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Gene Panel Testing In Syndromic Children with Autism Spectrum Disorder Trait in Bangladesh- A Cross-Sectional Study.

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ABSTRACT:-
Background: Autism spectrum disorder (ASD) is a neurobiologic disorder with onset in early childhood. The key features are impairment in social communication and social interaction accompanied by restricted and repetitive behaviors, interest and activities. There is no diagnostic biomarker currently for ASD. According to basis of etiological factor about 10-20 % ASD children are found as syndromic trait. The syndromic ASD are commonly associated with genetic mutation. Bat exact genetic etiologies of syndromic ASD are not easily identified phenotypically. In this case the diagnosis should be confirmed by targeted genetic testing.

Aims& objectives: To identify the underlying genetic mutation of syndromic children with ASD trait in Bangladesh
Method: This cross sectional study was conducted in Department of Paediatric Neurology, Bangabandhu Sheikh Mujib Medical University, and Dhaka from October 2019 to September 2020. All syndromic Children age 3 to 15 years with ASD trait, who fulfilled the diagnostic criteria of ASD according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) were included in this study. Total 23 syndromic children with ASD trait were enrolled in this study. Demographic characteristics, perinatal history, clinical presentations, family history with parental consanguinity were recorded. Then with all aseptic precaution blood sample was sent to genetic laboratory to see underlying genetic mutation for indexed case. Informed written consent was taken from their legal guardian or caregiver. Data were collected from their parents in structured pre-designed questionnaire. Data analysis was performed by Statistical Package for Social Science (SPSS), version-24.

Result: Among 23 ASD cases more two-third cases (16, 69.56%) were in 5-10 year age group. Males were outnumbered than females (17, 73.91% vs. 6, 26%). Male female ratio was 2.8: 1. Most of the ASD child had speech delay (86.95%) followed by poor social interaction (82.6%), poor eye contact (73.91%), and speech regression (69.56%). Intellectual disability was found about two-third cases (65.23%) of syndromic ASD children. TSC $_1$& TSC $_2$ gene mutation was most common finding (21.74%) among syndromic ASD children followed by MECP2 gene mutation (17.39%), positive methylation test for Prader willi syndrome (17.39%), and FMR_1 gene mutation for fragile-X syndrome (8.69%). Tuberous sclerosis complex (21.73%) was the most common genetic syndrome with ASD trait followed by Rett syndrome (17.39%), Pradder willi syndrome

(17.39%), PKU (13%), Fragile-X- syndrome (8.69%), Down syndrome (8.69%), Angelman syndrome (1, 4.34%), William syndrome(1, 4.34%), and KMT-2B associated syndrome (1, 4.34%).

Conclusion: In our study Tuberous sclerosis complex is the most common genetic syndrome with ASD trait followed by Rett syndrome, Pradder willi syndrome, PKU, and Fragile-X- syndrome. Common gene mutation were **T**SC_1 & TSC_2 gene mutation, MECP2 gene mutation, FMR_1 gene mutation and other genetic abnormalities for Pradder willi syndrome, Angelman syndrome, William syndrome and KMT-2B associated syndrome.

Key wards: ASD, Syndromic Child, Genetic testing

I. INTRODUCTION

Leo Kanner in 1943 first introduced the term autism as a diagnostic label to define a specific syndrome observed in young children manifested by early onset, characteristic symptomatology, and disrupted social and emotional relationships[1]. Since then, autism is now recognized as Autism Spectrum Disorder (ASD), which is classified as a developmental disorder as defined in DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition) by the American Psychiatric Association[2] and the ICD-10 (International Classification of Diseases, 10th Revision) by the World Health Organization[3] . Autism is characterized by significant impairment in social communication and atypical repetitive and/or restrictive behaviors or interests, with an onset in the early developmental period, prior to age 3 years.

ASD affects about 1 individual in 50–100 live births[4,5]. Genetic factors are estimated to contribute 40 to 80 percent of ASD risk.It is considered to be the most heritable neurodevelopmental disorder based on a large difference in concordance rates. Monozygotic twins having rates that are nearly three times higher than rates found in dizygotic twins[6].

Tordjman et al. provided a comprehensive review of diverse genetic disorders associated with autism[7]. Nearly 800 susceptibility, clinically relevant, or known genes for autism spectrum disorder collated by Butler et al[8].

In Bangladesh, the reported prevalence of autism in children (0.15-0.8%) is lower than the worldwide prevalence.[9-12] There are a few studies regarding non syndromic genetic aetiology in Bangladesh[13,14]. But there is dearth of studies regarding syndromic association of ASD.

According to basis of etiological factor about 10-20 % ASD children are found as syndromic trait. The syndromic ASD are commonly associated with genetic mutation. Bat exact genetic etiologies of syndromic ASD are not easily identified phenotypically. In this case the diagnosis should be confirmed by targeted genetic testing. So aim of this study to identify the underlying genetic mutation of syndromic children with ASD trait. This cross sectional study was conducted in the Department of Paediatric Neurology, Bangabandhu Sheikh Mujib Medical University to identify genetic cause of ASD in syndromic child.

II. MATERIAL AND METHODS

This was a cross sectional study was conducted in Department of Paediatric Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka from October 2019 to September 2020.

ALL syndromic Children age 3 to 15 years with ASD trait, who fulfilled the diagnostic criteria of ASD according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) were included in this study. Children with Intellectual disorders or other developmental disorders such as ADHD, Cerebral palsy, Down syndrome were excluded from the study.

A total 23 syndromic ASD children were included in this study on the basis of high index of clinical suspicion after evaluating appropriate history and performing relevant clinical examination. Demographic characteristics, perinatal history, clinical presentations, family history with parental consanguinity were recorded. Then for Indexed case with all precaution Blood sample was sent to genetic laboratory to see underlying genetic mutation. Informed written consent was taken from their legal guardian or caregiver.

Data were collected from their parents in structured pre-designed questionnaire. Data analysis was performed by Statistical Package for Social Science (SPSS), version-24. Results were presented as the frequency of different variables and expressed in %.

III. RESULT:

Among 23 ASD cases more two-third cases (16, 69.56%) were in 5-10 year age group.Males were outnumbered than females (17, 73.91% vs. 6, 26%). Male female ratio was 2.8: 1. They live mostly in urban area (17, 73.91%). Most of the ASD child had speech delay (86.95%) followed by poor social interaction (82.6%), poor eye contact (73.91%), speech regression (69.56%) and lake of pointing (65.21%). Intellectual

disability was found about two-third cases (65.23%) of syndromic ASD children. TSC $_1$& TSC $_2$ gene mutation was most common finding (5, 21.74%) among syndromic ASD children followed by MECP2 gene mutation (17.39%), positive methylation test for Prader willi syndrome (17.39%), FMR_1 gene mutation for fragile-X syndrome (8.69%) Abnormal karyotyping (8.69%) and positive clinical exome sequencing for other molecular genetic analysis (17.39%). Tuberous sclerosis was the most among associated syndrome (21.73%) followed by Rett syndrome (17.39%), Pradder willi syndrome (17.39%), PKU (13%), Fragile-X- syndrome (8.69%), Down syndrome (8.69%), Angelman syndrome (1, 4.34%), William syndrome(1, 4.34%), and KMT-2B associated syndrome (1, 4.34%).

Table 1- Baseline characteristics (n = 23)

category	Number	Percentage
Age of onset		
Age of presentation <5 years 5- 10 years >10 years	 6 16 1	 26 69.56 4.35
Sex Male Female	 17 6	 73.91 26
Residence Rural Urban	 6 17	 26 73.91
Socio-economic status Upper Middle Lower	 6 6 11	 26 26 47.82
Birth History Perinatal events: Absent Present	 4 19	 17.39 82.60
Consanguinity Present Absent	 3 20	 13 86.95
Sib affected Present Absent	 8 15	 34.78 65.22

Table 1 Showing most of the child with ASD were between 5-10 years of age (16, 69.56%). Among ASD child males were more than females (17, 73.91% vs 6, 26%). They live mostly in urban area (17, 73.91%) and have lower socio economic status (11, 47.82%). Majority of ASD child had perinatal events (19, 82.6%). Only 3 (13%) child with ASD had h/o consanguinity. H/O affected sib was present among 8 (34.78%) ASD cases.

Table 2 Clinical Presentation (n= 23)

Category	Number	Percentage
Speech delay	20	86.95
Speech regression	16	69.56
Poor eye contact	17	73.91
Pointing	15	65.21
Social interaction	19	82.60

Table 2 Showing most of the ASD child had speech delay (86.95%) followed by poor social interaction (82.6%), poor eye contact (73.91%), speech regression (69.56%) and pointing (65.21%).

Table 3 Associated co- morbidities (n= 23)

Category	Number	Percentage
Sleep disturbance	12	52.17
Gratification	2	8.69
Epilepsy	8	34.78
ADHD	7	30.44
Pica	3	13.04
GIT problem	3	13.04
Intellectual disability	15	65.23

Table 3 Showing intellectual disability was present in majority of the ASD children (65.23%) followed by sleep disturbance (52.17%), epilepsy (34.78%), ADHD (30.44%) as associated co-morbidities.

Table 4 Genetic testing (n = 23)

Category	Number	Percentage
FMR_1 gene analysis	2	8.69
$MECP_2$ gene analysis	4	17.39
Clinical exome sequencing KMT-2Bmutation (1) PAH gene mutation (3)	4	17.39
Methylation test	4	17.39
Chromosomal microarray test William syndrome (1), Angelman syndrome (1)	2	8.69
TSC_1 & TSC_2 gene analysis	5	21.74
Karyotyping	2	8.69

Table 4 showing type of genetic testing for diagnosis used among ASD child. TSC_1 & TSC_2 gene analysis among 5 (21.74%) cases followed by MECP2 gene analysis (17.39%), methylation test (17.39%), clinical exome sequencing (17.39%), FMR_1 gene analysis (8.69%) and karyotyping (8.69%) was done to diagnose genetic association of ASD.

Table-5 Associated syndrome with ASD(n=23)

Syndrome	Number	Percentage
Rett syndrome	4	17.39
Fragile –X- syndrome	2	8.69
Pradder willi syndrome	4	17.39
Angelman syndrome	1	4.34
PKU	3	13
Tuberous sclerosis	5	21.73
Down syndrome	2	8.69
William syndrome	1	4.34
KMT-2B associated syndrome	1	4.34

Table 5 showing Tuberous sclerosis was the most among associated syndrome (21.73%) followed by Rett syndrome (17.39%), Pradder willi syndrome (17.39%), PKU (13%), Fragile-X- syndrome (8.69%) and Down syndrome (8.69%). 1(4.34%) child had Angelman syndrome, William syndrome and KMT-2B associated syndrome each.

IV. DISCUSSION

ASD is one of the most prevalent neurodevelopmental disorder in paediatric age group. There are a variety of syndromic and non-syndromic causes of ASD.[7,8] Here we studied 23 ASD children with syndromic association.

In our current study most of the child with ASD were between 5-10 years of age (16/23, 69.56%). Males outnumbered female (17/23, 73.91% vs 6, 26%). Most of them residing in urban area (17/23, 73.91%). Bhuiyan MR et al. reported majority (73.4%) of the children were male and mean (±SD) age was 6.66±2.97

years with most (88.8%) of the children were from urban areas. This study had similar findings to the current study probably due to similar region of study area.

Majority of ASD child had perinatal events (19, 82.6%). Only 3 (13%) child with ASD had h/o consanguinity. H/O affected sib was present among 8 (34.78%) ASD cases. A study on ASD in Brazil evidenced a significant association between perinatal and postnatal factors and ASD[16].Consanguinity (OR=4; 95% CI [1.3-12.04]), familial history of ASD (6.7 [1.1- 39.3]) had also been significant association in another study[17]. Consanguinity and positive family history may be explained by genetic basis of ASD.

Intellectual disability was present in majority of the ASD children (65.23%) in our study followed by sleep disturbance (52.17%), epilepsy (34.78%), ADHD (30.44%) as associated co-morbidities. Hadjkacem I observed ADHD was the most common comorbidity, affecting more 1 in every 3 children with ASD (35.3%), much higher than 1 in 6 (16.8%) among non-ASD siblings. Learning disability (23.5%) and intellectual disability (21.7%) were the next most-common comorbid conditions among children with ASD[18]. A few literatures showed a wide range of epilepsy association of ASD as well from 5% to 46%[19,20]. A bit difference of results may be due to small sample size along with inclusion of syndromic cases of ASD.

Different type of genetic analysis was done in our study like TSC $_1$& TSC $_2$ gene analysis (21.74%), MECP2 gene analysis (17.39%), methylation test (17.39%), clinical exome sequencing (17.39%), FMR$_1$ gene analysis (8.69%) and karyotyping (8.69%). As ASD is also highly genetically heterogeneous, testing was advised by different studies in all cases of ASD with a view to address a wide range of variant types, including both large (historically detected by microarray) and small (detected by sequencing), at least across all genes (exome). Additional specialized testing important in ASD diagnostics includes fragile X, mitochondrial DNA, methylation study and whole genome sequencing needed to be included whenever needed [6-8,21]. However, a diagnostic rate of 25% for clinically and molecularly defined syndromes has been suggested[22].

In the current study, Tuberous sclerosis was the most among associated syndrome (21.73%) followed by Rett syndrome (17.39%), Pradder willi syndrome (17.39%), PKU (13%), Fragile-X- syndrome (8.69%) and Down syndrome (8.69%). 1(4.34%) child had Angelman syndrome, William syndrome and KMT-2B associated syndrome each.The strength of association or co-occurrence between a given genetic syndrome and ASD is variable, with prevalence estimates ranging from 5% in individuals with Down syndrome to 60% in individuals with Tuberous Sclerosis Complex[23]. However in a study by Niu M et al. over 90% of males with full mutations have autistic features, and up to 60% meet diagnostic criteria for ASD.[24]Recently, screening of 120 ASD cases via WES identified three patients (2.5%) with MECP2 mutations. [25]

A few genetic syndrome although has been identified beginning with systemic approach upto genetic analysis. But the sample size was short, along with lack of appropriate investigations for genetic analysis in our country. Additionally high burden of costs is also a drawback to study this perspective in a child with ASD. After alleviating these shortcomings large scale study needs to be conducted to draw appropriate conclusion to the genetic basis of ASD.

Conclusion: In our study Tuberous sclerosis complex is the most common genetic syndrome with ASD trait followed by Rett syndrome, Pradder willi syndrome, PKU, and Fragile-X- syndrome. Common gene mutation were **T**SC$_1$ & TSC$_2$ gene mutation, MECP2 gene mutation, FMR$_1$ gene mutation and other genetic abnormalities for Pradder willi syndrome, Angelman syndrome, William syndrome and KMT-2B associated syndrome.

Limitation

The data was not so large and data collected from single point source and so further larger multicentred genetic study regarding ASD should be done.

Conflict of interest: There was no conflict of interest.

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Author Contributions:

Conceive and study design, data collection and data analysis: Prof. Dr. Gopen Kumar Kundu. Clinical Help: Dr MD Arbab Sarker, Dr Md Abdul Quddus Mia

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