Chromosomal analysis and maternal age risk in some Down syndrome patients

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Abstract: Chromosomal analysis was done on 50 clinically diagnosed Down syndrome patients. The karyotype analysis revealed that 88% (44 of 50 cases) with trisomy 21, having three copies of chromosome 21. In 2% (one case) translocation was observed, one copy of chromosome 21 was translocated to another chromosome 21 and in 10% (5 cases) there was a mosaicism for a trisomic. The higher percentage (72%) of cases was found between 21-34 years. 26% of cases were found with ages above 35 years. Only 2% of cases were found with maternal age between18-20 years and Down syndrome is more frequent in males than in females.

Key words: down syndrome, aneuploidy, trisomy, translocation.

tحليل الكروموسوم وخطورة عمر الأمومة لبعض مرضى متلازمة داون

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الخلاصة: تم أجراء التحليل الكروموسومي لخمسين من مرضى متلازمة داون وقد بيبنت النتائج أن 88% من المرضى يملكون ثلاثية كروموسوم 21 و 2% بانتقال كروموسوم بين كروموسوم 21 و 10% مزدوجة لثلاثية كروموسوم 21. كما بيبنت النتائج أن 72% من المرضى بعمر 21-34 سنة و 26% بعمر أكثر من 35 سنة و 2% بعمر الأمومة 18-20 سنة و أن المتلازمة أكثر انتشارا بين الذكور من الإناث.
Introduction

Down syndrome (DS) is the most common autosomal aneuploidy in man associated with mental retardation, developmental delay, and characteristic physical findings (1). In addition, people with DS have an increased risk for leukaemia (2), congenital heart disease (3), gastrointestinal tract abnormalities, immune defects, and Alzheimer disease (4). The birth prevalence of Down syndrome is approximately 1 in 700 to 1 in 1000 which makes the syndrome the most frequent cause of mental retardation (5,6).

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Advanced maternal age at the time of conception is the most established significant risk factor for meiotic non-disjunction of chromosome 21 (7,8). Penrose, (1933)(9) was the first who noted the effect of advanced maternal age on the rate of DS. About 2% of recognized pregnancies of women under the age of 25 years are trisomic, this increases to 10% for women of 36 years and to 33% by the age of 42 years (10). The influence of maternal age has been observed in all population studies in respect to race, geography or socioeconomic factors. However, the basis for the effect of increasing maternal age on the nondisjunction rate is largely unclear. In human female meiosis starts in the 3rd month of fetal life and is arrested in prophase of MI from 6 months of fetal life onwards until ovulation which takes around 10 to 40 years (10,11). Altered recombination is another important factor after maternal age which is associated with non-disjunction error. Warren et al. (1987)(12) was the first who provided evidence that a proportion of maternal non-disjunction errors were associated with reduced recombination along chromosome 21. Further studies regarding the etiology of Down syndrome demonstrated a relationship between the non-disjunction event and altered recombination (13), most of these studies approved that the location of the recombination is a risk factor for non-disjunction of trisomy 21( 8).

Down syndrome is the most common genetic cause of mental retardation accounting for 25-30% worldwide (14). Cytogenetic studies have shown that approximately 95% of diagnosed Down syndrome cases have a complete trisomy 21 and the remaining 5% either have somatic mosaicism (~1%) or chromosome 21 translocations (~4%) (15). Faulty chromosome distribution leading to Down syndrome is more likely to occur at older maternal age. In almost all cases of translocation trisomy, one of the parents is carrier of a balanced Robertsonian translocation of the long arm of chromosome 21 to the long arm of a D- or G-group chromosome (16). De novo Robertsonian translocation are rare, one between chromosome 14 and 21 t(14;21) has been described originating from maternal germ cells (17). In contrast, most translocations between the long arms of two chromosomes 21, t(21;21) are isochromosomes due to a duplication of (21q) rather than a result of a Robertsonian translocation (18). In addition to that approximately 2-4% of DS patients are detected as mosaics (19).

In DS, approximately 95% of the cases are due to non-disjunction resulting in an extra copy of a chromosome 21 (trisomy 21) the remaining are due to
translocations involving chromosome 21 and somatic mosaicism (13). Most trisomy 21 cases are due to an error in maternal meiosis, whereby about 70% originate during maternal meiosis I and about 20% during maternal meiosis II, defective paternal meiosis is found for up to 8-10% of all cases (20). The present study was carried to determine the cytogenetic profiles of Down syndrome cases in correlation with maternal age and to study the frequency of Down syndrome in our community.

Materials and Methods
Fifty individuals (34 males and 16 females) (age between 6 months to 7 years) who attended the medical city teaching laboratories – genetic department during the period from April 2010 – September 2010 were enrolled in the present study. They were suspected to have Down syndrome. The diagnosis was based on a clinical examination, as well as, laboratory investigation (chromosomal analysis of peripheral blood lymphocytes).

Collection of Samples
Peripheral blood samples were collected from each patient. The blood (2 ml) was collected by venues puncture using a disposable 5ml syringe, and it was drawn into heparin tubes for the assessment of chromosomal abnormalities. The peripheral blood samples was cultured immediately and assessed for chromosomal abnormalities.

Blood culture assay and chromosomes preparation
The procedure of Rooney and Czepulkowski (1992)(21) was followed in which 10 ml of RPMI-1640 culture medium were supplemented with 0.2 ml of PHA, 10% Fcs, 0.5 ml (10 drops) of blood was added in a 10 ml culture tube. Then the following steps were followed:
   i. The culture tubes were incubated at 37ºC in a slant position (about 45º) to increase the surface area for 72 hours (long-term culture) with a gentle mixing twice a day.
   ii. Twenty-five minutes before the end of the incubation period, 0.1 ml of colchicine was added to each tube with mixing and the incubation was continued till the end of incubation period (72 hours).
   iii. After that, the tubes were centrifuged (1500 rpm for 10 minutes) and the supernatant was discarded.
   iv. The cell pellet was gently suspended with remain supernatant and 10 ml of warmed (37ºC) hypotonic solution (0.075M KCl) was added, the tubes mixed gently and incubated in a water bath (37ºC) for 20 minutes.
   v. The tubes were centrifuged (1500 rpm for 10 minutes) and after centrifugation, the supernatant was discarded.
   vi. The cell pellet was gently suspended in the remains of the hypotonic solution and few drops of chilled fixative were added and then the volume was made-up to 5 ml with the fixative with a continuous mixing.
   vii. The tube was incubated at 0ºC for 30 minutes, and then it was centrifuged (1500 rpm for 10 minutes). Steps vi and vii were repeated several times until the pellet became white.
   viii. The cell pellet was well-suspended in 2 ml of the fixative and 3-4 drops of the cell suspension were dropped on a clean slide (cleaned well with a detergent, washed several times
with distilled water and then kept in absolute methanol at 4°C overnight from a height of about two feet.

ix. Slides were air-dried at room temperature and stored for 4-7 days before staining.

x. Slides were then treated with 0.025% trypsin solution for 4-5 seconds, rinsed with PBS then stained with Giemsa stain for 8-10 minutes. The slides were washed with tap water and left to dry.

xi. Chromosomal abnormalities were examined using the magnification power 1000 (100 x 10) and at least 50 metaphase were examined for each sample.

Results and Discussion

Down's syndrome patients profile

Chromosomal analysis was done on 50 clinically diagnosed Down syndrome patients. The karyotype analysis revealed 88% (44 of 50 cases) with trisomy 21, i.e. having three copies of chromosome 21 in 2% (one case) translocation was observed, i.e. one copy of chromosome 21 was translocated to another chromosome 21 and in 10% (5 cases) there was a mosaicism for a trisomic and a normal cell line (Table 1).

Table 1: Cytogenetic profile of Down syndrome patients

<table>
<thead>
<tr>
<th>Cytogenetic Profile</th>
<th>NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free trisomy 21</td>
<td>44</td>
<td>88</td>
</tr>
<tr>
<td>Translocation (21,21)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Incidence of Down syndrome in different maternal age

It has long been recognized that the risk of having a child with Down syndrome increases with maternal age (22). The increase in risk for chromosomal abnormalities with relation to women age is gradual until the age of 33, after which the risk begins to rise at a faster rate (23). The American College of obstetricians and gynecologists recommends that all women above the age of 35 at the time of delivery be offered amniocentesis during pregnancy. Although the cut off limit of age 35 has been arrived at arbitrarily, it is still the traditional age at which a woman is considered to be at high risk for chromosomal abnormalities (24).

In this study the data of 50 child with clinical diagnosis of Down syndrome was correlated with three maternal age groups (Table 2). The higher percentage (72%) of cases was found between 21-34 years and this could be explained as most of pregnancies occur in this period of age. 26% of cases was found with ages above 35 years, these two results was slightly higher than those reported by Kothare et al., (2002)(25). Only 2% of cases were found with maternal age.
between 18-20 years and this low percentage may be due to the small number of patients in this study.

Table 2: Incidence of Down syndrome in different maternal age groups

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Maternal age range</th>
<th>Total No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18-20 years</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>21-34 years</td>
<td>36</td>
<td>72</td>
</tr>
<tr>
<td>III</td>
<td>≥ 35 years</td>
<td>13</td>
<td>26</td>
</tr>
</tbody>
</table>

Correlation of maternal age with cytogenetic profile
Nondisjunction (free trisomy 21)

Chromosomal nondisjunction is a random event that occurs more frequently as women get older. However, since it can occur at any time, children with trisomy 21 can be born to women of all ages (Figures 1 and 2). In fact because most pregnancies occur in younger women approximately 80% of all babies with trisomy 21 are born to women under the age of 35 (26). It is very much evident in this study as 37 cases (74%) had maternal age between 18-34 years of age (Group I and II together). This make up 74% of women to be less than 35 years. A study reported in India shows that 60% of Down syndrome is born to women less than 30 (25). The chromosomal profiles of Down syndrome cases having maternal age ≥35 showed 100% nondisjunction. Group I is neglected because of small number (only one patient) and those with maternal age between 21-34 years showed 83.3 nondisjunction (Table 3). Thus offering the evidence that advanced maternal age increases risk for a nondisjunctonal event in the ovum (24). Various hypotheses put forward to explain nondisjunction of chromosome 21 are:

1. Altered meiotic recombination patterns (27,28,29).
2. Grand maternal age effect implying fetal ovarian trisomy 21 mosaicism (30,31)
3. Fetal ovarian trisomy 21 mosaicism, the Oocyte mosaicism selection model (32).
4. Accumulation of toxic effects and environmental insults during fetal and post natal development (33,15).
Table 3: Correlation of maternal age and chromosomal aberration

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Maternal age</th>
<th>Total No.</th>
<th>Cytogenetic Profile</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18-20 years</td>
<td>1</td>
<td>Free trisomy</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Translocation (21,21)</td>
<td>Zero</td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mosaicism</td>
<td>Zero</td>
<td>Zero</td>
</tr>
<tr>
<td>II</td>
<td>21-34 years</td>
<td>36</td>
<td>Free trisomy</td>
<td>30</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Translocation (21,21)</td>
<td>1</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mosaicism</td>
<td>5</td>
<td>13.9</td>
</tr>
<tr>
<td>III</td>
<td>≥ 35 years</td>
<td>13</td>
<td>Free trisomy</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Translocation (21,21)</td>
<td>Zero</td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mosaicism</td>
<td>Zero</td>
<td>Zero</td>
</tr>
</tbody>
</table>

Figure 1: Metaphase of peripheral blood lymphocytes from patient with Down syndrome shows 47, XX+21 Karyotype (Trisomy 21)
Translocation

In the present study only one case had Down syndrome resulting from Robertsonian translocation (Figure 3). The translocations most commonly involve a D group chromosome (chromosome number 13, 14 and 15) (34). In this study the translocation happened between the long arms of two copies of chromosome 21 i.e.(21q,21q).This type of Robertsonian translocation which occur between two homologous chromosomes involving chromosome 21 is familial (inherited) in 25%of these patients and de novo in 75%. Gonadal mosaicism for isochromosome 21 has been reported and recurrence of Down syndrome is recorded which is about 1% (35). If it is inherited i.e. one parent is a carrier for a balanced Robertsonian translocation involving homologous chromosome 21, these carriers can’t produce any normal gametes so the recurrence rate will be 100%, while the risk of producing a child with Down syndrome in a carrier had a balanced translocation between two non homologous chromosomes involving chromosome 21 will mainly depend on the sex of the carrier parent (36), in female carrier it is about 15% while in male it is much lower (about 1% to 2%) (37). Maternal age also relates to the type of chromosomal abnormality. Translocation occurs 10% of the time in children born to mothers between 15 and 19 years of age (38). In our study only one case was seen between 21 and 34 of maternal age (Group II). Whenever chromosome analysis reveals a translocation, both parents should undergo karyotype study to check for a balanced translocation.
Figure 3: Metaphase of peripheral blood lymphocytes from patient with Down syndrome shows 46, XY, t (21;21)(q10;q10) karyotype (Translocation 21, 21)

Mosaicism
Mosaic (46/47, +21) is detected in 8.75% of Down syndrome cases (25) in present study higher incidence had demonstrated 10% (Table 1). The common concept is that advanced maternal age (≥ 35) is an increased genetic risk for Down syndrome baby. But as it has been mentioned before most pregnancies in Iraq occur in younger women, so trisomy 21 children are therefore born to women under the age of 35 years (Table 3). The maternal age less than 35 years observed in 74% of cases (Group I&II), really poses the question whether only advanced maternal age should be considered as criteria for selecting women for pregnancy monitoring or should Down syndrome pregnancy screening be done routinely for all pregnant women.

Sex ratio of DS cases with different types of trisomy 21
The results of the current study showed that Down syndrome is more frequent in males than in females (Table 4) and this is consistent with a recent study done by Petersen et al. (1993)(39), in which a multicolour FISH technique is used for detection of X and Y bearing sperm in disomy 21 sperms. Their results showed that among sperm disomic for chromosome 21 significantly more were Y bearing than X bearing, these findings are consistent with those of Petersen et al. (1993)(39) who found that trisomy 21 conspectuses that resulted from paternal meiotic errors were significantly more likely to be male than female (40). The basis for the association between chromosome 21 nondisjunction and the presence of a Y chromosome is unclear, but two possibilities similar to those proposed by Petersen et al., (1993)(39) are (1) there is aberrant exchange at prophase I, between a chromosome 21 and the Y chromosome leading to co-segregation at meiosis I and (2) the two chromosomes 21 fail to pair and/ or recombine and segregate against the X chromosome. These results pertain only to paternally delivered cases of trisomy
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21, which according to molecular studies of chromosome 21 nondisjunction constitute ≤ 10% of all cases of trisomy 21(7).

Table 4: Sex ratio of Down syndrome cases among different type of trisomy 21

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Males</th>
<th>Females</th>
<th>Male: Female Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free trisomy</td>
<td>31</td>
<td>13</td>
<td>2.38</td>
</tr>
<tr>
<td>Translocation (21,21)</td>
<td>1</td>
<td>Zero</td>
<td>Zero</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>2</td>
<td>3</td>
<td>0.67</td>
</tr>
</tbody>
</table>

References


