Cytogenetic Studies in Children with Developmental Delay

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Abstract

**Introduction:** 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.

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

**Methodology:** 50 children suffering from DD with or without mental retardation (MR) and/or congenital anomalies were subjected to the present study. Additionally, another 50 normally developed children were considered as control group. Peripheral blood samples were collected, cultured, harvested, metaphase spread and then chromosomes were stained for G-banding using Trypsin-Giemsa technique. Chromosomes were analyzed, metaphase spreads were captured, and karyotyping has been done.

**Result:** Seven cases (14%) out of the 50 affected children carried structural chromosomal rearrangements. Six (85.7%) out of the seven structural chromosomal abnormalities were detected in autosomal chromosomes and one (14.3%) in sex chromosome. Surprisingly, we have found a case (2%) carrying pericentric inversion of chromosome 3 within the normal control group.

**Conclusions:** 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

Development refers to how a child becomes able to do more complex things as he gets older. Human development runs in three parallel lines: physical, cognitive, and behavioral, and so, any defect of one of these parameters could affect the normal development. Developmental delay is characterized by cognitive impairment or mental retardation (MR); growth retardation (intra-uterine or extra-uterine); and/or behavior abnormalities. Developmental delay (DD) shows slower rate of development, in which a child exhibits a functional level below the norm for his/her age (Leonard et al., 2002). Significant delay in two or more of the developmental domains; gross/fine motor, speech/language, cognition, social/personal, and activities of daily living; is defined as Global Developmental Delay (GDD). The term GDD is usually reserved for young children (i.e., typically less than 5 years of age), whereas the term mental retardation (MR) is usually applied to older children.
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when IQ (intelligence quotient) testing is more valid and reliable (Lichten, et al., 2007).

DD can be categorized as syndromic, or nonsyndromic; and could or could not be associated with dysmorphic features. However, DD/MR is considered a big problem for family and community, where affected child needs not only financial support, but also special educational and medical services throughout life (Shevell, 2000).

The etiology of DD/MR could be genetics, metabolic, neurologic or others, where genetic disorders represent the major cause. Genetic disorders could be chromosomal, monogenic or multifactorial; however chromosomal abnormalities have been documented as a single most common cause. The frequency of chromosome anomalies detected by karyotyping in patients with DD/MR was variable and estimated between 9% and 36% (de Vries et al. 2001). Where as, 40% of patients with sever MR, and up to 10% of patients with mild MR are documented to have chromosomal abnor-malities. The frequency could be higher than what we expect when more accurate techniques are used in diagnosis such as high resolution banding, FISH, M-FISH, and others (Granzow et al., 2000).

The human genome is composed of ~25,000 -35,000 genes carried on chromosomes, approximately 50% of which are of paternal and 50% are of maternal origin. It is estimated that about 65% of human genes contribute to the development of the nervous system. Therefore; gene copy number alteration due to gene(s) and/or chromosome(s) deletion or duplication, or an abnormal pattern of allelic inheritance could affect neuronal development (Capone, 2001). On the other hand, multifactorial disorders are variable and cause the majority of birth defects e.g. diabetes, spina bifida, anencephaly, cleft lip and cleft palate, clubfoot and congenital heart defects (Leonard and Wen, 2002).

However, chromosomal disorders happened sporadically, in most cases the parent's karyotype is normal, but during cell division (gametogenesis) an error in segregation or recombination may occur. Moreover, inherited unbalanced chromosomal rearrangements are responsible for a large proportion of familial disorders (Gardiner and Sutherland, 2004).

Most cases of DD do not show clinical signs suggesting a particular chromosome abnormality, while in others there is a strong suspicion of underlying abnormality. On the other hand, small proportional of apparently normal individuals carry structural chromosomal anomalies, which sometimes runs in family throughout several generations (familial benign chromosome abnormality). Such cases are rare, and are only detected by chance, so the accurate population rate is unknown (Bourne et al., 2000; and Boyle and Cooper, 2001).

Screening of children with DD/MR for genetic disorders (chromosomal or genes) is of great value especially to those of unknown etiology. It will also help in offering an appropriate counseling for those parents owing to minimize the number of affected children.

Patients And Methods

50 children (27 girls and 23 boys) suffering from DD with or without MR and/or congenital anomalies were subjected to this study. Also, 50 apparently normally developed children were used as control group. Children were selected at the outpatient clinic of the Pediatric Department, Neurological Unit, Al-Azhar University Hospital, after their parent's consent. The selection criteria were based on positive family history, perinatal history, dysmorphic features, and neurological manifestations. Moreover, we have excluded all other causes associated with known syndromes and other neurological and/or metabolic disorders.

Our experiment was designed as follow: -

(1) Children evaluation: Children were selected and diagnosed separately through history, physical and clinical examinations. The history included the family pedigree; family history, as well as prenatal and natal history. The examination includes; measurement of growth parameters (by using the
percentile chart) and behavioral observations.

(2) Blood samples collection and setting up blood cultures: addition of 0.4 ml of heparinized blood to 8 ml of RPMI 1640 medium supplemented with L-glutamine, fetal calf serum, penicillin/streptomycin, and phytohaemagglutinin. Cultured samples incubated at 37ºC for 72h, harvested cultured samples, making slides, and then staining chromosomes for G-banding using Trypsin-Giemsa technique according to Fan,(2002).

(3) Chromosome analysis at 400-500 band per haploid set, captured metaphase spreads, and finally karyotyping was prepared for the spreading metaphases.

At least, 10 metaphases were scored for each case; two cells were captured and karyotyped per case. All chromosomal abnormalities were recorded according to the International System for Human Cytogenetics Nomenclatures (ISHCN).

Result

We found that 7 cases (14%) out of the 50 affected children carried chromosomal rearrangements; table (1) summarizes all data about patients, their karyotyping and their clinical presentations. Six (85.7%) out of the seven structural chromosomal abnormalities were detected in autosomal chromosomes and one (14.3%) was detected in sex chromosomes. Twenty-nine of the diseased children have some congenital anomalies (especially in face, hand, and feet), where five of them (24.1%) have structural chromosomal abnormalities. Moreover, two cases (9.5%) in the remaining twenty-one diseased cases were without dysmorphic features but carrying chromosomal anomalies. We found, in the control group a case (2%) with pericentric inverted chromosome 3 in an apparently normal boy aged 6 years. Unfortunately his parent, karyotyping was not available.

Table I: List of the chromosomal rearrangements associated with clinical presentation and phenotype in the current study.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Karyotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>13</td>
<td>Female</td>
<td>46, XX, del(x)(q12)</td>
<td>MR with short stature</td>
</tr>
<tr>
<td>Case 2</td>
<td>2</td>
<td>Female</td>
<td>46, XX, del(1)(q23;q25)</td>
<td>MR with multiple congenital anomalies and dysmorphic features including cleft lip, and palate, small hands and feet, short stature &amp; congenital heart disease.</td>
</tr>
<tr>
<td>Case 3</td>
<td>5</td>
<td>Female</td>
<td>46, XX, t(15;22)(q26;p12)</td>
<td>Mild MR and delay in motor function; she was not able to walk until the age of 2.5 years.</td>
</tr>
<tr>
<td>Case 4</td>
<td>2</td>
<td>Male</td>
<td>46,XY, t(5;18)(q43;p22)</td>
<td>Severe DD with hypotonia at birth associated with some dysmorphic features (low-set ear, microcephally, hypertelorism, and epicanthal folds).</td>
</tr>
<tr>
<td>Case 5</td>
<td>4</td>
<td>Male</td>
<td>46,XY, t(1;9)(q43;p22)</td>
<td>Growth retardation and mild delay of motor function with slight dysmorphic features (midface hypoplasia, trigonocephaly, upward-slanting palpebral fissures, and a long philtrum)</td>
</tr>
<tr>
<td>Case 6</td>
<td>3</td>
<td>Male</td>
<td>46,XY, ins(22)(q13)</td>
<td>Severe DD, hypotonia, epilepsy, dysmorphic features including round face, and small head.</td>
</tr>
<tr>
<td>Case 7</td>
<td>3</td>
<td>Female</td>
<td>46,XX, dup(16)(q11;q12)</td>
<td>MR with mild delay of motor function, dysmorphic features including upslanding palpebral fissures, high arched palate, small widely-spaced teeth, and bilateral clinodactyly.</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>Male</td>
<td>46, XY, inv(3)(p14;q11)</td>
<td>Apparently Normal boy</td>
</tr>
</tbody>
</table>
Case 1: 46, XX, del(x)(q12)

Case 2: 46, XX, del(1)(q23;q25)
Case 3: 46, XX, t(15;22)(q26;p12)

Case 4: 46,XY, t(5;18)(p15;q21)
Case 5: 46,XY, t(1;9)(q43;p22)

Case 6: 46,XY, ins(22)(q13)
**Discussion**

Evaluation of cases suffering developmental delay and mental retardation (MCA/MR) with multiple congenital anomalies is always a challenge to clinicians, as routine chromosomal analysis is the starting point to investigate such cases. Subsequent investigations might consider achieving an accurate diagnosis. Usually, visible loss or gain of chromosome material will lead to abnormal development, resulting in a malformed phenotype, but in a lesser instant they could show normal morphology. The morphologic defect is greatly variable, and depends on the size of chromosome lesion and gene content involved (McKinlay et al, 2004).

Our study showed that the percentage of autosomal anomalies (~ 80%) was much higher than those of the sex chromosomes (20%). 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, the numerical anomalies of sex chromosomes are more common than the structural anomalies (Schinzel, 2001). On the other hand, several studies based on phenotypic
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anomalies (Sungsoo et al., 1999; Clara et al., 2005) are in agreement with the present results.

The present study reported chromosomal abnormalities in seven cases (14%) out of 50 diseased children. In deed, 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.

Our study showed that the frequency of chromosomal anomalies was 9.5% out of 21 mentally retarded children without dysmorphic features, but it was much higher (24.1% out of 29) in cases associated with dysmorphic features. Also the current investigation and others (Tetsuji et al., 2003; Donnenfeld, et al., 2003) indicated that chromosomal anomalies are frequently associated with multiple malformations and MR. We found three patients had reciprocal translocation, where two of them had dysmorphic features and MR. Such translocations have been reported to be harmless, but they are commonly seen in mentally retarded individuals. Donnenfeld, et al., (2003) reported that abnormal phenotype is usually uncommon with balanced chromosomal translocations. But, some apparent cytogenetically balanced translocations could be molecularly unbalanced i.e. the break points could interrupt gene/s resulting in haploinsufficiency for the gene product in this region, which in turn result in unexpected phenotypic anomalies (Cohen et al., 2001; and Robert et al., 2006).

Moreover, we have found a case [case 3: 46, XX, t(15;22)(q26;p12)] with translocation between chromosomes 15 and 22, which need further investigation like M-FISH, or panel of FISH probes to hybridize all chromosomes in order to identify the origin of the extra-DNA material.

Finally, several case reports have been published since about 1980 of microscopically visible euchromatic (G-Band negative) chromosome deletions, duplications, or inversion in individuals with an apparently normal phenotype. Such cases are rare. Unfortunately, without an abnormal phenotype, individuals are not usually referred for karyotyping, and so cases are only detected by chance. In the present investigation we detected a case carrying chromosomal inversion but with apparently normal development and phenotype. Chromosomal inversion could be balanced, and usually is associated with normal phenotype, but carriers of such anomaly are at risk of producing abnormal gametes that may lead to unbalanced offspring. Lindberg, et al. (1992) has reported that balanced familial pericentric inversion in chromosome 3 (p 14; q 11) with no adverse effects and they have concluded that this chromosome aberration could be an example of a harmless chromosome polymorphism. Awareness of such situations without an abnormal phenotype is important for prenatal diagnosis/counselling; and reinforces the importance of parental cytogenetic analysis to interpret an abnormal karyotype, and estimate risks, to exclude the possibility of a familial benign chromosome abnormality.

Conclusion

Chromosome studies are relatively expensive thus; the consult of pediatricians, neurologists, and dysmorphologists could lead to exclude cases with single gene defects, and syndromes with known non-genetic etiology. This in turn will minimize the need for standard karyotyping. Our study concluded that chromosomal studies are a valuable diagnostic technique to evaluate cases with DD/MR. Investigating parents for chromosomal abnormalities is important as the risk of inheritance is usually high in such cases. It helps to provide proper genetic counseling.
References


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دراسات كروموسومية بين الأطفال ذوى النمو المتاخر

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النمو المتاخر المصحوب بوجود تخلف عقلي وخلال في كل من النمو والسلوك قد يبدأ مبكرا في الجينات نتيجة لخلال في الجينات الوراثية. وقد لاتظهر أي علامات أكليوبسكية على كثير من الأطفال المرضى بالخلال الكرموسموي و يتم اكتشاف هذا الخلل بالعديد من الاختبارات العملية و يوجد العديد من الاختبارات لعمل فحص جماعي لهذه الحالات مثل تحليل الكرموسمات و اختبار الوراثة الخلوية الجزيئية باستخدام مجز من الحمض النووي لكرموسم معين وكذلك اختبار الهرمونات والأملاس و هذه الاختبارات قد تساعد في التشخيص المبكر للحالات القابلة للعلاج وكذلك اعطاء النصيحة للآباء لتقليل فرص الإصابة فيما بعد. وكان الهدف من هذا البحث ينصب على تقييم الكرموسمات في هؤلاء الأطفال وتحديد نموذج الكرموسم الوراثي عن طريق فحص خص الكرموسمات الابيويه وايضا الكرموسمات من عائلات أخرى مصابها بالإضافة إلى خصائص أشخاص طبيعيين كمجموعة ضابطة و قد تم اخذ الموافقة من هذه العائلات على إجراء هذه الفحوصات و تم اخذ خمسين من الأطفال ذوى النمو المتاخر وابنهم وعدد من أفراد العائلة واخصائيم لتحليل كروموسومي. كما تم اخذ خمسين من الأطفال الطبيعيين كمجموعة ضابطة واخضاعهم لنفس التحاليل. وتم اخذ عينة دم من كل شخص ورئي في وسط خاص ثم تم جمع الخلايا وهي في مرحلة الانقسام الخلوي المتوسط ووضعها على شرائح زجاجية. ثم احضرت العينات الفحص الكرموسمي باستخدام الالتقيات العادية بعد تشريط الكرموسمات بواسطة أنزيم الترسيبين ثم صبغه بصبغة الجيما. و قد تم عمل اختبارات تاكيديا لبعض الخلايا التي تحتوى على خلل كروموسمي معقد. وفي خصائص حالات المصليين تبين وجود تسع وعشرين طفلا من بين الخمسين المصابين بالتخلف العقلي يعانون من تشابهات ظاهرة و بعض الخلايا وعشرين طفلا ويدينيون لا تظهر عليهم أي تشابهات ظاهرة. و في خصائص الحالات التسععة والعشرين تبين أن خمس حالات فقط ببعض عيب تركيبية واضحة في الكرموسمات كما أظهر خصائص الحالات الواحد والعشرين الأخرى وجود عيب تركيبية في الكرموسمات في حالتين فقط و في حالة واحدة فقط في الأطفال الخمسين الاصلحاءما. و توصي الدراسة بفحص الكرموسمات في الأطفال ذوى النمو المتاخر غير متلازم لأن ذلك يفيد في التشخيص المبكر للحالات القابلة للعلاج. أيضا يفيد اعطاء النصيحة للآباء مما يؤدي إلى تقليل فرص الإصابة في الأجيال القادمة.