Journal of American Science

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Multidisciplinary Academic Journal Publisher

Radiological and Laboratory Assessment of Children with Progressive Encephalopathy in Fayoum University Hospitals

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Abstract: Background: Progressive neurological disease in children poses an important challenge to health systems in terms of diagnosis and management. Progressive Encephalopathy is often used interchangeably with neurodegenerative encephalopathy. Both terms lack a firm definition, but PE is preferred because it encompasses clinically progressive conditions without demonstrable neuronal loss as well as those with a demonstrable loss of neural tissue. They are often detected by magnetic resonance imaging (MRI) examination. **Objectives:** The study aimed to determine the value of different radiological and laboratory studies as diagnostic tools for different etiologies of progressive encephalopathy (PE) and to investigate the relation between them. The study also aimed to illustrate the prevalence of different etiologies of PE among outpatient clinic attendants. **Methods**: Our study is a cross-sectional descriptive study. It included 79 patients aged between 3 months old up to 12 years old who sought medical advice at Neuropediatrics Clinic, Fayoum University Hospitals. They presented with progressive alteration of mental status with/without motor affection during a period of 18 months from December 2016 till June 2018. **Results:** Out of 79 cases showing manifestation of Progressive Encephalopathy: A-15 cases (19%) were diagnosed as Neurocutaneous diseases: 3 cases as SWS (3.8%), 6 cases as NF 1(7.6%) and 6 cases as TS (7.6%). B-Inherited metabolic diseases represented 55 cases (70%). fourteen cases were diagnosed as mitochondrial diseases (17.7%) and white matter diseases in 7 cases (8.9%) (1 case of Van der knapp, 2 cases of canavan disease, 2 cases of metachromatic leukodystrophy,1 case of krabbe and 1 case of adrenoleukodystrophy). Sixteen cases were diagnosed as storage diseases (20.2%): 3 cases as Wilson disease (3.7%),5 cases as Gaucher disease (6.4%), 4 cases as NP (5%) and 4 cases as Taysachhs disease (5%). Three cases were diagnosed as urea cycle disorders (3.7%). Five cases were diagnosed as Glutaricacidurea (6.4%) and 5 cases as PKU (6.4%). The study showed 2 cases of maple syrup (2.5%),1 case of propionic academia, 1 case of lysinuric protein intolerance and 1 case of Biotinidase enzyme deficiency (1.25% each). C- Epileptic syndromes were 4 cases (5%) (2 cases of Dravet syndrome and 2 cases of West syndrome). Five cases were undiagnosed and need molecular testing. The most common cause of PE was inherited metabolic diseases (70%) while mitochondrial diseases represented 17.7% of all cases. White matter diseases accounted for 8.9% of cases. Gaucher disease, Gluraticacidurea and PKU were present in 6.4% each. Neurocutaneous disorders accounted for 19%. The early age of presentation strokes alarms for Inherited Metabolic diseases as Neurometabolic and urea cycle diseases (17/18 cases of this group were diagnosed before 2 years of age and 7/18 cases were diagnosed during neonatal period), Mitochondial diseases (100% of cases during infancy), white matter diseases (5/7 cases diagnosed during infancy except for van der knapp and adrenoleukodystrophy. The late age of manifestations rise susceptibility of Neurocutaneous disorders and Wilson disease as well. Gaucher disease can be diagnosed at any age. **Conclusion**: Careful Examination is very important in all cases of PE. Imaging studies can be the next step to confirm diagnosis in case of neurocutaneous syndromes. Neurometabolic diseases can be diagnosed by laboratory and enzyme assay. Neonatal screening programs can spare time and effort. Molecular diagnosis can be a helpful diagnostic tool in case of doubtful or undiagnosed. MRS is helpful for diagnosis of mitochondrial diseases as well as Canavan disease. Epileptic syndromes can be diagnosed clinically together with EEG (west syndrome) and by molecular diagnosis. Undaignosed cases need molecular testing for diagnosis.

[Hadeer Mahmoud Gamal El Din Abdel Ghaffar, Eslam Rabie Abdel Aziz Abdel Bakky, Shahera Morsy El Shafaey, Ahmed Mohamed Magdy Mahmoud, Heba Ahmed El Awady. **Radiological and Laboratory Assessment of Children with Progressive Encephalopathy in Fayoum University Hospitals.** *J Am Sci* 2020;16(5):1-14]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 1. doi:10.7537/marsjas160520.01.

Keywords: Progressive Encephalopathy (PE), Inherited Metabolic Diseases, Magnetic Resonance Spectrometry (MRS)

1. Introduction

The term encephalopathy describes a diffuse brain disorder of the brain in which at least two of the following symptoms are present: (1) altered conscious state; (2) altered cognition or personality; (3) Seizures **[1].** Progressive neurological disease in children poses an important challenge to health systems in terms of diagnosis and management **[2].** Progressive Encephalopathy is often used interchangeably with neurodegenerative encephalopathy. Both terms lack a firm definition, but PE is preferred because it encompasses clinically progressive conditions without demonstrable neuronal loss as well as those with a demonstrable loss of neural tissue, most often detected by magnetic resonance imaging (MRI) examination. PE is predominantly caused by inborn errors of metabolism, hereafter called metabolic diseases. PE also contains a relatively large mixed group of neurodegenerative disorders without an identifiable metabolic deficiency, hereafter designated neurodegenerative disease. Infectious, inflammatory or toxic etiologies may also be considered as causes of PE (3). Epidemiological studies have most often focused on the cumulative incidence of known diagnoses associated with PE, particularly metabolic diseases. Cases with such diagnoses have either been discovered through screening programs for metabolic diseases or they have been registered after clinical presentation suggestive of metabolic deficiency **[4]**. The burden of disease may be heavily felt by patients and caregivers when the diagnosis remains unknown despite extensive investigation. It has been estimated that approximately one-third of all pediatric brain disorders without a known diagnosis, also called "anonymous" brain disorders, can be classified as PE **[5].**

In recent years, nonconventional techniques have been used to complement conventional MRI and overcome some of its limitations. Proton MR Spectroscopyhas been particularly useful in patients with metabolic disorders as it can simultaneously provide chemical-pathologic correlates of changes occurring within and outside visible MRI lesions. Thus, an expanding number of research groups have been using single-voxel and multi-voxel MR spectroscopic imaging in vivo to study patients with metabolic disorders. These H1-MRS techniques have demonstrated to increase diagnostic accuracy and the understanding of the evolution of pathology in many of these disorders. It must also be stressed, however, that H-MRS is complementary to MRI, except in a few cases where a disease-specific pattern could be detected **[6].**

The basic laboratory studies that should be obtained for an infant who has symptoms of a metabolic encephalopathy consistent with an inborn error of metabolism include: Complete blood count with differential, urinalysis, blood gases, serum electrolytes, blood glucose, plasma ammonia, urine reducing substances, urine ketones if acidosis or hypoglycemia present, plasma and urine amino acids, quantitative urine organic acids and plasma lactate **[7].** Tandem mass spectrometry (TMS/MS) is a powerful technology that allows a rapid and sensitive screening over more than thirty inborn errors of metabolism through measurement of amino acids and Acyl-Carnitine levels in a small quantity of blood **[8]**.

Aim of work: The study aims to:

*Determine the prevalence of different etiologies of progressive encephalopathy in Fayoum governorate.

* Determine the value of different radiological and laboratory studies as diagnostic tools for different etiologies of progressive encephalopathy and to investigate the relation between them.

2. Patients and methods

The current study is a cross-sectional descriptive study. It included 79 cases aged between 3 months old up to 12 years old out of 4320 patients who sought medical advice at neuropediatrics clinic, Fayoum University Hospitals, Egypt. The 79 cases presented with progressive alteration of mental status with/without motor affection during a period of 18 months from December 2016 till June 2018.

Inclusion criteria: infants and children

* > 3 months of age

* Progressive alteration of mental status with/without motor affection.

Exclusion criteria: All causes of stationary Encephalopathy:

Post-infectious (postmengitis-postencephalitis), postkernictures, post hypoxic- ischemic, poisoning, electric shock, post vaccination and Rabies.

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and after the approval of the local ethics committee. Informed consent was obtained from all study subjects after the nature of the study was explained.

All patients were subjected to the following: I. History taking: personal history including demographic data (age and sex), perinatal history, past history (previous neonatal ICU admissions), medication history (antiepileptic; types, duration of treatment and response), developmental history

including motor and mental development, family history including consanguinity, history of neurological problems, history of similar illness, disease history in family…. etc. II. Thorough clinical examination included: anthropometric measurements height, weight of the patients and head circumference was recorded, regional examination head and neck (dysmorphic features, eyes, pupils, ears, mouth, head shape, neck), extremities, skin, back and spine examination, local examination cardiac, chest, abdominal examination, neurological assessment to detect degree of spasticity and extrapyramidal movements; motor system examination muscle power assessment and reflexes and cranial nerve examination III. Investigations included.

Lab assessment:

- ✓ Serum Creatinine kinase levels in 14 cases
- ✓ Serum lactate level in 64 cases
- ✓ Plasma Ammonia level in 64 cases
- ✓ TMS/MS was done in 52 cases: A mass spectrometer is a device that ionizes molecules and separates the charged products, or ions according to their mass-tocharge ratios (m/z). An Acylcarnitine is a fatty acyl ester of L-carnitine. The family of acylcarnitines analyzed by TMS/MS includes fatty acyl groups for saturated and unsaturated species ranging from the 2-carbon acetylcarnitine (C2) through the 20-carbon aliphatic groups. Blood samples were spotted on Whatman filter paper cards (Schleicher and Schuell 903; Dassel, Germany) and sent to the screening lab for screening by TMS/MS.
- ✓ Organic acid profile in urine was done in 44 cases: Urinary acylcarnitine profiles by GCM S/MS were done for selected cases. Samples belonging to cases clinically suggestive of having organic acidemias, but showing normal TMS/MS profile as well as those with positive ESI-LC-MS/MS results were diagnosed and confirmed by gas chromatography mass spectrometry using the GCQ, Finnigan Mat, USA.

- **Electrophysiological studies:**

Nerve Conduction velocity (NCV) and Electromyography (EMG) was done for 5 cases suspected to be mitochondrial encphalo-myopathy.

- **Imaging studies:**

✓ MRI Brain was done for all patients: MR imaging was performed with a 1.5 Tesla magnet using Titan closed machine (Toshiba medical systems). Cases are imaged in the supine position using Head phased-array coil. Routine axial and coronal T1, T2, Flair weighted images in addition to a protocol of DW images. MRS was applied to all patients and recorded using TE short 80 mSec and TE long 180mSec.

MR Protocol

Sequence	TR (msec.)	TE (msec.)	FOV (mm)	Matrix	Slice thickness (mm)
T2 Sagittal	3000	90	290*290	208*205	4
T2 Axial	3700	100	288*350	292*180	5
T1 Axial	500	10	261*216	263*171	5
T2 Coronal	5000	90	300*300	272*200	4.5
DWI (b 0,500,1000,1500)	5000	77	240*240	124*100	5

Statistical Analysis

Data was collected, coded, translated to English to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 under windows 7.

Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test:

For quantitative parametric data:

In-depended **student t-Test** used to compare measures of two independent groups of quantitative data.

For qualitative data

Chi square test to compare two of more than two qualitative groups.

The level **P ≤ 0.05** was considered the cut-off value for significance.

Ethical Consideration

This study was reviewed by the Faculty of Medicine Research Ethical Committee. The researcher was informed the participants about the objectives of the study, examination, investigation that were done. Also, we explained the confidentiality of their information and their right not to participate in the study. Written consents were taken from all patients.

3. Results

The study included 79 cases aged between 3 months old up to 12 years old out of 4320 patients who sought medical advice at neuropediatrics clinic, Fayoum University Hospitals.

Demographic data of the study patients (N=79):

Males represented 45/79 (56.9%) while female were 44/79 (43.1%) with ratio of 1.3:1. Consanguinity was positive in (60/79) cases representing (76%) of all cases.

Distribution of the study cases among different groups:

The study cases were classified into 4 main groups depending on their clinical presentation as shown in (Table 1).

Table (1): classification of cases in the current study

Groups	Diagnosis	N	% From group	% from total
1-Neurocutaneous Syndromes (N=15/79)	Neurofibromatosis	6	40.0	**19%**
	Sturge Weber Syndrome	3	20.0	
	Tuberous Sclerosis	6	40.0	
2-Inherited Metabolic Diseases (IMD) (N=55/79)				**70%**
A-Neuromtabolic and urea cycle disorders (N=18/79)	**(Amino acid, Organic acid and Urea cycle Disorders)**			23%
	Bioitindase deficiency	1	5.6	
	Citrullinemia	1	5.6	
	Glutaricacidurea	5	27.8	
	Lysinuric protein intolerance	1	5.6	
	Maple syrup	2	11.1	
	Oroticacidurea (OTC deficiency)	2	11.1	
	PKU	5	27.9	
	Propionocacedima	1	5.6	
B-Mitochondrial Diseases (N=14/79)	Leigh disease	2	14.3	18%
	LHON disease	1	7.1	
	MELAS	1	7.1	
	Mitochondrial encephalopathy	8	57.1	
	Mitochondrial encephalomyopathy	2	14.3	
C-Storage disease (N=16/79)	Gaucher disease	5	31.25	20%
	Neiman Pick disease	4	25	
	Tay Sacchs disease	4	25	
	Wilson disease	3	18.75	
D-White matter diseases (N=7/79)	Adrenoleukodystophy	1	14.25	9%
	Canavan disease	2	28.6	
	Van der Knapp Disease	1	14.25	
	Krabbeleukodystrophy	1	14.25	
	Metachromatic leukodystrophy	2	28.6	
3-Epileptic syndromes (N=4/79)	Dravet syndrome	2	50.0	**5%**
	West syndrome	2	50.0	
4-Miscellaneous (N=5/79)	?? Vanishing white matter diseases	1	20.0	**6%**
	Undiagnosed cases	4	80.0	
Total		**79**		**100%**

The study cases (N=79) were classified into 4 main groups as mentioned in the abovementioned table: 55/79 cases belonged to Inhreited metabolic diseases group (70%), 15/79 cases belonged to Neurocutaneous disorders group (19%) and (4/79) cases were diagnosed as epileptic syndromes and (5/79) cases were undiagnosed cases. In this study, (55/79) cases were diagnosed with Inhreited metabolic diseases (70%). This group (N=55) was classified into the following subgroups: (18/55) cases were diagnosed with Neurometabolic and urea cycle disorders (33%), (14/55) cases were diagnosed as Mitochondrial diseases (25%), (16/55) cases were diagnosed as Storage diseases (29%) and (7/55) cases were diagnosed as White matter diseases (13%).

Pattern of clinical presentation in patients with progressive encephalopathy (table 2)

As suspected from the study, progressive mental deterioration was the most prominent clinical picture (100% of cases) with variable degrees. Motor delay was present in 68% of cases. Seizures were present in 56% of cases while extrapyramidal movements were present in 7.5% of cases.

Distribution of Seizures among different study groups:

Table 2: Clinical presentation of children with PE

Clinical presentation	N (%)
Low body weight (below 3rd percentile)	49(79%)
Abnormal head circumference	24 (30.4%)
DCL	40 (53.1%)
Mental delay	79 (100%)
Motor delay	54 (68%)
Seizures	44 (56%)
Extrapyramidal movements	6 (7.5%)
Hypertonia	36(45.6%)
Floppiness	9(11.3%)
Ocular findings	29(36.7%)
Skin manifestations	25 (31.6%)
Cardiac manifestations	5(6.3%)
Renal manifestations	2(2.5%)
Hepatic manifestations	11(13.9%)

Seizures were present in 44/79 cases (56%). Intractable seizures were present in 30/44 cases with seizures (68%). Among IMDs group (N=55), seizures were present in 24/55 cases (43.6%) and were intractable in (14/24) cases (58%). Seizures were common among mitochondrial diseases (N=14) and were present in (9/14 cases) (64% of Mitochondrial diseases group). Intractable seizures were noted in 6/9 cases representing more than 65% of mitochondrial patients (N=14). Among Storage diseases (N=16), seizures were present in (5/16) cases. Seizures were present in all 4 cases of Taysacchs disease. Seizures were present in (6/18) cases (33%) diagnosed with Neurometabolic and Urea cycle disorders (N=18).5/7 cases of white matter diseases (N=7) showed intractable seizures. As regards Epileptic syndromes, all 4/4 cases showed intractable seizures. Seizures were present in more than 60% of cases with Neurocutaneous syndromes. Tuberous sclerosis and Sturge Weber Syndrome were associated with more seizures (6 out of 6 cases are on antiepileptic medications) compared to Neurofibromatosis (only 2cases).

Ocular findings among different study groups (table 3):

In the current study, ocular assessment was performed in (50/79) cases and (18/50) cases showed normal results representing about 44% of cases performed the assessment. ERG and VEP were diagnostic for LHON disease.

Table 3: Abmormal Ocular Findings:

Abnormal ocular findings	N (%)
Cherry red spot	8 (10%)
Pale optic disc	6 (7.5%)
Nystagmus	15 (18.9%)
Squint	23 (29%)
Lisch Nodule	4 (5%)
Kayser Fleischer ring	2 (2.5%)

Radiological findings among study groups (table 4)

Regarding Neuroimaging value, MRI was more important than other methods of neuroimaging as it gave more detailed pictures. Positive findings were present in more than 55.6% of cases (44/79) and were normal in about 44% of cases. MRS was done as a complementary diagnostic tool in all cases with positive findings in 28/79 cases (34%).

Table (4): Characteristic MRI findings of study groups

Characteristic MRI findings	N (%)
Optic gliomas as hypointense masses on T2 in variable areas along optic pathway **(NF 1)**	3 (3.7 %)
Ependymal and cortical tubers **(TS)**	6 (11.3%)
Hypointese as Hemangiomas **(SW)**	2 (2.5%)
Megacephaly with subcortical cysts **(Van der knapp disease)**	1 (1.2%)
T2 white matter hyperintensity involving subarcute U fibers and internal capsule **(Canvan disease)**	2 (2.5)
Bilateral widened opercula (bat-wing configuration) **(Glutaricacidurea 1) as called wide and deep Sylvain fissure (temporal lobe atrophy)**	4 (5%)
T2 white matter hyperintensity sparing subcortical U fibers (butterfly pattern) *bilateral symmetrical and confluent) **(Meta chromatic leukodystrophy)**	2 (2.5%)
T2 posterior white matter hyperintensity (**Adrenoleukodystrophy)**	1 (1.2%)
T2 hyperintense periventricular areas with atrophy **(Krabbeleukodystrophy)**	1 (1.2%)
T2 hyperintensity involving medulla, midbrain, putamen, thalalmi and substansianigra **(Leigh Disease)**	2 (2.5%)
Extensive Diffusion Weighed restriction anteriorly with T2 Hypointensity **(Lysinuric protein intolerance +Citrullinemia)**	2 (2.5%)
Diffuse Brian atrophy	31(39.2%)

Concerning Neurocutaneous syndromes (N=15), 12/15 cases (80%) showed positive results and were diagnosed by MRI brain.6/6 cases of Tuberous Sclerosis (Ependymal and/or cortical tubers+/- atrophy) and 3/3 cases of Sturge.

Weber Syndrome (atrophy and /or calcification) were diagnosed by MRI brain.

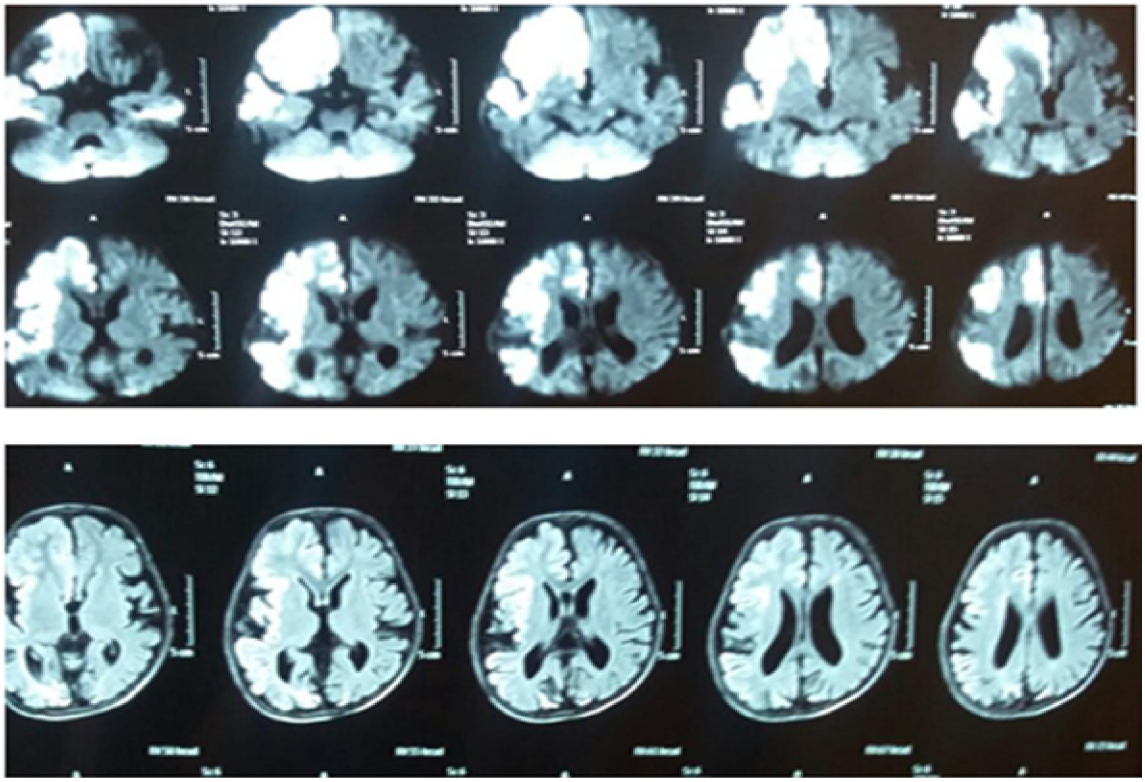

Figure 1: NF type 1 with recurrent TIAs

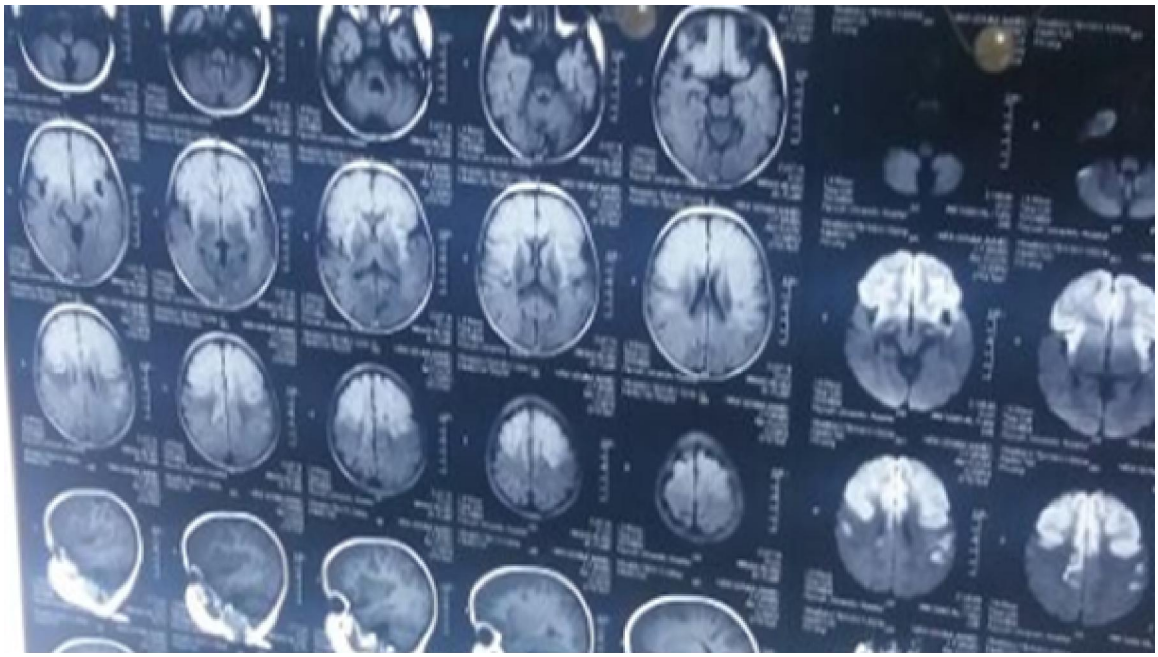

Figure 2: DW restriction and flair hyperintensity is noted due to cytotoxic edema in acute hyperammonemia crisis (a case of Citrullinemia)

1-DW images showing right frontal and temporal hyperintesity with acute ischemic attack

2- Flair images showing frontal and temporal hyperintensity with acute ischemic attack.

3/6cases diagnosed as tNF type 1 showed optic gliomas. one case diagnosed with NF I showed atrophic changes associated with recurrent (TIAs). MRS was of low value in diagnosis and showed low metabolites in one case of Tuberous sclerosis. Elevated lactate peak was present in one case of Neurofibromatosis with TIAs.

In the IMDs group (N=55), 28/55 cases showed positive results. About 60% of cases of Neurometabolic and urea cycle disorders (10/18 cases) showed positive MRI findings. 4cases of Glutaricacidurea showed significant and diagnostic data (wide and deep sylvian fissure (atrophy) with bat wing configuration).

One Citrullinemia case and one Lysinuric protein intolerance case showed positive significant findings (Frontal atrophy following DW restriction in the acute stage) as shown in the following figure.

PKU cases including atypical type showed atrophic changes in 3 cases (60%) which denoted the effect of poor compliance on the brain tissue. These 3 cases showed decreased all metabolites on MRS as well.

Only 1/16 cases of Storage disease group (N=16) showed MRI findings, one case of Gaucher disease (atrophy) as shown in the following figure.

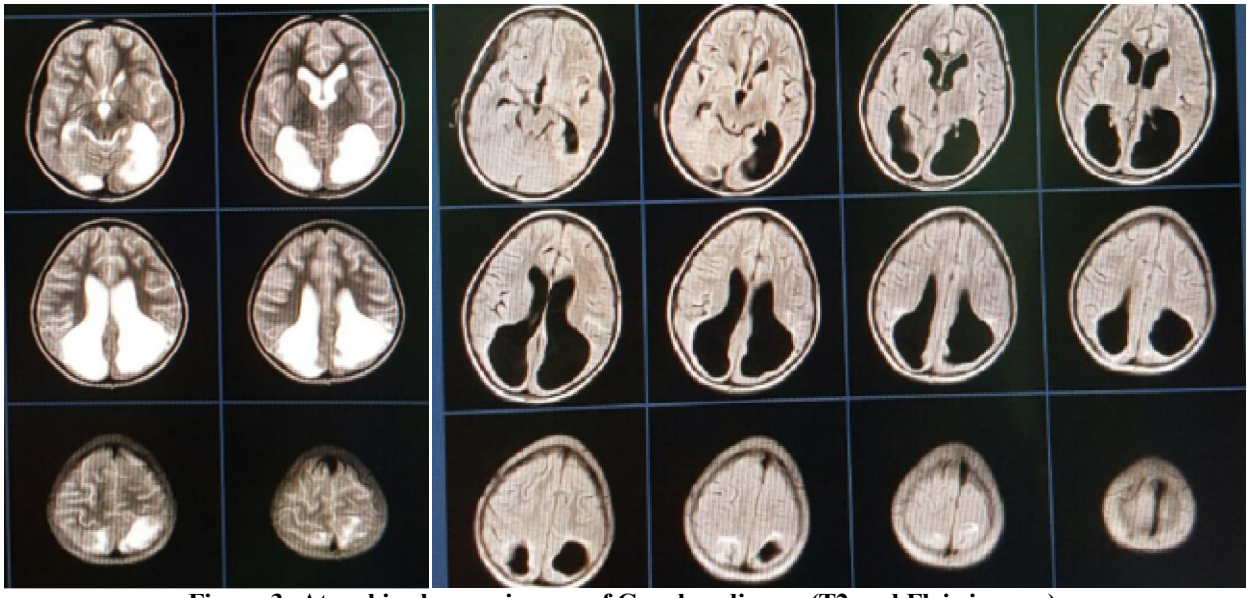

Figure 3: Atrophic changes in case of Gaucher disease (T2 and Flair images)

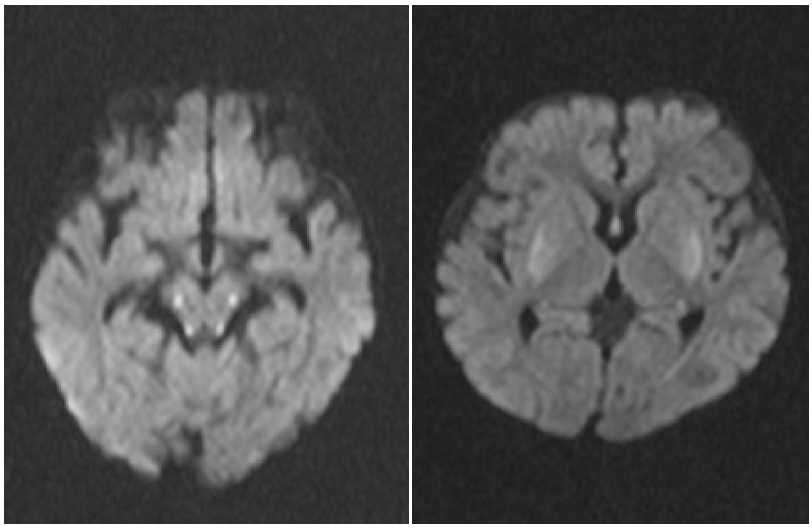

Fig 4: DW images of Leigh disease

Regarding Mitochondrial disorders (N=14), 10/14 cases showed prominent ventricular and extra axial spaces (Brain atrophy). 2 /14 cases showed MRI brain findings. Elevated Lactate peak was characteristic in 9/14 cases.

MRS findings were also of high importance in diagnosis of Canavan Disease (Elevated NAA).

Metachromatic leukodystrophy as well as drenoleukodystrophy showed high lactate and low metabolites.

In case of white matter diseases, MRI showed high grade of importance. 100%of cases showed diagnostic findings.

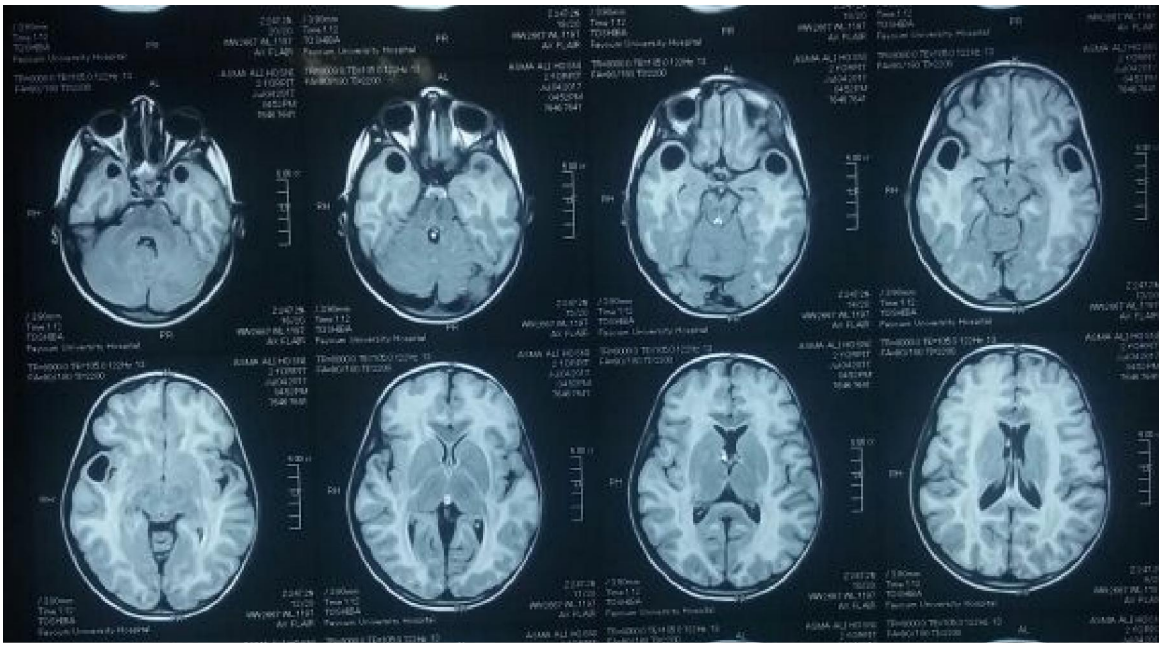

Fig 5: Flair images of a case of Van der Knapp disease (Megancephaly with subcortical cysts)

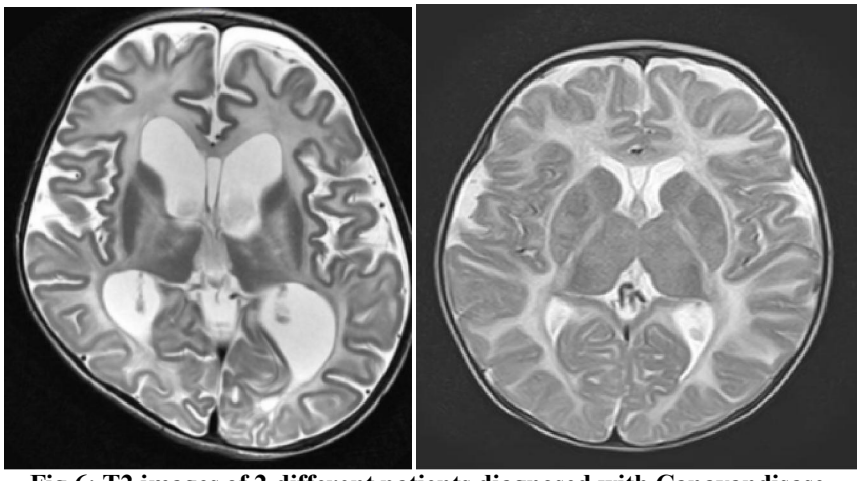

Fig 6: T2 images of 2 different patients diagnosed with Canavandisase

Laboratory findings among different study groups

As regards Serum Lactate, High levels were noted in 78% of mitochondrial diseases (11/14 cases) representing 20% of all cases performed the test (11/79). Serum Ammonia levels were elevated in 5 cases (Lysinuric protein intolerance, OTC deficiency (2 cases), Citrullinemia (1 cases) and Propionic academia (1 case)). S.CK showed high levels in 6 mitochondrial cases. Concerning Aminogram and Acylcarnitine profile, (53/79) cases performed

TMS\MS. Concerning Neurometabolic and Urea cycle disorders group, 11/18 cases were diagnosed by TMS/MS. 3/18 cases showed normal results but were diagnosed by OA profile in urine (Lysinuric protein intolerance (1 case), OTC deficiency (2 cases)).3/18 cases were diagnosed by neonatal screening programs (1 case of GA and 2 cases of PKU).1/18 cases was diagnosed by specific enzyme assay (Biotinidase enzyme deficiency). Concerning Epileptic syndromes, only one case showed false positive results (misdiagnosed as methymelonic academia but by molecular testing was confirmed as Sodium channelopathy 1(SCN1)). Organic acid profile in urine was performed in (44/79) cases. (12/44) cases showed positive results and was confirmatory for diagnosis representing 27% of cases performed the test. The 3 cases showed normal results by TMS/MS and were diagnosed by specific OA profile in urine included: Orotic acid profile in urine was positive in 2 cases showed normal results by TMS/MS. COLA in urine was positive in one case showed normal TMS/MS.

N.B. OA profile in urine should be complementary to TMS/MS especially in cases of hyperammonemia.

Table 4: Neurometabolic and Urea cycle disorders and different methods of diagnosis

Disease	Method of diagnosis	No. of cases
PKU	Neonatal Screening Prog.	2
PKU	TMS/MS	3
Glutaricacidurea	Neonatal Screening Prog. (KSA)	1
Glutaricacidurea	TMS/MS	4
Lysinuric protein intolerance	COLA in urine Cystiene-Ornithine-Lysine-Arginine	1
OTC deficiency	Orotic acid in urine	2
Citrullinemia	TMS/MS	1
Biotinidase deficiency	Enzyme Assay	1

Specific enzyme assays were done on need and dependent on suggestion of clinician.19/79 cases were diagnosed by specific enzyme assays as shown in table 5-18. High grade of experience is needed to avoid heavy expenses on parents of the patient. Specific enzyme assays were done in a total of 21/79 cases and false positive results were present in 1 case of Gaucher disease. All 4 cases of white matter diseases and all 4 cases of Taysach disease were diagnosed by specific enzyme assays. All 5 cases of Gaucher disease were diagnosed by enzyme assay and confirmed by molecular testing. one Gaucher case was misdiagnosed by enzyme assay and confirmed by molecular testing as Neimann Pick type C. 2 cases of Neiman pick type B were diagnosed by enzyme assay and 2 cases by Molecular testing.

Table 5: Different enzyme assays for diagnosis of study groups

Disease and no. of cases	Confirmatory enzyme assay	Other complementary tests
Biotinidase enzyme deficiency (1 case)	Biotinidase enzyme assay	--
Wilson disease (3 cases)	-----	S. Ceruloplasmin and 24 hour urinary cooper
Gaucher Disease (5 cases)	Glucocerebrosidase enzyme assay	Chitotriosidase enzyme assay
Neimann pick type B (2 cases)	Sphingomylinase enzyme assay	Chitotriosidase enzyme assay
Neimann pick type C (2 cases)	Normal enzyme assay	Molecular diagnosis (2 cases)
Taysacch disease (4 cases)	Hexosaminidinase A enzyme assay	Hexosaminidinase B enzyme assay
Adrenoleukodystrophy (1 case)	VLCFA	----
Metachromatic leukodystrophy (2 cases)	Arylsulfatase enzyme assay	---
Krabbeleukodystrophy (1 case)	Galactosylceramidase enzyme assay	-----

Table 6: Different gene mutations among different study groups

Disease	Gene mutations
Gaucher Disease (4 cases)	GBA gene mutation,D448H Homozygous
Gaucher disease (1 case)	GBA gene mutation,Leu483 Pro Heterozygous
Neimann Pick type C 1 (2 cases)	NPC1 gene mutation 607623 Homozygous
Dravet Syndrome (1 case)	SCN1 related disorder, 607208,604403 Heterozygous
Mitochondrial Myopathy (1 case)	MTTE gene mutation,590025 Homozygos

Electrophysiological studies among different groups:

In our study, EEG study was done in (76/79) cases. EEG findings showed normal results in (45/76) cases performed EEG. EEG study showed abnormal findings in (31/76) cases representing (40.1%). Epileptic syndromes showed positive findings as hypsarrythmia in (2/4) cases while (1/2) dravet cases showed Epileptogenic EEG. Encephalopathic findings on EEG were present in (9/76) cases. (8/9) cases with encephalopathy was diagnosed with Inhreited metabolic diseases. Concerning EMG, it was done in suspicious mitochondrial cases as a method for confirmation of diagnosis especially with elevated S.CK and S. Lactate. Three cases showed myopathic findings on EMG and all of them were mitochondrial diseases. Neuropthic potentials on EMG were present in 1 case of metachromatic Leukodystrophy and 1 case of adrenoleukodystrophy.

Table 7. Different diagnostic tools

	Clinical		**Laboratory (S. Lactate, TMS/MS, OAI, S. Ammonia and S. CK)**		**Imaging**		**Molecular**		**Enzymes assay**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
1-Neurocutaneous syndromes	**15**	**100**	**0**	**0.0**	**12**	**80.0**	**0**	**0.0**	**0**	**0.0**
2-Inhreited metabolic Diseases	**1**	**1.8**	**21**	**38.2**	**31**	**56.4**	**8**	**14.5**	**18**	**31.7**
A-Organic	0	0.0	13	72.2	12	66.7	0	0.0	1	5.6
B-Mitochondrial	1	7.1	8	57.1	12	85.7	1	7.1	0	0.0
C-Storage	0	0.0	0	0.0	3	15.8	7	36.8	13	68.4
D-White matter diseases	0	0.0	0	0.0	4	100.0	0	0.0	4	100.0
3-Epileptic syndromes	3	75	0	0.0	0	0.0	1	25.0	0	0.0
4-Miscellaneous	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	19	24	21	26.6	43	54.4	9	11.4	18	22.8
P-value	**<0.0001 (S)**		**0.034 (S)**		**0.001 (S)**		**0.237 (NS)**		**0.006 (S)**	

N.B. P- Value can be deceiving especially with a small field of study; being a very expensive tool of diagnosis in a developing country, Molecular diagnosis was not done routinely and was done in only 9 cases mostly Gaucher disease and discovered 1 misinterpetation showing the importance of such test and the importance in undiagnosed 5 cases as well.

4. Discussion

In the current study, the cases were classified into 2 main age groups below 2 years of age (65/79 cases =82% of cases) and above 2 years of age (14/79 cases=18% cases). In a Norway population based study (2007) (2), the age of onset was defined as neonatal (0–4 weeks) (32% of cases), infantile (1–12months) (40% of cases), late infantile (1–5 years) (24% of cases) and juvenile (6–12 years) (4% of cases). While the present study extended to a period of 18 months, the Norway study (2) extended to a period of 18 years. Another Egyptian study conducted in Cairo University children hospital (CUCH) (2009) (9) by Selim et al included 800 patients showing manifestations of progressive encephalopathy during a period 9 months. Onset was in the first month of life in 1/19 patient (5.2%), within first year in 12/19(63.1%) and second year in 4/ 19(21%).

In the current study, positive consanguinity was in 76% of all cases. In a Bangladesh study by Mahbub,

et al (2015) (7), positive consanguinity was in 31% of all cases. In an Iranian study by Karimzadeh (2015) (10), 71.4% of all cases showed positive consanguinity. In another Egyptian study by Selim et al (2009) (9), positive consanguinity was present in 40% of all cases.

In this study, 79 cases were diagnosed with progressive encephalopathy and the prevalence rate was calculated in relation to outpatient clinic attendants (N=4320) during a period of 18 months to be 18 patients per 1000 outpatient case seeking medical advice at Neuropediatrics clinic, Fayoum University Hospitals. A Norway population based study (2007) (2) showed 84 PE cases representing 28 diagnoses among 1,305,997 person years, giving an incidence rate of 6.43 per 100,000 persons.

In the present study, Neurocutaneous diseases accounted for 19% of all 79 cases. Inherited metabolic diseases represented 70% of all cases (14% Mitochondrial-8.75% white matter diseases-5%Tay sacchs disease- 5% Neimann pick-6.5% Gaucher-3.5% Wilson- 6.5%PKU-6.5%Glutaric acidurea-2.5%Maple syrup disease-1.25% propionic acideima-3.5%Urea Cycle disorders-1.25%Lysinuric protein intolerance-1.25% Biotinidase enzyme deficiency). In another Egyptian study by Selim et al (2009) (9), 90 patients out of 800 cases were diagnosed as organic acidemias (1/42): three cases were diagnosed as MMA, three cases as Ketothiolase deficiency (BKT),2 cases as 3-methylcrtonylglycinuria (MCG), 3cases as biotinidase deficiency,2cases as Canavan disease and for each of the following one case: GA type 1, IVA, PPA, ASA. An Iranian study by Karimzadeh et al (2016) (11) showed Overall, 213 patients with 34 different neurometabolic disorders were diagnosed and classified in the 7 sub classes: 1- organic acidemia and aminoacidopathy (122 patients), 2-storage disease (37 patients) 3- leukodystrophy (27 patients), other classes consisted: lipid oxidation disorders, urea cycle disorders, progressive myoclonic epilepsy; and peroxisomal disorders (27 patients).

Regarding Clinical manifestation, in the current study, mental delay was present in 100%of cases, motor delay was present in 68.3% of all cases, seizures were present in 56% of cases (44/79 cases), hypertonia was present in 36 cases (45.6%), hypotonia was present in 9 cases (11.3%) while. while Extrapyramidal movements were present in 7.5% of all cases (6 cases) (Dystonia was present in 3 cases). Ataxia was present in one case. In another Egyptian study by Selim et al (2009 (9) Developmental delay was present in 84% of cases, seizures were present in 42% of cases, ataxia in 2 cases, extrapyramidal movements in 2 cases and abnormal muscle tone in 57.8% of cases. In a Bangladesh study by Mahbub et al (2015) (7), the common presentations were seizures (75%) and delayed milestones (51%). Karim Zadeh, et al (2016) (11) mentioned, out of 213 patients diagnosed with neurometabolic disorders, (87%) of patients had developmental delay (or/and) regression and (55.5%) of them had different type of seizures.

In the current study, (44/79) cases (56% of cases) showed variable degrees of seizures and 68% of them showed intractable seizures difficult to treat (6 /15 cases of Neurocutaneous group, 3/4 PKU cases, 6 /14 cases of mitochondrial diseases, 4/4 cases of epileptic syndromes, 3/4 cases of Taysacchs, 4/7cases white matter diseases, 3 un diagnosed cases, 1 maple syrup case and 1propinic academia case). GTC seizures were present in 70% of cases (33cases). In a Bangladesh study by Mahbub et al (2015) (7), Seizures were present in 96 (75%) children and abnormal EEG findings recorded in 83 (65%). An Iranian study by Karimzadeh et al (2016) (11) showed a total of 55.5% of patients had seizures and intractable seizures were present in 20.8% of all cases. The most common type was tonic and then GTC. Abnormal EEG was present in 38% of cases.

In the current study, EEG was positive in 45% of 76 cases (20.2% showed epileptogenic activity while 11.5% showed encphalopathic findings and brain insult was present in 6.3% of cases). In an Iranian study published by Narjas et al (2018) (12), EEG findings were positive in 23% of cases. In another Iranian study by Karimzadeh et al (2016) (11), 20.8% of cases showed abnormal EEG results. In a Bangladesh study (2015) by Mahbub et al (7), a total of 128 patients showed positive EEG findings in 60% of cases. 28% of cases showed encephalopathy picture while 35% showed epileptogenic activity.

In the present study, abnormal head circumference was present in 24 cases (30.4% of all cases) with 13cases showed microcephaly (16.4%) mainly PKU and Mitochondrial diseases while 11 cases (13.9%) showed macrocephaly mainly Taysacch disease, Canavan and glutaricacidurea disease (4/5 glutaric cases). In another Egyptian study by Selim et al (2009) (9), 26.3% showed microcephaly while 15.8 % of cases showed macrocephaly. In An Iranian study Published by Karimzadeh et al (2016) (11), a total of 16% of patients were micro cephalic and 12.7% were macro cephalic.

In the current study, out of 79 cases, 50 cases performed ocular assessment and 55% of them showed abnormal findings (N=28/50): 40% of cases with neurocutaneous disorders (Lisch nodules in 4 cases of NF1); 22% of cases with amino acid disorders, 57.2% of mitochondrial cases (squint in all cases and pale optic disc as well as LHON disease) and, 50% of storage diseases (Kayser Fischer ring for Wilson as well as cherry red spot for NP type B and Taysacchs disease) and 28.5% of white matter diseases (cherry

red spot for metachromatic leukodystrophy). In an Iranian study, Narjas et al (2018) (12) mentioned that ophthalmological abnormalities were observed in 33.5% of patients in a study performed over a period of 10 years and involving 213patients with a distribution of 17.2% of cases with amino acidopathy; 78.4% of cases with storage disorders; 33.3% of cases with urea cycle disorders; 55.6% of cases with paroxysmal disorders; 9% of cases with fatty acid oxidation defects; 44.4% of cases with leukodystrophy; and 25% of cases with progressive myoclonic epilepsy. In another Iranian study by Karimzadeh et al (2016) (11), ophthalmological abnormalities were present in 32.9% of cases.

In the current study, Diagnosis was dependent on clinical criteria leading towards the accurate diagnosis; being in a low socioeconomic country, this way of diagnosis was obligatory rather than a choice. In a Bangladesh study (2015), there was no standard battery of laboratory tests; thus, experience and multidisciplinary teamwork were of the outmost importance. In a Norway study (2007) (2) as well as a German one (2000), There was a trend towards identifying an exact molecular cause in patients with known diseases. In the current study, Serum Ammonia was performed in 64 cases and showed high results in 5 cases (7.9%) (Highly specific) if performed in a right situation while Serum Lactate was performed in 64 cases and showed high results in 17 cases (26.5%), in (78%) of mitochondrial diseases and in (90%) of high lactate peak on MRS. Serum C.K. was performed in suspected mitochondrial cases and positive in 3cases of Myopathic EMG findings as well. In the current study, TMS\MS was performed in 53 cases and 11 cases were accurately diagnosed. One case of Dravet syndrome showed false positive results. 3 cases performed the test (TMS/MS) and showed negative results (OTC deficiency and Lysinuric protein intolerance). Special urinary assessment was helpful and complementary to TMS\MS as to diagnose these 3 undiagnosed cases (Orotic acid in urine and COLA (Cysteine-Ornithine-Lysine and Arginine) in urine. Neonatal screening program for PKU was added in 2015 in Egypt and was of great value for early detection and proper management. Neonatal screening programs can be of great benefit for minimizing hazards and bad sequences of late diagnosis and management. Specific enzyme assays were essential for diagnosis of storage diseases, biotinidase enzyme deficiency and others not detected by TMS\MS or OA profile in urine. Molecular Diagnosis still is the most diagnostic test but still expensive and cannot be done on routine basis. In a Bangladesh study by Mahbub et al (2015) (7), Plasma ammonia was done in 98 cases and found to be increased in 53 (54%) cases. Plasma lactate was done in 94 cases and found high in 40 (43%). TMS were done in 111 (85%) children and abnormality was found in 70 (63%) cases. Considering on the nature of abnormal values, biotinidase deficiency was suggested for 41 (59%) and urea cycle disorder for 9 (13%) cases. Another Egyptian study by Selim et al (2009) (9) shows among 800 Outpatient cases presenting to the neurometabolic clinic, 24 cases with IEM were detected (1/ 23; 4.3%) 19 cases with amino acid disorders (1/28) and 5 cases with organic acidemias (1/110). In a Syrian study (2015) (14) conducted by Shennar et al, Out of 134 suspected OA patients, 70 patients (52.2%) underwent advanced diagnostic biochemical metabolic investigations and were diagnosed with one of the organic acids disorders, 54 cases (77.1%) were confirmed by urine organic acid analysis, and 16 cases (22.9%) were confirmed by acylcarnitine profile tandem mass spectrometry. No advanced biochemical metabolic investigations were performed in the remaining 64 patients (47.6%) to differentiate between organic acidemias and other causes of clinical manifestations as non-specific laboratory results indicated a possible metabolic disorder. Among these cases, seven were considered and treated as organic acidemias patients according to their clinical manifestation and known family history of confirmed OA cases in siblings and/or relatives, without performing any specific confirmatory tests. A delay in diagnosis similar to that reported in Brazil (13) and Egypt (14) was noted in the Syrian study (14), which might be attributed to the lack of newborn screening programs in Syria. High rates of parental consanguinity and positive family history among OA patients were reported in the Syrian (14) and in the Egyptian studies as well. In an Iranian study by Karimzadeh et al (2016) (11), Plasma ammonia was elevated in 13.5% of cases while serum Lactate was elevated in 18.3%.

In the present study, Brian MRI and complementary MRS were done in all 79 cases. Normal MRI findings were present in 44.3% of cases (35 cases). MRI brain showed atrophy in 39% of cases while positive MRS findings were present in 38% of cases roughly. White matter involvement was present in 7.5% of cases. High signal intensity on T2 images was present in 13.7% of all cases. Basal ganglia involvement was present in 6% of cases. While 86 % of cases showed positive findings in Neurocutaneous disorders, 50% of Inherited Metabolic Disorders showed positive findings. Clinically, MRI was helpful in diagnosis of Glutaricacidurea (75%), white matter diseases (100%). MRS showed diagnostic criteria of high lactate peak for mitochondrial diseases in more than (70%) of mitochondrial diseases and was diagnostic for Canavan disease. Normal findings were present in 67% of cases. In another Egyptian study (2009) (9), Brain MRI was performed in 15/ 19

patients with OA. It showed brain atrophic changes in 16 patients (40%) more prominent in patients with GA type 1and extensive white matter demylinations in 2 cases of Canavan Disease. In an Iranian study by Karimzadeh et al (2016) (11), Brain imaging in 36% of patients was normal; 20.4% of patients had brain atrophy; 25.1% had white matter involvement; 4.3% had basal ganglia involvement; 2.8% had basal ganglia and white matter involvement; 2.4% had basal ganglia involvement and brain atrophy. Brain atrophy with white matter involvement was seen in 5.7% of patients. In the Iranian study, MRS was not done in all cases and positive findings were present in 11% of cases done the test.

In this study, Confirmation of Gaucher disease diagnosis after enzyme assay was done; 1 case was falsely diagnosed as Gaucher disease at first and Neiman Pick type C was diagnosed. In a study published by M. Lo et al (2010), there have been experimental data that glucocerebrosidase (GCase) is also reduced in NPC, and that the glycolipid that accumulates in GD (glucosylceramide) also accumulates in NPC.

In the current study, the delayed diagnosis for most of our patients was associated with many severe and irreversible complications. This indicates the absolute necessity for early detection and early intervention for favorable outcomes in such disorders. Global developmental delay, unexplained neurological abnormalities and disturbed conscious level together with abnormal clinical, laboratory and radiological findings should strike the alarm for neurodegenerative diseases especially IMDs.

Limitations of the study:

The time period was not long enough which led to some negative findings and molecular testing was expensive for many patients' especially undiagnosed cases.

Conclusion:

The study offers a good description for many causes of Progressive encephalopathy and strikes alarms for paying attention towards these diseases as they are not as rare as suspected with prevalence rate of 18/1000 cases seeking medical advice at Neuropediatrics clinic, Fayoum University hospitals. A Fact can be attributed to positive consanguinity in a developing country. So, cheap diagnostic tools should be searched for. The most common cause of PE was Inherited metabolic diseases (70%) followed by Neurocutaneous diseases (19%) then Epileptic syndromes (5%). The Neurometabolic and urea cycle disorders were in the first place (22.7%) due to large number of PKU cases detected by the Egyptian national screening program. The storage diseases were in the second place (20.2%) due to high prevelance of Gaucher and Neiman Pick diseases in Egypt and Mitochondrial diseases were in the third place (18%).

Neonatal screening programs are important diagnostic tools as they spare time, effort and can prevent neurodegenerative sequel. Basic and cheap laboratory tests as S. Lactate, S. Ammonia and S. CK can be helpful especially in cases of low socioeconomic states. Brain imaging as MRI and Complementary MRS can be very helpful as a next step. Advanced Laboratory Testing should be asked after interpretation of all of the above to spare high expenses.

Molecular testing is an important tool for diagnosis especially in doubtful cases as well as cases of more than one suspicion.

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4/2/2020