Clinical and karyotypic aspects of Down's syndrome in Sudanese patients

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Abstract

Introduction Trisomy 21 or Down syndrome is the most common type of autosomal chromosome abnormality it has three chromosomal patterns the free copies of chromosome 21, translocation and mosaics. The aims of this study were to evaluate the clinical and the karyotypic pattern of Sudanese Down's syndrome patients and to compare the data with what have been reported before.

Material and Methods A total of 230 patients referred during 2009 and 2013 for confirmation of Down syndrome by cytogenetic analysis at the Cytogenetic Unit at Al Neelian Medical Research Center, faculty of medicine, Al Neelian University, Sudan.

Cytogenetic analyses performed in peripheral blood samples that cultured in RPMI 1640 medium for three days, the clonality criteria and the karyotypic descriptions were according to the ISCN 2009 recommendations.

Result Out of the 230 cases of Down's syndrome male to female ratio was found to be (1.2:1) in the referred cases, free trisomy 21 was present in 221cases (96. 4%), 6 patients had translocation (2.6%) and three cases were mosaics (1.3%) The median maternal age of the Sudanese mothers at the birth of the affected child was 35.9 years the average age at presentation was 15.6 months (range 1 days to13years), 43.9% of the cases had congenital heart disease.

Conclusion the identification of specific types of chromosomal abnormalities in Down's syndrome patients showed that regular trisomy 21 is more common than translocation and Mosaics which is important to assists in patient management and family counseling.

Key words:

Cytogenetic analysis; karyotype pattern; Genetic counseling; Down syndrome, mosaic, nondisjunction, translocation

Introduction

Down syndrome is a common genetic disorder found in about one in 600 to one in 1,000 livebirths [1]. This can be due to nondisjunction, in which genetic materials fail to separate during a crucial part of the formation of gametes, resulting in an extra chromosome (trisomy 21). The cause of nondisjunction is not known, although it correlates with a woman's age reference.

The additional material present influences development and results in the state of Down syndrome. It is the single most common genetic cause of mental retardation and is thus of major socioeconomic concern [2]. The phenotype of Down syndrome is complex and variable in severity among individuals; including mental retardation and cognitive deficits, heart defects, hypotonia, motor dysfunction, immune system deficiencies, an increased risk of leukemia [3].There are three different types of Down syndrome, the Trisomy 21 also called no disjunction Syndrome is the most common type of Down syndrome and is the cause of approximately 84.6-95% of all cases of this syndrome [4]. Trisomy Down syndrome is not hereditary.mosaic type, this type is very rare and is caused by non-disjunction of the chromosome pair 21 shorterly after fertilisation. The result is that tow cells line one contain an extra chromosome 21 while the other cells contain only 46 chromosomes, mosaicism is reported in 1.42% of DS cases [5]. The translocation type of Down syndrome. When a portion of one chromosome21 is transferred to another chromosome, translocation are attributed to 3-4% of DS cases [2]. This risk differs greatly between the cases as nondisjunction, and mosaicism rarely recur in siblings of people with DS; translocation may be recurrent, depending on the type of translocation [2,6]. A clinical diagnosis of Down syndrome may be unconfirmed in one third of cases. [7,8] .The diagnosis is normally confirmed through conventional karyotyping, through evidence of extra material from chromosome 21. [9] Karyotyping is essential for confirmation of the clinical diagnosis, determination of recurrent risk and to provide a basis for genetic counseling [1].

Objectives

The aims of this study were to describe the cytogenetic pattern, to study the link between maternal age and trisomy 21 in cases referred to the Genetic Unit. Aleelian Medical Research Center Alneelian University, faculty of medicine Sudan and we compared our findings with those of previous studies.

Materials and methods

Out of several cases referred to the Cytogenetic Unit at Aleelian medical research center Alneelian University, Faculty of Medicine. This is the referral laboratory for cytogenetic Investigation in sudan) during the period from 2009 to July 2013, 230 patients with a cytogenetically-confirmed diagnosis of Down syndrome were included in this study .For routine cytogenetic analysis, 0.3- ml peripheral blood samples were collected from the patients into heparinized test tubes, and then were incubated in complete lymphocyte culture medium (10% fetal bovine serum in RPMI-1640 with 0.15% phyto hem agglutinin and 1%Penstrept) in 5% CO2 incubator at 37 °C for 72h. Metaphases are harvested by adding colcemid for 45 min followed by hypotonic KCl treatment for 5 min and fixation using standard 3:1

methanol-acetic fixative (all the reagents were from Gibco Life Technologies Ltd., Paisley UK).A high-resolution study was done by synchronization using methotrexate (10–7*M*) for 17 h and thymidine (10–5*M*) for 5.5 h before harvesting, as mentioned elsewhere. The karyotype of each patient was determined by G-banding using trypsin and Giemsa (GTG) technique according to Seabright M1971 [10]. At least 30 cells were routinely analyzed; in cases of mosaics, this number was increased to approximately 100 metaphases. The best metaphases were photographed to determine the karyotypes. If the case was carrier of a translocation or unusual karyotypes, their parents or other family members were also tested [4]. Cytovision imaging applied photography and karyotype were done for documentation of cases, the karyotypic descriptions were reported according to the International System for Human Cytogenetic Nomenclature recommendations ISCN 2009 [11]. The relative frequency of each diagnostic group was calculated, and the percentage of abnormal cases and the distribution of the numerical and structural abnormalities were determined in each group. The frequencies were compared to similar studies using the Z test for Comparison of two frequencies with unequal variance. The findings in these cases are summarized in the Results section .A detailed interview was conducted with all cases before cytogenetic analysis, and a detailed medical history was obtained. Informed consent for genetic testing was obtained from all patients.

Results

Two hundred and thirty cases (127 males and 103 females) male to female ratio, (1.2:1) included in this study were cytogenetically confirmed cases of Down syndrome. The rate of parental consanguinity among Dawn's syndrome in this study is (34.7%). The median maternal age at the time of birth of the affected child was 37.8 years-old. Mothers aged older than 40 year were 114 patients (49.6%), from 30 - 39 years were 105 patient (45.2%), and Those from 20 -29 year-old accounted for 5.3% of Down's syndrome patients . The average age of patients presentation was 15.6 months; ranged between 1 day and 13-years. One hundred twenty patients (52%) ranked as first and second child.Congenital heart disease seen in 43.9% of the cases; most common cardiac defects observed were ventricular septal defect (17.%) and atrial septal defect (15%).

The vast majority of cases 221 patients (96.4%) had trisomy21, followed by translocation in 6 patients (2.6 %), and mosaic seen in 3 patients (1.3%). The frequency of down syndromes was presented in Table (1). The frequency distribution of maternal age, chromosomal aberration, sex ratio and consanguinity in Down syndrome presented in Table (2).

Table (1) The frequency of down syndromes

Karyotype	frequency	Percentage%
46,t(14;21)	5	1.50%
46XY,t(21;21)	1	0.43%
47,XX,+21 and 47,XY,+21	221	95.9%
47,XY,+21/46,XY and 47,XX,+21/46,XX	3	1.29%
Total	230	100%

Table (2)The frequency distribution of maternal age, chromosomal aberration, sex ratio and consanguinity in Down syndrome

Maternal age	No=230	Ratio(male: female)	consanguinity	Cytogenetic profile	No.of cases	Total percentage (%)
20-29 year	12	1.5	10.5%	Trisomy	9	5.3%
				Translocation	2	
				Mosaic	1	
30-39 year	104	1.2	14%	Trisomy	99	45.%
				Translocation	3	
				Mosaic	2	
40-49 year	114	2.1	12%	Trisomy	113	49.7%
				Translocation	1	
				Mosaic	0	

Discussion

The excess of males appears to be universal and was reported in all studies in different countries that the sex ratio ranged from 1.1:1 to 2.3:1 [12]. However, it varied according to the type of down syndrome, in the current study Down syndrome was identified especially in males which observed a similar gender ratio as 1.1% males by Balkan et al. (2010) [14]. 1.14:1 reported by Abdel-Hady ElGilany et al. (2011) [2]. 1:1.1 Azman et al. (2007) [1]. 1.24:1Mokhtar et al. (2003) [12]. And 2.3:1 Sheth et al. (2007) [13]. The distribution of different anomalies

associated with DS in the present study is very similar to earlier studies worldwide. Regular trisomy 21 was range from 84.6-95% [12]. The frequency of regular trisomy 21 in present study was 96.08%. This was relatively compatible with 96% reported by other others [14].

Previous study have reported that the frequency of down syndrome translocation varies from 0%-4.6% [15]. The Robertsonion translocation Down's syndrome included in the recurrent study was similar to 2.7% reported by Nazmy et al.(2003) [12]. 2.3% by Al-awadi et al(1985) [16]. And it was little less when compared with 3.6% by Stoll c et al.(1990) [17]. 3.1%, by Goud et al.(2005) [4]. 3.8% by Mutton et al.(1996) [18]. 3.1% reported by Abdel-Hady El-Gilany et al, (2011) [2] .And higher than 1.5% by English CJ et al.(1989) [19].The result also are lower than 8.9% by Mohamed M. Mokhtar,8.5% by Sheth et al.(2007) [13].

Translocation may arise as a sporadic event *de novo* or may be transmitted by a carrier parent (familial) [20]. 25% of Robertsonian translocation result from familial transmission in Down's syndrome and three quarter are *de novo* [15]. The five cases of translocation observed in the present study was the result of a Robertsonian translocation between chromosome 14 and 21, four of them had arisen *de novo.* Both parents of this translocation Down's syndrome child showed normal karyotype, the last case was familial translocation, the balance translocation from maternal original. An interesting observation in this study was seen in one case where karyotypic analysis showed *t*(14;21), the father was the carrier of a balanced translocation the interested in this case that the paternal karyotype showed mosaic translocation in one cell line *t*(14;21) the other cell line was normal.

The recurrent risk is less than 1% if the translocation is *de-novo*. In case of familial Robertsonian translocation Down's syndrome, the genetic risk for female carrier to have a live born child with translocation Down's syndrome is about 10-20% which increases to 15% at amniocentesis [21]. For male carrier the recurrence risk to have a child with translocation Down's syndrome is about 1%, most *de-novo* rearrangements (21q21q) are isochromosomes derived from a single parental chromosomes 21 and only small proportion isconsistent with true Robertsonion translocation [21]. In this study one case (16.7%) of the translocation cases was the de novo t (21;21) the pervious study have reported that according to molecular studies many of this *de novo* cases originate at an early post zygotic mitosis so the recurrence risk is low [15]. The geneticist will inform the couple of the possibilities of antenatal diagnosis, if desired. In rare cases, trisomy 21 by translocation involves two chromosomes 21. If one of the parents is carrying this translocation in a balanced state, the risk of recurrence is 100 [20]. Detection of structural abnormalities is very important, since in these cases there is a need for parental karyotyping evaluation for assessment of future reproductive risk.

The pervious study have reported that

mosaics accounted from 0%-4.6% [22]. However, the present study found only 1.3% with ratio of men to female 2:1The value of this frequency is similar to a study performed in the Sultanate of Oman. by Goud et a l (2005) [4]. 1.3%, and 1.42% by Aboussair et al.(2011) [5]. And 1.57 %by Vasilica Plaiasu11 (2012) [15]. Higher than 0.7% reported by Mahrous et al(2003) [12]. lower than 3.95 Sheth et al.(2007) [13]. This study found only one cases combination mosaic and translocation 46,XY/46,XY,t (14.21) Most mosaic cases result from a trisomic zygote with mitotic loss of chromosome 21(21). It was reported that despite extensive studies it is not possible to clinically differentiate patients with mosaicism or translocation from those with regular trisomy [15].

Although it is well known that consanguinity increases the risk to offspring, particularly for autosomal recessive conditions, the definite effect of consanguinity on chromosomal abnormality is unknown [23]. A previous Egyptian study reported that about 17% of the patients with DS were products of consanguineous marriages the proportion of consanguineous marriage did not differ according to karyotype [12]. Iran study reported parental consanguinity in 10.4%, among children with DS [12]. Some authors postulate a direct relationship of consanguinity with a higher incidence of dawn syndrome [22]. Which agree with the finding of this study.

In this study The mean maternal age at the time of birth of the affected child was 37.8 years old The frequency of distribution the maternal age above 40 year old was significantly more frequent than another category was found in 113 patients (50%), from 20-30 year old was found in 12 patients (5.3%) and from 30-40 year old was found in 103 patients (45.2%) (the p-value of goodness of fit using chi square =0.000) .This agrees with the previous studies 36.8 years old in Egypt, 33.48 years old in Dubai, 39 year old inMorocco,38.8 year old in Alexandria [2,9,7,12]. On the other hand, a study in India reported that the median maternal age was 25 year [21]. The risk of Down's syndrome and other trisomies increases with the mother's age. The risk of having a baby with Down's syndrome is about1 in 1,300 at age 25 year,1 in 1,000 at age year1 in 400 at age 35 year 1 in 100 at age 40 year and1 in 35 at age 45 year [24,25]. Previous study reported that Down syndrome is the most common autosomal syndrome shows a strong association between increasing incidence and advancing maternal age [5,9,12,17,21,22]. In the present study Mothers of children with nondisjunction Down's syndrome are significantly older than those of translocation and mosaic children this finding within the line with observation of different investigator [2,9,12,15,21].

The age at referral ranged from 1 days after birth up to the age of 14, with a mean of 12.4 months the mean age at referral was 12.2 months in Egypt [2]. And 10.6 months in a Malaysian study [1]. And 19.4 in India [13]. This may reflect low awareness of the family as well as health-care provider for early suspicion of affected newborns .on the other hand an

earlier study in England and Wales revealed that 37.8% of DS cases were diagnosed prenatally, 59.9% were diagnosed postnatally and 2.3% were diagnosed among spontaneous miscarriage [12]. Early intervention programs, which are individualized programs designed to meet the specific needs of each Down's syndrome patient, should be implemented at any time shortly after birth, early intervention helps in each of the four main areas of development: gross motor and fine motor skills, language, social development and self-help skills. Taking this fact under consideration, the age of diagnosis and the age of presentation are important factors for the success in managing Down's syndrome patients [26]. Also early case detection is important for early intervention to the patient's families by genetic counseling and helping in planning care to these children to improve their life's quality. It is important to educate women at high risk of recurrence (e.g., advanced maternal age) to go for screening during pregnancy. Thus in although .Nondisjunction, translocation, and mosaicism are the classical anomalies of DS in the last few years, non-classical DS karyotype (structural or numerical) have been reported in major DS studies with frequency ranging from 0-1.2% [13,18,21,22]. It is important to consider such non-classical DS cases in genetic counseling and provide precise recurrence risk for such distinct groups.

Conclusions

This study highlight the important of identification specific frequencies of the different karyotype patterns of Down syndrome to confirm the clinical diagnosis of Down syndrome, especially with a proper cell count, because of the low-grade mosaicism, and determination of the recurrence risk, as well as to provide a basis for genetic counseling. Advanced maternal age is the principal risk factor for maternal meiotic nondisjunction and trisomy 21. Improvement in medical care, early intervention, special education, and vocational counseling and training, will improve the quality of life for Down syndrome patients and increase their life expectancies**.**

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