in easing out the situation by causing less discomfort to the patients. After drying the blood spot cards, these samples can be sent by post to Metabolic Laboratory, Department of Neurochemistry, NIMHANS. So, patients who are unable to come to our hospital could also be screened for X-ALD, currently for free of cost but comparatively at a much lesser cost than the plasma VLCFA estimation by GC-MS.

After the test was established and validated in our laboratory, the clinicians called Binodini and convinced her to bring her son for clinical check-up along with DBS based biochemical screening for X-ALD by LC-MS/MS, done free of cost. Unfortunately, his symptoms had worsened by then as he had severe behavioural problems which were difficult to manage. He threw childish tantrums, had stopped eating food, remained attached to his mother always, became violent, used curse words, wanted to talk to only girls and constantly asked his mother to get him married. He was facing issues because of the adolescent onset of this condition. His test results turned out to be positive which correlated with his MRI findings. Since they were not able to pay for genetic testing, the clinicians had to continue his evaluation based on our test reports only. The other two boys

aged 7 and 12 years were also screened positive for X-ALD by our DBS-based LC-MS/MS method and were also confirmed positive by genetic testing. We have been able to screen 21 such patients as positive for X-ALD by our method. We hope that the progression of their disease condition could be clinically managed because after a certain age even bone marrow transplant would not work as a method for treating X-ALD.

DBS method of sample collection for measuring VLCFA-LPCs – the better markers for biochemically screening patient for X-ALD by LC-MS/MS will be a potential method to screen children at birth for this disorder. It could be added as a mandatory test to be conducted for every newborn along with the other metabolic screening tests which are conducted post birth. One blood spot has a potential to test for many disease conditions which could be managed with clinical intervention right from the time a child is born. Some of these inherited metabolic diseases, if left untreated, undiagnosed or misdiagnosed can lead to severe neurological conditions which when left unmanaged can also cause death. This could avoid financial and emotional distress to the family whose child is suffering. Continuous research initiatives like these which are patient-centric need to be given utmost priority in our country where so many diseases are yet to be managed and many are left undiagnosed. Our testing technique could provide a ray of hope especially to those families who cannot even afford a bare minimum meal. As citizens of this country it is our responsibility to contribute towards research that is able to bring a positive impact on the lives of the citizens belonging to all sections of the society.