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Projection of Need for Pathogenetic Testing for Mitochondrial Dysfunction in Autistic Spectrum Disorder (ASD) Children of India

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Abstract:

Background

Autistic Spectrum Disorder (ASD) is a neurodevelopmental disorder. There is a large quantity of evidence which point towards a positive correlation between Autism and Mitochondrial disorders (MD). In addition to that, several published reports, indicate that people with neurological disorders exhibit pathological signs of mitochondrial disorders and vice versa. Screening for underlying MD is essential in ASD as the children (clinically) appear and behave the same way in the both instances; however, their management is very different.

Materials and Methods

The current study examined biochemical, neuroimaging and genotyping technique in ASD patients to see which technique would be easier to interpret and indicate underlying MD. The analysis of the screening was based on several objectives like clinical, histological, biochemical, molecular, neuroimaging and enzymatic findings.

Results

We found out that pathogenetic analysis based on clinical and genotyping gives spontaneous results to analyse the possibility of MD in ASD patients.

Conclusion

It does not necessarily require blood samples from ASD patients to accomplish this type of analysis.

***Key Words*:** Autistic Spectrum Disorder, Children, Mitochondrial DNA.

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1- INTRODUCTION

ASD encompasses neurodevelopmental disorders that are defined by behavioural observations, in particular dysfunctions in social interaction and communication skills, as well as repetitive behaviours (1). Many children with ASD have associated underlying medical comorbidities, like epilepsy, sleep disruptions, mitochondrial dysfunction (MD) and gastrointestinal (GI) abnormalities (2). Few studies have hypothesized that individuals with ASD may have an abnormality in carbohydrate metabolism should be tested for disorder of impaired Mitochondrial function (3). Many studies has provided evidence (19-43%) that individuals with ASD have concomitant MD and proposed a "Mitochondrial autism subgroup" (4). There are no reports of any tests to be done to find any association of these two disorders from central India (5). Many explorations have been done to uncover the mitochondrial problems and relationship to Autism and suggested that these children clinically look and act same in both, but their management would be very different if diagnosed (6).

Screening for MD is based on several objectives clinical, histological, biochemical, molecular, neuroimaging and enzymatic findings. Usually biochemical tests require blood sample or sedative for Magnetic resonance spectroscopy (MRS) (7). The major cause of MD is mutations in mitochondrial DNA (mtDNA).The presence of mtDNA mutations in ASD children may indicate an etiological role of MD in autistic patients. Previously done research on study 810 patients with ASD identified two individual (2.2%) with same mutation (8). Mousavizadeh et al. has showed a significant relationship between the point mutation of the mtDNA and the risk of autism (6).

In the current study, we examined three widely used biochemical, neuroimaging and genotyping techniques to see which technique would be easy to interpret/or indicate towards underlying MD in ASD patients.

2- MATERIALS AND METHODS

2-1. Patient selection

Ten Autistic patients registered at AIIMS paediatrics department, Bhopal, India, where contacted. Bhopal is the capital city of Madhya Pradesh and is located in the central part of the Indian Subcontinent. Institute Human Ethical Committee (IHEC) AIIMS Bhopal, has reviewed and approved the research study.

2-2. Biochemical parameters

1mL blood sample was taken. Serum Lactate, Pyruvate, Creatine kinase (CK), Aspartate transaminases (AST), Alanine transaminases (ALT), Amylase, Glucose (9) on automatic auto-analysers at the department of Biochemistry, AIIMS Bhopal- India.

2-3. Neuro Imaging

Three parameters were taken into consideration for the MR spectroscopy which is N-Acetylaspartic acid, or *N*-acetylaspartate (NAA), Choline and creatinine (10).

2-4. Genotyping of mtDNA

2-4-1. mt DNA isolation from cheek cells (11)

DNA from cheek cells is considered as ideal for mtDNA isolation and Single nucleotide polymorphisms (SNPs) by Polymerase chain reaction (PCR) for conducting MD (10). 5 µl of each primer.

- 5-TTAACTCCACCATTAG-. CACC-3
- 5'-GAG. GATGGTGGTCAAGGGAC-3

3- RESULTS

3-1. Biochemical testing:

Results of the biochemical tests are shown in (Tables.1- 3).

3-1-1. MR spectroscopy results and their interpretation

Proton MR spectroscopy was done and chemical metabolites were recorded at 4 specific areas: the right and the left amygdala, Anterior Cingulate Cortex and Posterior Cingulate Cortex. The level of NAA was elevated in sample ID *humtDNAKA002* on both sides of the amygdala while the other 2 remained the same (Fig 4 &5). NAA is an amino acid synthesized in the mitochondria of neurons and is a marker for neuronal integrity. It correlates with cognitive function. Dorsal anterior cingulate cortex (dACC) is a part of a functional network and has a role in reward based decision making. It is involved in both monitoring and control. Posterior cingulate cortex (PCC) is a part of the default mode network and helps in self-reflection and autobiographic memory.

3-1-2. mtDNA sequencing results

mtDNA isolated from dispersed cheek cells to perform genotyping by Polymerase chain reaction (PCR) to get 320bp product (**Figure.1**). Partial sequencing data was analysed. Sample *ID humtDNAKA002* for their nucleotide amino acid showed two point mutations in D-loop of mtDNA at 16068 G>A and 16623 T>C and translated amino acid show change of Aspartic acid to Glycine and Phenylalanine to Leucine (**Figures.2, 3**). SNPs were not seen two other mtDNA isolated from ASD patient.

Table 1: Patient ID number humtDNA001

Investigation		Result	Unit	Reference Range*
Plasma glucose	Random	91.0	mg/dl	
Liver function test	ALT	17.0	U/L	12-38
	AST	34.0	U/L	42-362 (male); 51-332(female)
Creatine Kinase *(*CK*)*		312.0	U/L	51-294 (male) ; 39-239(female)
Serum Amylase		81.0	U/L	28-96
Serum Lactate		16.7	mg/dl	4.5-19.8

*Children (5-15 years).

Table 2: Patient ID number humtDNAKA002

Investigation		Result	Unit	Reference Range*
Plasma glucose	Random	91.0	mg/dl	
Liver function test	ALT	20.0	U/L	12-38
	AST	35.0	U/L	42-362 (male); 51-32(female)
Creatine Kinase *(*CK*)*		63.0	U/L	51-294 (male) ;39-239(female))
Serum Amylase		91.0	U/L	28-96
Serum Lactate		21.2	mg/dl	4.5-19.8

*Children (5-15 years).

Table 3: Patient ID Number humtDNA003

Investigation		Result	Unit	Reference Range*
Plasma glucose	Random	102.0	mg/dl	
Liver function test	ALT	78.0	U/L	12-38
	AST	40.0	U/L	42-362 (male); 51-32(female)
Creatine Kinase *(*CK*)*		58.0	U/L	51-294 (male) ;39-239(female))
Serum Amylase		131.0	U/L	28-96
Serum Lactate		16.6	mg/dl	4.5-19.8

* Children (5-15 years).

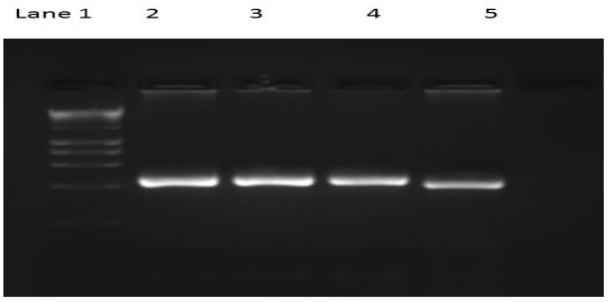

Fig.1: Gel Photo showing amplified MtDNA isolated from blood sample and cheek cells. Photo-: Gel Lane 1 Molecular weight marker (1kb) lane 2, 3, 4 &5 MtDNA isolated from ASD patients from cheek cells (320bp).

Fig.2: SNP were identified in D-loop at 16068 G>A and 16623 T>C. Nucleotide blast (NCBI analysis) (Query is *ID humtDNAKA002* (Genbank Accession no. KU534601) with Ref no ID: gb|KC990651.1 is subject|).

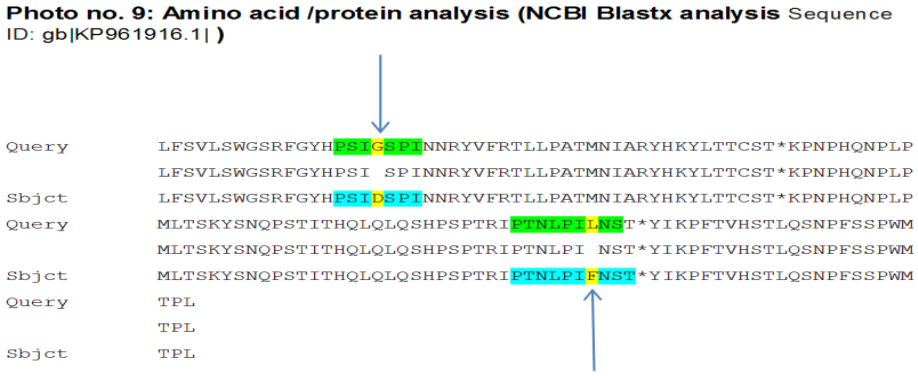

Fig.3: Translated Amino acid /protein analysis (NCBI Blastx) showing amino acid change of Aspartic acid to glycine and phenylalanine to leucine (Query is *ID humtDNAKA002* (Genbank Accession no. KU534601) with Ref no ID: gb|KC990651.1 is subject|).

4- DISCUSSION

In the present study we could see the biochemical tests performed indicated abnormal values of tests for lactate, CK, ALT, AST, serum amylase and random glucose (**Tables. 1-3**). Previous research study reported, different plasma lactate and/or pyruvate may be seen in a range of conditions other than primary MD, including spurious elevations due to poor collection or handling techniques, physiological elevation due to wide range of systemic disease or nutritional deficiency (12). This limits the value of this laboratory test in absence of other investigations (**Tables, 1-3**).

MRS of Patient ID *humtDNAKA002*, revealed increased concentration of NAA with normal lactate levels (**Table.4, and Figures. 4, 5**), whereas other two patients were showing normal NAA levels. Previous research reports has indicated that the elevated, decreased or absence of lactate (13), decreased NAA is not very specific for MD or even metabolic disease, because in other disorders there is possibility of altered NAA or lactate signal (14).

Similarly compound choline signal changes and there interpretations have not been well reported for patients with MD (15). An interpretation of biochemical and MRS is dependent on many other technical factors.

By genotyping in patient ID *humtDNAKA002* mtDNA (Genbank accession no.KU534601, two mutations at 16068 G>A and 16623 T>C (**Figures. 2, 3**) identified. Similarly many studies have also indicated presence of point mutations in mtDNA analysis of ASD patients indicating towards MD. Study reports has also indicated towards significant mutations with the ASD risk were reported are 16126 T> C (P=0.01), 14569 G>A (P=0.02) and 1811A>G (P=0.04). The mutation 16126T>C was in the mtDNA control regional (6).

Table 4: MRS results of all four parameters

Areas	Metabolite	PPM	Value
Right amygdala			
	NAA	2	612
	CR	3	495
	CHOLINE	3.5	396
Left amygdala			
	NAA	2	288
	CR	3	341
	CHOLINE	3.5	396
ACC(Anterior Cingulate Cortex)			
	NAA	2	44
	CR	3	37
	CHOLINE	3.5	33
PCC(Posterior Cingulate Cortex)			
	NAA	2	1944
	CR	3	825
	CHOLINE	3.5	891

PPM: Part per million; NAA: N-acetyl L- aspartate; CR: total Creatine; ACC: Anterior cingulate cortex; PCC: Posterior cingulate cortex.

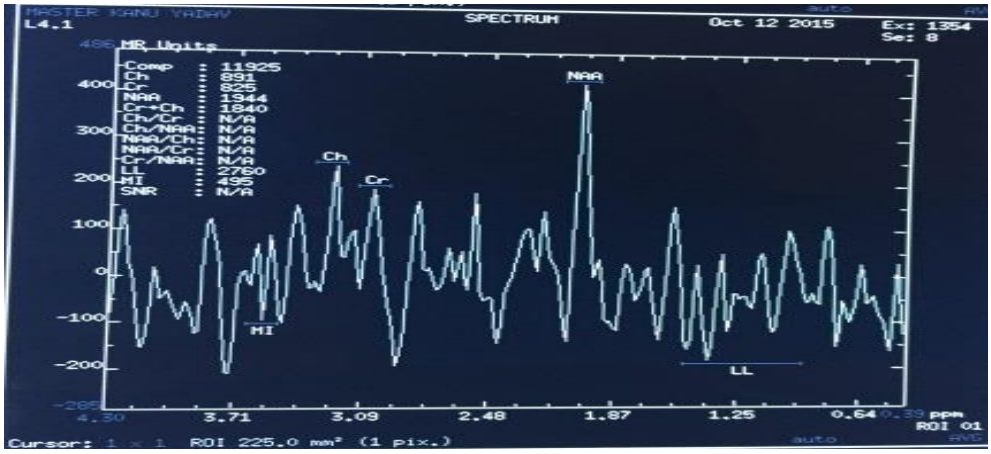

Fig.4: MRS spectrum image showing levels of the various metabolites at the ACC.

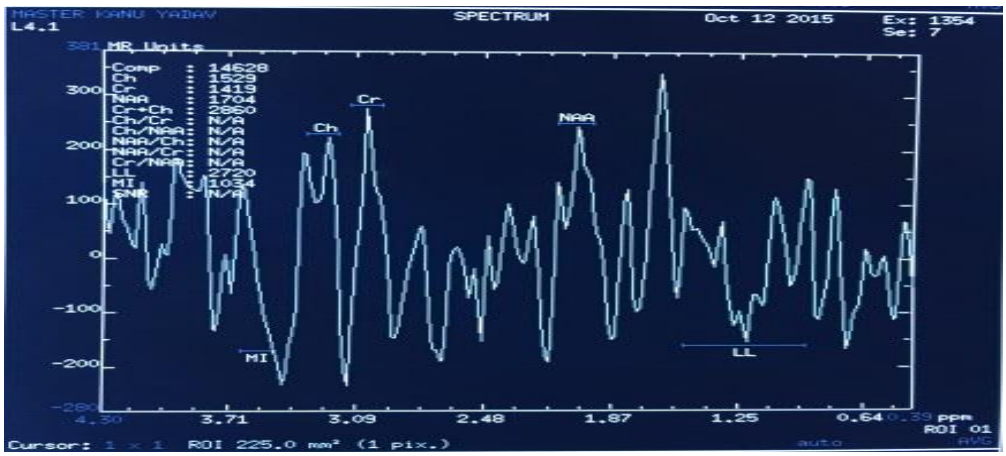

Fig.5: MRS Spectrum image showing the 4 parameters tested at the PCC.

5- CONCLUSION

Conducted pathogenetic testing has its own limitations and strengths. Since it is easy to perform, does not necessarily require blood sample or use of any sedative or aesthesia, it can be used as first diagnostic tool to review MD in ASD patients. The results of pathogenetic tests would provide basis of ASD screening of "Mitochondrial autism subgroup" in ASD patients in India for their better treatment and management rather than keeping them undiagnosed for MD.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENTS

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