CYTOGENETIC STUDY OF CHILDREN WITH DEVELOPMENTAL DELAY AND ASSOCIATED ANOMALIES

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ABSTRACT

***Background**: Developmental delay (DD) could be syndromic or non-syndromic, and collectively it affects 10% of all children. There are numerous causes of DD that could be genetical, hormonal and/or neurological. The frequency of defected chromosomal anomalies in patients with DD is variable and estimates between 9% and 36%. However, the accurate diagnosis needs further tests based on the information gather from parents and the findings on physical examination.*

***Objectives**: We aim to evaluate the pattern of chromosomal abnormalities in children with DD, in order to detect the treatable cases, and offering an appropriate genetic counseling.*

***Patients & Methods:** This is a cohort study comprised 40 children with developmental delay and associated congenital anomalies were referred from the outpatient clinic of the pediatric department, El Sayed Galal Hospital, to the Human Cytogenetics department, National Research Centre (NRC), Cairo, Egypt. During the period from December 2015 till June 2018.*

The patients were subjected to the present study. Peripheral blood samples were collected, cultured, harvested, metaphase spread and then chromosomes were stained for Gbanding using Trypsin-Giemsa technique. Chromosomes were analyzed, metaphase spreads were captured, and karyotyping has been done.

***Results:** 2 cases out of the 40 affected children have structural chromosomal rearrangements, and 3 out of them carried numerical chromosomal abberations .*

***Conclusion:** Chromosomal studies are valuable in detecting such cases with DD. Prenatal genetic diagnosis is of clinical importance to prevent and offer genetic counseling. Additionally, small proportion of apparently normal population could carry some types of structural chromosomal anomalies.*

***Key words:** developmental delay, mental retardation, congenital anomalies, chromosomal anomalies.*

INTRODUCTION

Global developmental delay (GDD) is the preferred term to describe intellectual and adaptive impairment in children younger than five years of age, based on failure to meet expected developmental milestones in several areas of intellectual functioning. Not all children with GDD will meet criteria for intellectual disability (ID) as they grow older.

The term intellectual disability (ID) usually is applied to children five years or older, when the clinical severity of impairment is more reliably assessed **(Pivalizza and Lalani, 2013).**

Children with developmental delay (DD) usually are brought to the attention of a pediatrician because of parental concerns of language delay, immature behavior, immature self-help skills, or difficulty in learning.

GDD can occur in isolation or with neurological abnormalities such as epilepsy or structural brain defects, or with other congenital anomalies.

The causes of GDD are extensive and include any disorder that interferes with brain development and functioning. The majority of GDD causes are due to genetic abnormalities **(Moeschler, 2008).**

A minority of cases have environmental causes such as teratogens, toxins, infections, trauma, birth asphyxia, and nutritional deficiencies **(Leonard and Win, 2002)**.

Genetic conditions are increasingly being diagnosed by technological advances in genetic testing; a specific genetic cause can be identified in more than 50% of cases of DD **(Karnebeek; et al., 2005)**.

Different cytogenetic techniques are used by genetic testing laboratories to investigate the possibility that an individual has a genetic or chromosomal alteration.

Karyotyping;

Separating individual chromosomes in ametaphase spread and arranging them systematically in a karyotype for examination.

Fluorescence in situ hybridization (FISH);

FISH can demonstrate submicroscopic deletions and is important for precise identification of translocations, marker chromosomes and precise detection of mosaicism. Different probes can be used including locus

specific probes, whole chromosome painting probes and centromeric probes.

AIM OF THE WORK

- The aim of the work is to identify the chromosomal abnormalities in children with developmental delay and multiple congenital anomalies.
- Phenotype / karyotype correlation will be performed in cases with chromosomal abnormalities.

PATIENT AND METHODS

Patients: This is a cohort study comprised 40 children with developmental delay (delayed motor, speech, social or behavioral milestones) and associated congenital anomalies (eg; micro/macro cehaly, dysmorphic facies, etc.).

The patients were referred from the outpatient clinic of the pediatric department, El Sayed Galal Hospital, to the Human Cytogenetics department, National Research Centre (NRC), Cairo, Egypt. During the period from December 2015 till June 2018.

Methods: All patients included in the study were subjected to the following:

I- Careful history taking:

1) Family history:

Careful family history should be obtained and include consanguinity, previous pregnancy outcomes: miscarriages, stillbirths, neonatal or childhood deaths, and other affected family members with similar or relevant neurologic impairments.

The family history can be nearly recorded in the form of a pedigree. Often, seeing the family history in pictorial form makes the pattern of inheritance more apparent

2) Perinatal history:

▪ Prenatal history;

- Potential teratogens including alcohol, medications, vitamins, maternal infection (rubella, cytomegalovirus, toxoplasmosis, varicella), maternal diabetes, hyperthermia, maternal phenylketonuria.

▪ Natal history;

- Gestation, mode of delivery, Apgar scores, resuscitation.
- Birth weight, length, head circumference.

▪ Postnatal history;

- History of jaundice, bleeding, convulsions or respiratory distress.

- Hitory of admission to NICU.

3) Developmental history:

Developmental milestones should be reviewed and the age at the time the problem emerged should be documented.

Clinicians should be alert to the loss or regression of previously acquired developmental skills, which suggests other possible etiologies such as inborn error of metabolism (IEM) or neurodegenerative disease.

II- Physical examination:

Detailed clinical examination with special emphasis on:

- Craniofacial dysmorphic features, other congenital anomalies
- Anthropometric measurements including height, weight, head circumference, were assessed and compared with the age and sex matched Egyptian controls **(Ghalli et al., 2002)**.
- A careful neurological examination, noting abnormalities of muscle tone and strength, ataxia, abnormal movements, etc.

III- Laboratory and radiological investigations:

Were carried out whenever indicated including:

- Complete blood cell count, Chest X-ray, ECG, and echocardiogram, MRI and EEG, when indicated

IV- Cytogenetic analysis (karyotyping):

In this study, G-banding technique was used as cytogenetic marker in blood lympocytes from patients.

The technique was done according to **(Verma and Babu, 1995)**.It consists of four essential steps as following:

I- Peripheral blood culture technique:

- 4 - 5 ml of venous blood was drown aseptically in a heparinized sterile tube, mixed well by gentle inversion.
- 0.5 ml drops of the whole blood were added to each culture tube, the two tubes were incubated at 37ºC for 72 hours.

II- Harvesting:

- After 72 hours from starting culturing, 0.1 mg/ml of 0.05 colchicine solution was added to the culture and left for 45 minutes.
- The cells were regimented by centrifugation at 1000 rpm for 10 minutes; the supernatant fluid was removed leaving 0.5 ml of it above the cell sedment.

- 5ml of hypotonic solution warmed at 37ºC were added and the tubes were then left for 30 minutes at 37ºC.
- 5ml of freshly prepared Carney's fixative (one part glacial acetic acid: 3 parts methanol), then kept in refrigerator were added to each of the tubes.
- Spreading of cells was carried out by splashing on clean slides followed by 2-4 times of short, hard blowings directly perpendicular to the slides.

III- Banding:

G-Banding for human chromosomes was done according to **(Seabright, 1972)** and **(Verma and Babu, 1995)**.

IV- Staining:

The treated slides were stained in phosphate Giemsa buffer for 1 to 3 minutes.

VI- Chromosomal study:

- Slides were examined with a low power research microscope for the presence of spread metaphases.
- Chromosomal analysis then carried out using the oil immersion lens (100xeye piece). Twenty five metaphases were analyzed for each case.
- Metaphases with good banding quality and those with abnormalities were katryotyped using image analysis system **(Applied Imaging USA)**. Individual chromosomes were identified and arranged according to the **(ISCN, 2005)**.

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.
- Chi-square (x2) test of significance was used in order to compare proportions between two qualitative parameters.
- The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:
- Probability (P-value)
  - P-value <0.05 was considered significant.

- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

The study included 40 cases collected from the outpatient clinic of the pediatric department, El Sayed Galal Hospital, Al-Azhar University to the Human Cytogenetics department, National Research Centre (NRC).

We found that 5 cases (12.5 %) out of the 40 affected children Presented with abnormal karyotyping, three (60%) out of the five have numerical aberrations, where two of them (20%) have structural chromosomal abnormalities.

Table (1): Demographic data distribution of the study group

Demographic Data	No.	%
Gender		
Female	11	27.5%
Male	29	72.5%
Age (years)		
Range [Mean±SD]	0.67-14 4.44 ± 3.50]	

This table shows male and female distribution and the mean age of presenting patients.

Table (2): Risk factors distribution of the study group

Risk factors	No.	%
Family history of similar conditions		
No	37	92.5%
Yes	3	7.5%
Consanguinity		
No	26	65.0%
Yes	14	35.0%
Recurrent Abortions		
No	36	90.0%
Yes	4	10.0%
Hypoxia		
No	26	65.0%
Yes	14	35.0%
Maternal factors		
Bleeding	3	7.5%
IDM	2	5.0%
PIH	2	5.0%
Radiation	1	2.5%
No	32	80.0%
Mode of delivery		
SVD	15	37.5%
CS	25	62.5%
GA (wks.)		
Pre-term	9	22.5%
Full-term	31	77.5%
History of NICU admission		
No	26	65%
Yes	14	35%

This table shows that C.S delivery (62.5%), Hypoxia (35%), +ve consanguinity (35%), and NICU admission (35%); were the most common risk factors in our cases.

Table (3): IQ distribution among the study group

IQ	No.	%
Normal	28	70.0%
Mild MR (50-69%)	5	12.5%
Moderate MR (35-49%)	1	2.5%
Severe MR (20-34%)	6	15.0%
Range [Mean ± SD]	20-86 [62.90 ± 20.70]	

This table shows that severe MR was present in 6 cases (15%), while mild MR was present in 5 cases (12.5%) among the study group.

Table (4): distribution of clinical abnormalities among the study group

Examination	No.	%
Microcephaly		
No	19	47.5%
Yes	21	52.5%
Neurologic signs		
Hypotonia	3	7.5%
Convulsions	2	5.0%
Associated disorders		
ASD	2	5.0%
ADHD	5	12.5%

This table shows that microcephaly (52.5%) and ADHD (12.5%) are the most common clinical abnormalities among the study group.

Table (5): Distribution of MRI findings among the study group

MRI findings	No.	%
Normal	23	57.5%
Abnormal (e.g.; PVL, demyelination)	17	42.5%

This table shows that PVL and demyelination disorders are the most common MRI findings among the study group.

Table (6): Distribution of karyotype abnormalities among the study group

Karyotype	No.	%
Normal	35	87.5%
Abnormal (Numerical & Structural)	5	12.5%

This table shows the numerical and structural chromosomal abnormalities distribution among the study group.

Table (7): Summarizes all data about patients, their karyotyping and their clinical presentations

This table shows that 3 cases were have numerical chromosomal

Case no.	Age (Y:M)	Sex	Clinical presentation	Karyotype
1	2 3/12	M	psycho-motor delay, hypotonia, Hypoplasia of corpus callosum (CC)	47, XY, + mar
2	1 6/12	M	Severe MR, psycho-motor delay Hypoplasia of CC	47, XY, + mar 46, XY (30%) (47, XY, +13 (70%)
3	1 yr.	F	Global develop-mental delay with failure to thrive, Generalized Tonic-Clonic seizures Microcephaly and highly arched palate.	46,XX,del(22q11) .ish del (22) (q11.2q11.2) (N25-)
4	4 5/12	F	Microcephaly, hypertelorism, Low-set ears, severe mental retardation	46, XX, del (5p15).
5	10 yr.	M	microcephaly, Triangular face, large ear, high arched palate, wide spaced nipples	47, XY, +21

abnormalities while 2 cases have structural aberrations.

Table (8): Correlation between karyotype and demographic data

Demographic Data	Karyotype		t/x2#	p-value
	Normal (N=35)	Abnormal (N=5)		
Age (years) **Mean ± SD** **Range**	 4.56 ± 3.51 0.67-14	 3.60 ± 3.73 1-10	0.444	0.509
Gender **Female** **Male**	 9 (25.7%) 26 (74.3%)	 2 (40.0%) 3 (60.0%)	0.448#	0.503

This table shows no statistically significant difference between normal and abnormal karyotype according to demographic data.

Table (9): Correlation between karyotype and risk factors

Risk factors	Karyotype		x2	p-value
	Normal (N=35)	Abnormal (N=5)		
Family history				
No	32 (91.4%)	5 (100.0%)	5.463	0.049*
Yes	3 (8.6%)	0 (0.0%)		
Consanguinity				
No	21 (60.0%)	5 (100.0%)	6.077	0.029*
Yes	14 (40.0%)	0 (0.0%)		
Recurrent Abortions				
No	32 (91.4%)	4 (80.0%)	0.635	0.426
Yes	3 (8.6%)	1 (20.0%)		
Hypoxia				
No	21 (60.0%)	5 (100.0%)	6.077	0.029*
Yes	14 (40.0%)	0 (0.0%)		
Maternal factors				
Bleeding	3 (8.6%)	0 (0.0%)	1.429	0.921
IDM	2 (5.7%)	0 (0.0%)		
PIH	2 (5.7%)	0 (0.0%)		
Radiation	1 (2.9%)	0 (0.0%)		
No	27 (77.1%)	5 (100.0%)		
Mode of delivery				
NVD	13 (37.1%)	2 (40%)	0.137	0.711
CS	22 (62.9%)	3 (60%)		
GA (wks)				
Pre-term	8 (22.9%)	1 (20%)	0.184	0.668
Full-term	27 (77.1%)	4 (80%)		
History of NICU admission				
No	23 (65.7%)	3 (60%)	0.063	0.802
Yes	12 (34.3%)	2 (40%)		

This table shows statistically significant difference between karyotype findings and positive consanguinity, hypoxia and family history.

Table (10): Correlation between karyotype and IQ

IQ	Karyotype		t/x2#	p-value
	Normal (N=35)	Abnormal (N=5)		
Mean ± SD **Range**	64.58 ± 19.83 20-86	31.00 ± 0.00 31-31	3.723	0.016*
Category **Normal** **Mild MR** **Moderate MR** **Severe MR**	 26 (74.3%) 5 (14.3%) 1 (2.9%) 3 (8.6%)	 2 (40.0%) 0 (0.0%) 0 (0.0%) 3 (60.0%)	9.306#	0.025*

This table shows statistically significant difference between karyotype findings and IQ %.

Table (11): Correlation between karyotype and clinical abnormalities

Examination	Karyotype		x2	p-value
	Normal (N=35)	Abnormal (N=5)		
Microcephaly **No** **Yes**	 17 (48.6%) 18 (51.4%)	 2 (40.0%) 3 (60.0%)	0.129	0.72
Neurologic signs **Hypotonia** **Convulsions**	 2 (5.7%) 1 (2.9%)	 1 (20%) 1 (20%)	0.313	0.576
Associated disorders **ASD** **ADHD**	 2 (5.7%) 5 (14.3%)	 0 (0%) 0 (0%)	0.571	0.449

This table shows no statistically significant difference between karyotype findings and microcephaly, neurologic signs and associated disorders.

Table (12): Correlation between karyotype and MRI findings

MRI findings	Karyotype		x2	p-value
	Normal (N=35)	Abnormal (N=5)		
Normal MRI	20 (57.1%)	3 (60.0%)	0.015	0.904
MRI	15 (42.9%)	2 (40.0%)		

abnormaities				

This table shows no statistically significant difference between karyotype findings and MRI findings.

DISCUSSION

Developmental delay (DD)/ intellectual disability (ID) is a unpredictable manifestation of central nervous system dysfunction, and its incidence rate reaches 3% in the general population. Around 40% of patients suffering developmental delay and /or intellectual disability have a genetic underlying cause, involving chromosomal aberrations (e.g., Trisomies, microdeletions and microduplications) and monogenic etiologies (e.g., fragile X syndrome).

Chromosome abnormalities are revealed and displayed in 25% of pediatric patients with Developmental delay / intellectual disability issues. Traditional cytogenetic investigations have been the standard First line genetic investigation for the detectability and diagnosis of genetic abnormalities in cases clinically presenting with Developmental delay and / or intellectual disabilities for more than three decades , and it permits the diagnosis of numerical and structural chromosomal abnormalities present within in the entire genome but has a restricted resolution of 5 - 10 Mb. Fluorescence in situ hybridization (FISH) could reveal particular cytogenetic aberrations with a higher sensitivity than traditional cytogenetic testing;

On the other hand, FISH couldn’t cover up whole chromosomal regions. Besides, only a somewhat small percentage of cases (around 6%) could be diagnosed by traditional cytogenetic testing and FISH **(Rongyue et al., 2018)**.

In the scientific gene era, accurate recognition of breakpoints within DNA could supply useful clues to the underlying genetic diseases that assist in precise estimation of recurrence risk for a particular case. Meticulous pedigree analysis and obtaining of family history, in conjunction with implementation of FISH after that chromosomal microarray analysis, could detect cryptic imbalance in atypical cases.

Approximately 5% of the general population is estimated to be carrier of a balanced

chromosomal rearrangement. Such rearrangements could result in meiotic errors and non-disjunction causing production of unbalanced gametes. The resulting unbalanced chromosome composition of gametes could lead to the delivery of children with malformations. The majority of reciprocal translocations could be revealed via usage of traditional cytogenetic testing **(H. sheth et al., 2017)**.

In the current research, study involved 40 pediatric cases with delay of developmental milestone and associated congenital abnormalities. The cases were referred from the outpatient clinic of the pediatric department, El Sayed Galal Hospital, Al-Azhar University to the Human Cytogenetics department, National Research Centre (NRC). All cases have been subjected to Careful history taking, pedigree analysis, physical examination, laboratory and radiological investigations, involving CBC, Chest X-ray, ECG, and echocardiogram, MRI and EEG, as indicated.

Cytogenetic analysis (karyotyping). **Fluorescence in situ hybridization (FISH):** have been carried out (if necessary) on peripheral blood lymphocytes for the proper characterization of rings, marker chromosomes, translocations or an additive chromosomal material and for detection of microdeletions when suspected.

As regards to demographic research, data distribution of the study cohort in which female cases represented 27.5% of the cases (n = 11) and male cases represented 72.5% of cases (n = 29) of gender, also age (years) ranged 0.67-14 with mean 4.44 ± 3.50 years.

As regards to risk factors distribution within the research cohort **Consanguinity was detected in** (35.0%), **Hypoxia in** (35.0%), **Recurrent Abortions** (10.0%), **Family history** (7.5%), **Maternal factors** Bleeding (7.5%), DM (5.0%), pregnancy induced hypertension (5.0%), Radiation exposure (2.5%), **Mode of delivery**; spontaneous vaginal delivery (37.5%), cesarean section (62.5%), **gestational age**; pre-term (22.5%), full-term (77.5%) and history of NICU admission (35%).

Concerning mentality, IQ (was found normal in 70%), indicated mild intellectual disability in (12.5%), moderate intellectual disability in (2.5%) and severe retardation (15%) of mentality IQ, and score ranged 20-86 with mean score ± SD= 62.90±20.7.

As regards to clinical presentation distribution within the research cohort: microcephaly was present in 52.5% of cases, neurologic signs such as hypotonia existed in 7.5% of cases and convulsions in 5% of cases, in addition associated disorders ASD (5%) and ADHD (12.5%).

Negative MRI findings were present in 57.5% of cases (n=23) and 42.5% of cases (n= 17) had positive findings, on the other hand 87.5% had normal karyotype results (n= 35) and 12.5% of cases had abnormal karyotype results (n= 5). However there was no statistically significant difference between normal and abnormal karyotype according to demographic research data. (P values =0.509, 0.503). Statistically significant difference between normal and abnormal karyotype was present as regards consanguinity, hypoxia and family history. (P values =0.029, 0.029, 0.049 consecutively). Statistically significant difference between normal and abnormal karyotype concerning mentality IQ. (P values =0.016, 0.025).

Interestingly no statistically significant difference between normal and abnormal karyotype according to dysmorphic features (microcephaly), neurologic signs and associated disorders. (p values =0.72, 0.576, 0.449 consecutively). In adittion no statistically significant difference existed between normal and abnormal karyotype according to MRI findings (p value=0.904).

The present study reported chromosomal abnormalities in five cases (12.5%) out of 40 diseased children. Indeed, our result could be similar, higher or lower than those of other investigators. **(Berry, 1995)** has reported a frequency of 15.8% out of 114 cases, **(Verma et al., 1980)** reported a frequency of 27% out of 357 cases, while **(Singh, 1977)** has reported a frequency of 28.8% out of 451 patients. However; much lower frequencies (1% to 6%) have been reported in other studies **(Kenue, 1995;** and **Hook et al., 1977)**. The variable frequencies shown could contribute to the size of the population sample, patient selected criteria, and /or to the techniques used in investigation.

In comparison to current research study , a study done by **(Rajasekhar et al., 2011)**, to identify the chromosomal abnormalities in children with mental retardation and associated anomalies.in which 420 children (237 males and 183 females) diagnosed with developmental delay, multiple congenital anomalies were subjected to

clinical and G-banded cytogenetic evaluation.

In such study, 246 (58.5%) children were clinically diagnosed as Down syndrome. Of these, 208 (84.55%) cases were with trisomy 21, 11 (4.47%) cases with Robertsonian translocation and 5(2.03%) cases were mosaic down syndrome with instances of duplication, inversion, and reciprocal translocation 5 (2.43%) were also observed. Rest of the children 17 (6.91%) were found to have normal chromosomal karyotypes although they were diagnosed with developmental delay and associated malformations. In conclusion, this study suggests that G banded karyotyping is a routine clinical test for Mental retardation (MR) patients with or without congenital anomalies, albeit molecular karyotyping needs to be applied for detection of submicroscopic chromosome alterations.

Our study showed that all cases with chromosomal abnormalities are carried on autosomal chromosomes. This could be due to the fact that sex chromosome defect has a much lesser deleterious effect on the phenotype than autosomal anomalies do **(Brown et al., 2004)**. In contrast to this chromosomal study in neonates showed that autosomal chromosome anomalies are usually as common as sex chromosome anomalies **(Gardner and Sutherland, 2004)**. Also, Our study showed that the numerical anomalies of chromosomes (60 %) are more common than the structural anomalies (40 %), which is in agreement with the study done by **(Schinzel, 2001)**.

Similar research study aimed to evaluate value of chromosome microarray analysis for clinical diagnostic testing within the Chinese population in which Whole-genome high-resolution single nucleotide polymorphism (SNP) arrays was applied on 489 cases with unexplained developmental delay / intellectual disability. The research group obtained the following results in which a total of 489 children were categorized into three research categories: isolated DD/ID (n = 358 cases), DD/ID with epilepsy (n= 49 cases), and DD/ID with other structural anomalies (n = 82 cases). They revealed 126 cases (25.8%, 126/489) having pathogenic copy number variants (CNVs) by CMA, involving 89 cases (24.9%, 89/358) with isolated DD/ID, 13 cases (26.5%, 13/49) with DD/ID with epilepsy,

and 24 cases (29.3%, 24/82) with DD/ID with other structural abnormalities. Among the 126 cases of pathogenic copy number variants, 79 cases were diagnosed as microdeletion/ microduplication syndromes, While 47 cases were diagnosed as non-syndromic pathogenic copy number variants. **(Rongyue et al., 2018).**

Another research genetic team conducted a study in using advanced genetic testing in comparison to current research study in which they assessed the diagnostic usefulness of chromosomal microarray testing in a large research cohort of cases with developmental delay or intellectual disability in Korea. In which they performed a genome-wide microarray analysis of 649 consecutive cases with developmental milestones delay and /or mental disabilities at the Seoul National University Children's Hospital. The hospital medical records and investigations were gathered and research data was obtained in retrospective manner. Pathogenicity of detected copy number variations (CNVs) was assessed by using previous reports as reference or parental testing implementing FISH or quantitative PCR. The genetic research team revealed the following results in which 110 cases had pathogenic copy number variations, which involved 100 deletions and 31 duplications of 270 kb to 30 Mb. The diagnostic test yield obtained was 16.9%, displaying the diagnostic value of chromosomal microarray testing within the hospital clinic. Parental testing was conducted in 66 cases, 86.4% of them carried de novo copy number variations. In 8 cases, pathogenic copy number variations have been inherited from healthy parents having a balanced translocation, and genetic counseling made for those families. Interestingly they demonstrated five rarely reported chromosomal deletions on 2p21p16.3, 3p21.31, 10p11.22, 14q24.2, and 21q22.13.

The research group displayed the clinical value of CMA testing in the genetic diagnosis of cases suffering developmental milestones delay or mental disabilities. CMA genetic testing should be implemented as a clinical diagnostic testing protocol for all pediatric patients with delay in developmental milestones or mental disabilities **(Jin Sook Lee et al., 2018)**.

Another Case presentation by **(H. sheth et al., 2017)** In which the research group reported a case of five gestational weeks primigravida married since four years have been refered for genetic counselling. There was a

family history of developmental delay and dysmorphic clinical features in the proband's nephew as observed and was inherited from a normally phenotypical carrier mother - proband's sister - who had a cryptic balanced translocation involving #2 and #17 i.e. 46, XX.ish t(2;17)(RP11-321A15-,CTB-50C4+;CTB-50C4-;RP11-321A15+). Normal genetic test results were obtained from traditional banding of the proband necessitated the authors to conduct FISH, which revealed breakpoints at 2q37.3 and in 17q25 region; therefore, the karyotype for proband and her sister has become reassigned as 46, XX, t(2;17)(q37.3;q25). Chromosomal microarray analysis was conducted at 16 weeks of pregnancy after observing increased nuchal translucency and single umbilical artery using fetal fetal sonography.

Genomic imbalance was evident with 4.9 Mb deletions in 2q37.3 region and 8.2 Mb duplication in 17q25.1q25.3 region. That emphasis the significance and value of genetic pedigree determination and advanced genetic testing implementation in modern practice .

CONCLUSION

The most significant updates include the inclusion of CMA as a first-tier diagnostic test for individuals with developmental delay (DD)/intellectual disability (ID). Any genetic testing approach should be individualized for a child's specific clinical history, physical examination findings, and family history. Collaboration with clinical geneticists may be helpful in determining the optimal test strategy, particularly when progressing beyond first-tier analyses, and in interpreting abnormal results.

RECOMMENDATIONS

1. For any child with unexplained DD, even in the absence of dysmorphic features, other clinical features or positive family history, routine chromosome analysis (minimum 550-band resolution) is indicated.
2. For children with clinical features of known chromosomal abnormality syndromes (e.g., Down syndrome), cytogenetic analysis should be performed. The identification of a translocation may affect the family's recurrence risk.
3. For children with clinical features suggestive of a particular microdeletion / microduplication syndrome, FISH or other molecular

techniques should be performed prior to or concurrently with chromosome analysis.

REFERENCES

1. **American Psychiatric Association. Intellectual Disability (Intellectual Developmental Disorder) in (2013):** Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, and American Psychiatric Association. (Ed), Arlington, VA (2013). p.33.
2. **Moeschler JB. (2008):** Genetic evaluation of intellectual disabilities. Semin Pediatr Neurol 15:2.
3. **Leonard H, Wen X. (2002):** The epidemiology of mental retardation: challenges and opportunities in the new millennium. Ment Retard DevDisabil Res Rev 8:117.
4. **VanKarnebeek CD, Scheper FY, Abeling NG, et al. (2005):** Etiology of mental retardation in children referred to a tertiary care center: a prospective study. Am J Ment Retard 110:253.
5. **Berry R. (1995):** Benefit of repeat cytogenetic studies in a high-risk pediatric population. Americal jurnal of human genetic, 49(3) 128-134.
6. **Verma RS, Dosik H, and Conte RA. (1980):** Incidence of major chromosomal abnormalities in a referred population for suspected chromosomal aberrations: a report of 537 cases. Clinical genetics, 17: 305-308.
7. **Singh DN (1977):** Cytogenetic study of individuals suspected of chromosomal anomalies. Clinical pediatrics, 16: 619-22.
8. **Hook ED, Hamerton JL, and Loman CE (1977):** The frequency of chromosomal abnormalities detected in consecutive newborn studies- difference between studies results by sex and severity of phenotypeic involvement. In: Hook ED, parter IH, Eds. Population cytogenetics studies in humans. New York, Academic press, inc., 63-79.
9. **Kenue RK (1995):** Cytogenetic analysis of children suspected of chromosomal abnormalities. Journal of tropical pediatrics, 41:77-80.
10. **Brown, C.J., L. Carrel, H.F. And Willard (2004):** Expression of genes from the human active and inactive X chromosomes. Am. J. Hum. Genet. 60: 1333-1343.
11. **Gardner R.J.M and Sutherland G.R. (2004):** Chromosome Abnormalities and Genetic Counselling. Oxford Univ. Press, Oxford, New York 342:411.
12. **Schinzel A. (2001):** Catalogue of unbalanced chromosome aberrations in man. 2nd ed. W. de Gryter, New York, Berlin.
13. **M. Rajasekhar, Neetha John, P M. Gopinath and K. Satyamoorthy. (2011):** Int J Hum Genet, 11(2): 89-92. Manipal Life Sciences Centre, Manipal University, Manipal 576 104, Karnataka, India.
14. **Rongyue Wang, Tingying Lei , Fang Fu, Ru Li, Xiangyi Jing, Xin Yang, Juan Liu, Dongzhi Li, Can Liao (2018):** Application of chromosome microarray analysis in patients with unexplained developmental delay/intellectual disability in South China Pediatrics and Neonatology (2018) xx, 1-8 https://doi.org/10.1016/j.pedneo.2018

.03.006.

15. **Jin Sook Lee, Hee Hwang, Soo Yeon Kim, Ki Joong Kim, Jin Sun Choi, Mi Jung Woo, Young Min Choi, Jong Kwan Jun, Byung Chan Lim , and Jong-Hee Chae, (2018):** Chromosomal Microarray With Clinical Diagnostic Utility in Children With Developmental Delay or Intellectual Disability Ann Lab Med 2018;38:473-48 https://doi.org/10.3343/alm.2018.38.5.473

16. **H Sheth, S Tewari, K Shah, T Liehr, J Trivedi - Int J Pregn & Chi Birth (2017):** Volume 3 Issue 4 2017.

دراثة وراثية خلوية للاطفال المصابين بالأعاقة الذهنية والتشوهات الخلقية المصاحبة

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الهدف من البحث: تقييم نمط التشوهات الصبغية لدى الأطفال المصابين بإعاقة ذهنية وتشوهات خلقيه مصاحبة . من أجل اكتشاف الحالات القابلة للعلاج ، وتقديم المشورة الوراثية المناسبة.

منهج البحث: تم اجراء الدراسة على ٤٠ طفلا يعانون من تأخر فى النمو مصاحب بتشوهات خلقيه والمترددون على عيادة اعصاب الاطفال فى مستشفى باب الشعريه الجامعى وذلك بعد اخذ موافقه كتابية من اباء الاطفال وموافقة لجنة الاخلاقيات بقسم الاطفال وكلية الطب جامعة الازهر.

وقد تم اخذ عينة دم من كل شخص وزرعها فى وسط خاص ثم تم جمع الخلايا وهى فى مرحلة الانقسام الخلوى المتوسط وفردها على شرائح زجاجيه ثم تم اخضاع العينات للفحص الكروموسومى باستخدام التقنيات العادية بعد تشريط الكروموسومات بواسطة انزيم التريبسين ثم صبغه بصبغة الجيمسا.

النتائج: حملت 3 حالات من أصل 40 طفلاً مصابًا انحرافات صبغية عددية ، وحمل اثنان منهم إعادة ترتيب كروموسومي.

الإستنتاجات و التوصيات:

تعتبر الدراسات الكروموسومية ذات قيمة في الكشف عن مثل هذه الحالات من التأخرالذهنى والتشوهات الخلقية المصاحبة.