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CLINICO-RADIOLOGICAL FINDINGS AND GENETIC ANALYSIS OF CHILDREN WITH HEREDITARY NEUROLOGICAL DISORDERS

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ABSTRACT

Objective: To determine the clinical profile, radiological findings and genetic analysis of children with inherited neurological disorders.

Study Design: Cross sectional study.

Duration and Place of Study: Department of Neurology, The Children's Hospital Lahore, from March 2013 to September 2016.

Material and Methods: Ninety two patients upto 20 years of age were selected. Blood sample was taken from patients and both parents. Samples were tested for whole exome sequencing

Results: There were 51% males. Age ranged from 5 months to 20 years. A total of 55.4% patients had developmental delay and 44.5% had neuroregression. Hearing impairment was found in 7.6% patients while vision was affected in 11.9%. Speech was affected in 66.3% patients while 43.4% patients had seizures. Autistic spectrum of disorders were observed in 20.6% patients. Microcephaly was seen in 19.5% cases. Out of 60 families, 23 (38.3%) had more than one offspring affected. Upper motor neuron signs were present in 50% patients while 15.2% patients had lower motor neuron signs. Leukodystrophy was seen in 19.5% patients. Cerebellar atrophy was observed in 15.2% patients while 17.3% patients had cerebral atrophy. Neuroimaging was normal in 34 patients. On genetic analysis, 48.3% families had known gene mutation while 51.6% families had novel gene mutation.

Conclusion: Degenerative brain disorder (DBD) and Global developmental delay/intellectual disability (GDD/ID) are entities with diverse clinical presentations. Whole exome sequencing is a helpful diagnostic tool. In addition to previously discovered genes, many novel genes have been identified.

Keywords: Consanguinity, Intellectual disability, Neuroimaging, Seizures, Whole Exome Sequencing.

INTRODUCTION

Herditary neurological disordes (HND) are a group of disorders which have a genetic origin and follow Mendelian inheritance pattern which later on affects the nervous system[1]. The overall incidence of hereditary disorders is about 2 to 5% of live births while majority of these hereditary disorders have neurological dysfunction[2,3].

Most of the HND have autosomal recessive inheritance pattern. Consanguinity among the parents is the main etiological factor for most of these[4]. Worldwide 20-25% of all the marriages are cousin marriages[5] while in pakistan consangunity is more prevalent which is about 80% of all the marraiges[6].

Hereditary neurological disorders include global developmental delay (GDD) or intellectual disability (ID), degenerative brain disorders (DBD), movement disorders, epileptic encephalopathies, neuropathies, myopathies, structural brain malformations and some forms of epilepsy. GDD/ID and DBD account for the majority of the cases of HND.

GDD is defined as when a child is significantly delayed in two or more domains of development i.e. gross motor, fine motor, speech, cognition, social and daily life activities[7]. This term is usually used for children <5 years of age. For children older than 5 years we use the term mental retardation or intellectual disability (ID). ID is defined as marked defficiency of a person in IQ and adaptive behaviours[8].

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DBD are those disorders in which a child gradually loses all previously acheived developmental milestones after reaching a certain age. They are further classified into grey matter degeneration, white matter degeneration, basal ganglia degeneration and spinocerebellar degeneration[9].

The diagnostic tools for these HND are limited. Clinical data, laboratory workup and neuroimaging have many shortcommings and pose major diagnostic challenge for clinicians[10].

The tremendous advancement in the field of molecular genetics has uncovered many mysteries regarding most of these HND and now it has become gold standard for most of these HND[11,12]. Our group recently adopted a 'whole exome' sequencing approach to reach genetic diagnosis in HND cases, in order to advance knowledge and identify new genetic causes of disease. Also no comparable data is available in Pakistan.

The objective of this study is to see the various clinical features and neuroimaging findings in patients with inherited neurodegeneration or developmental delay and their genetic analysis.

MATERIAL AND METHODS

This cross sectional study was conducted in the Department of Neurology, The Children's Hospital, Lahore from March 2013 to September 2016. Study was approved from ethical review committee of the institute. Sample size was calculated with the help of WHO sample size calculator. It was 90 by keeping prevalance at 18.3%[13], precision at 8% and confidence level of 95%. We selected ninety two patients of consaguinous parents having age upto 20 years with clinically defined DBD or GDD/ID by random sampling. While children having non-consanguinous parents or acquired causes for neurological illness like history of birth asphyxia, meningoencephalitis, head injury or TORCH infections were not included. Informed written consent was taken from parents. Detailed history, thorough clinical examination and pedigree analysis was done. Eye examination and hearing assessment was carried out. Neuroimaging (CT/MRI) was done. All the clinical data was entered into a pre-designed proforma. Photographs and videos of affected members were taken to document key findings including key aspects of the neurological examination such as gait and coordination. A 10 ml venous blood sample was taken and labeled. It was used for whole exome sequencing from one or two affected members per family for multiplex families, or from trios for simplex families. Skin biopsy samples were taken in selected patients for detailed functional work on cultured skin fibroblasts. These samples were analyzed at Laboratory for neurogenetics, University of California, San Diego USA. The data were analyzed and frequencies of different variables were calculated (table-I). Genetic analysis data were matched with OMIM and novel genes were also identified (table-II, III).

RESULTS

During this period, a total 92 patients from 60 different families were enrolled. There were 47 (51%) males and 45 (49%) females. Age of these patients ranges from 6 months to 20 years with mean age of 78.6 months. Out of these 92 patients, 51 (55.4%) patients had GDD/ID and 41 (44.6%) were having neuroregression.

Hearing impairment was found in 7 (7.6%) patients while vision was affected in 11 (11.9%) patients. Speech was affected in 61 (66.3%) patients whereas 40 (43.4%) patients had a history of seizures. Autistic spectrum of disorders (ASD) were observed in 19 (20.6%) patients. Microcephaly was seen in 18 (19.5%) cases. Out of 60 families, 23 (38.3%) had more than one offspring affected. Upper motor neuron (UMN) signs were present in 46 (50%) patients while 14 (15.2%) patients had lower motor neuron (LMN) signs.

On neuroimaging, 34 (36.9%) patients had normal study. Leukodystrophy was seen in 18 (19.5%) patients. Cerebellar atrophy was obsereved in 14 (15.2%) patients while 16 (17.3%) patients had cerebral atrophy. While there was

one case each of lissencephaly, basal ganglia calcification and agenesis of corpus callosum. A total of 10 (10.8%) patients expired during the study.

On further genetic analysis, 29 (48.3%) families had known gene mutation while 31 (51.6%) families had novel gene mutation. The separate results of DBD and GDD/ID are shown in table-I. Table-II shows the different known genes for DBD and GDD/ID as well as the disease caused by these genes. The identified novel genes in both categories are shown in table-III.

Table-I: Results of Global Developmental Delay (GDD) / Intellectual Disability (ID) and Degenerative Brain Disorders (DBD) Groups Independently.

	GDD/ID	DBD
Total Patients	51	41
Total Families	33	27
Male	29	18
Female	22	23
Seizures	23 (45%)	17 (41.4%)
ASD	17 (33.3%)	2 (4.8%)
Hearing Impairment	3 (5.8%)	4 (9.7%)
Vision Affected	2 (3.9%)	9 (21.9%)
Speech Affected	45 (88.2%)	16 (39%)
More than 1 Offspring Affected	15 (45.4)	8 (29.6%)
Microcephaly	18 (19.5%)	-
UMN Sign	27 (52.9%)	19 (46.3%)
LMN Sign	4 (7.8%)	10 (24.3%)
Normal Neuro Imaging	22 (43.1%)	12 (29.2%)
Leukodystrophy	5 (9.8)	13 (31.7%)
Cerebral Atrophy	9 (17.6%)	7 (17%)
Cerebellar Atrophy	5 (9.8%)	9 (21.9%)
Lissencephaly	1	-
Basal Ganglia Calcification	1	-
Agenesis of corpus callosum	1	-
Expired	5 (9.8%)	5 (12.19%)
Novel Gene	19 (57.5%)	12 (44.4%)
Known Gene	14 (42.4%)	15 (55.5%)
Abnormal MRI/ Neuroimaging	22 (43.1%)	29 (70%)

DISCUSSION

Degenerative brain disorders and developmental delay are commonly occuring neurological diseases which are always difficult to diagnose clinically. Results of our study are comparable with observations made by Karimzadeh and his colleagues in Iran[13]. We found almost equal gender distribution in these inherited neurological disorders (1:1) as seen in Karimzadeh's study. Seizures were observed in 43.4% in our study group while slightly more incidence of seizures was observed in the other group. About 19% of our patients were having microcephaly while it was 16% in Iran. Similarly 55.4% of our patients have GDD/ID and 44.5% have degenerative brain disorder which are comparable (47.4% and 39.4% respectively). Neuroimaging was normal in 36% of patients in both studies[13].

Zaki *et al*[14] conducted a large trial on 9400 participants of more than 5000 families having neuro developmental disorders. Consaguinity was present in >80% of families. About 63% of families had >1 affected members as compared to 38.3% in our study.

In a study done by Elanchezhian and colleagues, MRI of children with GDD were evaluated and it was found that 61.9% of patients had abnormal MRI while in our study, 43.1% of GDD patients have abnormal MRI[15].

Table-II: Known genes.

Known genes of DBD		Known genes of GDD/ID	
Gene	**Diagnosis**	**Gene**	**Diagnosis**
MFSD8	Neuronal Ceroid Lipofuscinosis-7	PLA2G6	Infantile Neuroaxonal Dystrophy 1
ARG1	Argininemias	SLC13A5	Early Infantile Epileptic Encephalopathy, 25
CLN6	Neuronal Ceroid Lipofuscinosis-6	PYCR2	Hypomyelinating Leukodystrophy, 10
MICU1	Myopathy with extrapyramidal signs	BSCL2	Spastic Paraplegia 17, AD
ARSA	Metachromatic Leukodystrophy	KIF7	Acrocallosal Syndrome
ARSA	Metachromatic Leukodystrophy	TMEM5	Walker Warberg Syndrome
CLN5	Neuronal Ceroid Lipofuscinosis-5	AP4M1	Hereditary Spastic Paraplegia
CLN6	Neuronal Ceroid Lipofuscinosis-6	EIF2B2	Leukoencephalopathy with Vanishing White Matter
SLC1A3	Episodic Ataxia-6	ANK3	Mental Retardation AR, 37
MTHFR	Homocysteinuria	AHI1	Joubert Syndrome, 3
SPG11	Hereditary Spastic Paraplegia	WDR62	Microcephaly 2, primary AR, with or without cortical malformation
ATM	Ataxia Telengiectesia	ATRX	Mental Retardation-Hypotonic Facies Syndrome XL
ATM	Ataxia Telengiectesia	GPR56	Polymicrogyria, B/L, Fronto-parietal
ARSA	Metachromatic Leukodystrophy		
CLN6	Neuronal Ceroid Lipofuscinosis-6		

The results of our study do not match with results of Kwok *et al* where they found 18% of patients with GDD to be deaf but we have only 5.8% of our GDD patients deaf[16]. This disparity in the result is due to larger and more diverse study group.

An interesting observation in our study is the presence of cerebellar atrophy in 15.2% of our patients in the absence of cerebellar signs. Now it has been proven that in addition to control of balance, cerebellum especially olivo-cerebellar tract are important key factors for acheivement of many higher mental skills such as cognition[17,18]. The classical example of known disease of GDD with cerebellar hypoplasia is Joubert Syndrome for which gene has been identified. There are still many neurometabolic diseases which are undiagnosed and has cerebellar involvement. This is also evident in the work of Bolduc *et al* in two trials. In one trial they observed that all patients with cerebellar malformations on MRI had GDD. In other trial they highlighted the topographic involvement of different cerebellar

Table-III: Novel genes.

Novel Genes in GDD/ID	Novel Genes in DBD
ACTL6B	HSD17B4
PLEKHG6	METTL20
KIF24	SNX2
TSEN15	CSMD2
ODF2L	ECHS1
MBOAT7	MAML1
GDPD3	TRAPPC2L
ZMYM6	MYEF2
MTMR7	MBLAC2
FAM122C	PITRM1
SLC16A13	NUP93
SEC14L1	LYPD3
SLC9A7	
SP1	
MRPL49	
SBNO1	
MKLN1	
KIAA2013	
FIGF	

areas in different affected higher mental skills[19]. Similarly in the work of Bonkowsky *et al* on pediatric leukodystrophies, they found crebellar atrophy in 14% of their patients[20].

Recent techniques in genetic analysis of such patients are diagnosing not only the gene for already known inherited neurological disorder but also discovering many novel genes. In the last decade, the number of genes causing GDD/ID has increased. Uptil 2006, only 3 genes were known for causing ID[21-22]. With the help of next generation sequencing, a large number of new genes have been discovered. Najamabdi and his co-workers identified 50 (36.7%) novel genes when they did next generation sequencing on 136 families having sibling with ID with cousin marriage[23]. Here we have found 19 (57.5%) novel genes of GDD/ID. Similarly in Saudi Arabia, Alazami and colleagues applied whole exome sequencing to 143 consangunous families having children with ID. They reported 33 (23%) novel genes[24].

Recently in Pakistan, Riazuddin *et al* has done a trial on 121 families with ID having consanguinity and reported 30 (24.7%) novel genes[25].

Previously most of such patients were mislabelled as having cerebral palsy and were not offered any kind of specific treatment, investigation and counselling thus creating a hopeless condition for the families. Moreover there have been multiple hospital admissions of such patients with different complications, adding financial burdon to healthcare system and the families. There has been multiple affected children in a family as in our study 38.3% of families have more than one affected offsprings. The results of our study prove the genetic basis of these HND. We have also found a large number of new genetic mutations as well. With the help of genetic studies, new clinical data will be included in the literature. With the help of genetic analysis, early diagnosis and timely treatment of some of these diseases can stop the progression of disease and improve the neurological status.

Our study has many limitations. It is a single centre study with small sample size. A more comprehensive multicentre trial is needed to further understand the disease penetration in our country. It will be helpful both in screening and counselling of families. Alongwith previously discovered genes, many newer genes have been revealed necessitating their detailed evaluation. Genetic confirmation of the diseases is considered mandatory now a days. Pakistan being a limited resource country is unable to establish even a single diagnostic centre meeting international requirments. We strongly recommend establishment of genetic laboratory fulfilling the latest requirements.

CONCLUSION

DBD and GDD/ID are entities with diverse clinical presentations. Whole exome sequencing is helpful diagnostic tool. In addition to previously discovered genes, many novel genes have been identified.

Author's Contribution

AOV analysed the data and wrote the manuscript. TS conceived the idea, designed the proposal, provided the supervisory role and made the final approval. FZ worked on acquisition and interpretation of data as well as final revision of the manuscript.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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