



Cytogenetic Study of Down Syndrome in Algeria: Report and Review

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Background: Down syndrome (DS) is the most common type of chromosomal trisomy found in newborn. It is associated with mental retardation and characteristic facial features. A clinical diagnosis of DS may be unconfirmed in one-third of cases. **Objective:** This study was conducted to confirm the clinical diagnosis of suspected cases with DS by a cytogenetic analysis and to evaluate several risk factors associated with trisomy 21 in a group of patients from West region of Algeria, Tlemcen. **Materials and Methods:** Karyotype analysis was carried out for 22 patients with the clinical diagnosis of DS. GTG-band and RTG-band have been made according to the standard protocols. **Results:** Among the 22 cases with DS, free trisomy 21 was presented in 20 cases (91%). One case (4.5%) had translocation DS. One other case had mosaic DS. There was an excess of male than female; sex ratio was 1.75:1. The mean maternal age at birth of the affected children was 36.27 ± 7.59 years. It was significantly higher than this of mothers of nontrisomic children (27.83 ± 6.34 years; $P = 0.0002$). Higher parity was an important risk factor associated with trisomy 21, 81% of affected children were of last or second last birth order. Paternal age and consanguinity had no effect. **Conclusion:** The identification of specific types of chromosomal abnormalities in DS children is very significant. It greatly helped in the management of these children and to make aware the affected families about the recurrence risk and the options available.

Key words: Cytogenetic analysis, down syndrome, karyotype, maternal age, Algeria

INTRODUCTION

Down syndrome (DS) is the most common autosomal abnormality and is the most genetic cause of mental retardation, appearing in about 1 of every 700 newborns.^{1,2} DS can be caused by three types of chromosomal abnormalities: Trisomy 21, translocation, or mosaicism.² Trisomy 21 is characterized by the presence of three copies of chromosomes 21, generally resulting from nondisjunction during maternal meiosis whereas the extra chromosome 21 in mosaic DS arises from mitotic nondisjunction in a chromosomally normal zygote.³ For DS by translocation, the extra chromosome 21 translocated to other chromosomes or to the acrocentric chromosomes of D and G group that is, 13, 14, 15, 21, and 22.⁴

The cause of the nondisjunction error is not known, but there is a definite connection with maternal age. Advanced

maternal age remains the major well-documented risk factor for maternal meiotic nondisjunction. The incidence of trisomy 21 conceptions increases with maternal age.⁵ Subsequently, maternal parity was established as an additional independent risk factor⁶ and genetic predisposition as third independent risk factor.^{7,8} An increase risk for DS may be the result of an autosomal recessive gene mutation, particularly in the Middle East where the rate of consanguinity is increasing.⁹

Karyotype analysis by chromosome banding remains the standard method to identify the cytogenetic variants of DS and to provide appropriate genetic counseling. Most cytogenetic studies in the world indicate that the most frequent type of chromosomal abnormalities in DS is free trisomy 21 with frequency ranges from 93% to 96%, mosaic DS presents a frequency between 2% and 3%, and translocation DS presents

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a frequency ranges from 2% to 5%.¹⁰ However, these values show a geographical variation from the Eastern to the Western countries. In Algeria, the number of children with DS is about 80,000 cases.¹¹ No data is yet available about cytogenetic variants of DS in the Algerian population.

The aim of this study was to describe the cytogenetic profile of children with DS in the west region of Algeria, Tlemcen, study the impact of maternal age and other risk factors associated with this disorder, and then review and compare the findings of previous international studies with our results.

MATERIALS AND METHODS

Sample

The study was carried out on 42 children (22 with DS, 20 control subjects) aged 1–19 years old. They were recruited from the Pediatric Department of Maghnia Hospital and the psychomotor center for mentally challenged children of Maghnia during a period of 8 months (2013–2014). Information on age, birth order, parity parental age, parental consanguinity, and family history of DS at presentation were documented using a questionnaire. All tested individuals were voluntary donors which parents gave consent in compliance with ethical norms get by international conventions.

Karyotype analysis

Chromosome preparation was carried out from 2 to 5 ml of peripheral blood collected in sodium heparin in all cases with clinical features of DS. Chromosomal culture was done according to standard protocol.¹² Peripheral blood lymphocytes were stimulated for 72 h in incubator at 37°C with phytohemagglutinin-M (5 ng/l). Then, metaphases are harvested by adding colcemid (10 mg/l) for 120 min, followed by hypotonic KCl (0.075 M) treatment for 30 min, and fixation using stand 3:1 methanol-acetic acid. Finally, cells obtained were dropped on distinct slides.

The karyotype of each patient was determined by direct staining with Giemsa or by G-banding using banding trypsin solution and Giemsa for staining (GTG)¹³ or by R-banding using phosphate buffer heated to 87°C, then Giemsa for staining (RTG).¹⁴

In each case, 25–50 metaphases were examined and 3–5 cells were photographed and karyotyped. In cases of mosaicism, 50–100 metaphases were scored. Karyotype description was done according to the ISCN (International Standard Committee on Human Cytogenetics Nomenclature).¹⁵

Statistical analysis

Data were analyzed using the software SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as the number and percentage, when

the quantitative variables were presented as mean $\pm$ standard deviation. Student's *t*-test was used for comparison of means. $P \leq 0.05$ was considered statistically significant.

RESULTS

A total of 42 children were included in this study. Twenty-two patients with DS, among them, 14 (63.3%) were males and 8 (36.4%) were females, with male to female ratio of 1.75:1. The mean age at referral was 11.2 years. About 81% of cases were of the last or second last birth orders. Parental consanguinity was reported in 22.7% of the cases. Only 1 patient has a similar case in his family [Table 1].

Mean maternal age at first birth was significantly higher in (1990–2005) (27.2 ± 5.24 years) than in (1974–1989) (22.7 ± 3.92 years) ($P = 0.016$) in our studied population. The age at first parity increase in these last years [Table 1].

For the mean maternal age of mothers at birth of DS children was 36.27 ± 7.59 years (ranges 21–52 years), of which 54.5% were in the advanced age group (≥ 35 years). This mean was significantly higher than the maternal age of mothers of nontrisomic children, whose age was around 27.83 ± 6.34 years ($P = 0.0002$) [Figure 1].

The chromosomal analysis were undertaken in 22 cases, out of which 20 (91%) cases had free trisomy 21, 1 case had trisomy 21 with translocation (46, XY, der (21;21)(q10;q10),+21), and 1 case had mosaic trisomy 21 (47, XY,+21/46, XY) [Table 2].

A comparison of the frequencies of trisomy 21, mosaicism, and translocation DS of the current study with results of previous international studies was carried out. The frequencies of different countries, including Algeria are summarized in Table 3.

DISCUSSION

Trisomy 21 is a common birth defect and can be diagnosed easily on the basis of clinical features. However, karyotyping is necessary for the confirmation of free trisomy 21, mosaicism, and translocation in DS children to determine the recurrent risk and to provide genetic counseling. The data reported in this study represent the first work of DS in Tlemcen, Algeria. All cases were diagnosed postnatally, where a karyotype analysis was done for all studied cases.

In this study, the overall sex ratio was 1.75:1. The excess of males to be universal and was reported in many studies in different countries. Our results are similar to those found by Kolgeci *et al.* in Kosovo (1.72:1),⁴² and near to those of Amayreh *et al.* in Jordan (1.61:1).²⁸

The higher male sex ratio may be the inherent tendency of Y belonging to the G group chromosome to be closer to its other members, 21 and 22, especially the smallest acrocentric

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Table 1: Sociodemographic features of Down syndrome cases

Parameters	Values
Age of children (years)	
Minimum-maximum	5-19
Mean±SD	11.23±4.12
Sex	
Male	14 (63.6)
Female	08 (36.4)
Sex ratio	1.75:1
Birth order (%)	
First and second	04 (18.2)
Third and more	18 (81.8)
Maternal age at first birth (years)	
Minimum-maximum	16-39
Mean±SD	25.18±5.13
Years of birth: 1974-1989	
Mean maternal age±SD	22.7±3.92
Years of birth; 1990-2005	
Mean maternal age±SD	27.2±5.24*
*P	0.016
Maternal age at birth of DS child (years)	
Minimum-maximum	21-52
Mean±SD	36.27±7.59
*P	0.0002
Paternal age at birth of DS child (years)	
Minimum-maximum	27-62
Mean±SD	41.45±8.09
Consanguinity (%)	
Consanguineous	05 (22.7)
Not consanguineous	17 (77.3)
Similar case in the family (%)	
No	21 (95.4)
Yes	01 (04.6)

*Student's *t*-test, SD = Standard deviation; DS = Down syndrome

Table 2: Karyotype analysis of 22 Down syndrome cases

Karyotype	n	Percentage
Regular trisomy 21		
47,XY,+21	12	54.6
47,XX,+21	8	36.4
Translocation DS		
46,XY,der(21;21)(q10;q10),+21	1	4.5
Mosaic DS		
47,XY,+21/46,XY	1	4.5
Total	22	100

DS = Down syndrome

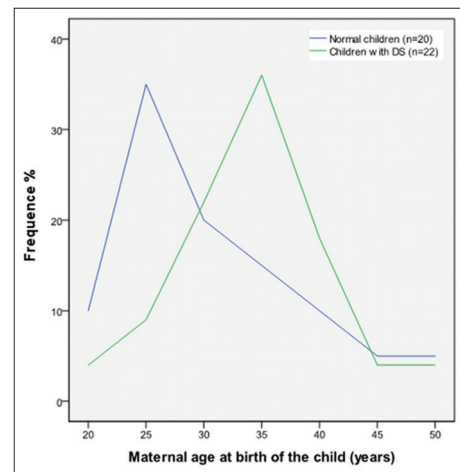


Figure 1: Prevalence of normal and trisomic newborns according to maternal age at term

the 21. The reasons for the excess of male DS-associated to the paternal errors are not yet clearly known.⁴⁷

The birth order of children with DS ranged from 1 to 10. Overall, 81% of them were of the last or second last birth orders. This result agrees with previous studies in the UAE and Dhaka.^{23,48} Several studies suggest an increased risk of DS with increasing parity⁶ that is the same as our result, but at the same time, other studies reported that there is no increased risk with increasing parity.⁴⁹

Age of marriage in this last years became higher than the age in the earliest years, so the age at the first birth became higher. This increase the maternal age for the last births and consequence, the age at birth of DS children became higher in our results where 54.5% of births were over 35 years old.

The mean maternal age at birth of all studied DS children was 36.27 ± 7.59 years, this result agrees with the study of El-Gilany *et al.* in Egypt,¹⁹ where the mean maternal age was 36.8 years, and the study of Jaouad *et al.* in Morocco;⁵ the mean maternal age was 35.39 years. Also agrees with the result found by Verma *et al.* in Libya (35.62 years).¹⁷

Advanced maternal age remains the principal risk factor for trisomy 21. It was reported in many previous studies in different countries: India,⁵⁰ Turkey,⁵¹ Malaysia,³¹ England and Wales,³⁸ Jordan,²⁸ Saudi Arabia,²⁰ Tunisia,¹⁶ and Dubai.²³

Many other studies had shown increased number of DS babies born to the young mothers, like the study of Kava and his collaborators in India, the maternal age at birth of affected children was 26.8 years.⁵⁰ Other study in the same country reported a mean of 24.95 years.⁵²

For older mothers, the maternal age effect may be due to differential selection and accumulation of trisomy 21 oocytes in the ovarian reserve of older women.⁵³

For younger mothers, the mechanism behind the nondisjunction is not well understood. One of the reasons could

Table 3: Karyotype frequencies among studied Down syndrome cases and pooled data from worldwide surveys

Country	Author	Total number	Regular T21 n (%)	Translocation DS n (%)	Mosaic DS n (%)	Nonclassical n (%)
North Africa						
Algeria	Current study	22	20 (91.0)	01 (04.5)	01 (04.5)	-
Morocco ⁵	Jaouad <i>et al.</i> , 2010	852	820 (96.2)	27 (03.1)	05 (00.6)	-
Tunisia ¹⁶	Chaabouni <i>et al.</i> , 1999	500	456 (91.2)	20 (04.0)	24 (04.8)	-
Libya ¹⁷	Verma <i>et al.</i> , 1990	150	144 (96.0)	04 (2.67)	01 (0.67)	01 (0.67)
Sudan ¹⁸	Ellaihi <i>et al.</i> , 2008	05	04 (80.0)	01 (20.0)	-	-
Egypt ¹⁹	El-Gilany <i>et al.</i> , 2011	712	684 (96.1)	22 (03.1)	06 (00.8)	-
Middle East						
Saudi Arabia ²⁰	Qahatani <i>et al.</i> , 2011	72	68 (94.4)	03 (04.1)	01 (01.5)	-
Yemen ²¹	Al-Maweri <i>et al.</i> , 2015	50	50 (100)	-	-	-
Oman ²²	Al Harasi, 2010	680	640 (94.1)	20 (2.94)	19 (2.79)	01 (0.15)
UAE ²³	Murthy <i>et al.</i> , 2007	141	138 (97.9)	01 (00.7)	01 (00.7)	01 (0.7)
Qatar ²⁴	Wahab <i>et al.</i> , 2006	146	143 (98.0)	-	03 (02.0)	-
Bahrain ²⁵	Al-Arrayed, 1999	89	86 (97.0)	01 (1.12)	01 (1.12)	01 (1.12)
Kuwait ²⁶	Al-Awadi <i>et al.</i> , 1991	1024	985 (96.2)	24 (02.3)	09 (00.9)	06 (0.6)
Iraq ²⁷	Al-Mefraji, 2012	39	33 (84.6)	01 (2.56)	05 (12.8)	-
Jordan ²⁸	Amayreh <i>et al.</i> , 2012	80	74 (92.5)	02 (02.5)	03 (03.8)	01 (1.3)
Turkey ¹⁰	Demirhan <i>et al.</i> , 2015	1103	1020 (92.5)	28 (02.5)	28 (02.5)	27 (2.4)
Iran ²⁹	Mehdipour <i>et al.</i> , 1996	150	132 (88.0)	01 (0.63)	17 (11.3)	-
Asia						
China ³⁰	Wang <i>et al.</i> , 2010	86	80 (93.0)	03 (03.5)	03 (03.5)	-
Malaysia ³¹	Azman <i>et al.</i> , 2007	149	141 (94.6)	01 (00.7)	07 (04.7)	-
India ³²	Verma <i>et al.</i> , 1991	2410	2207 (91.6)	98 (04.1)	98 (04.1)	07 (0.3)
India ³³	Mandava <i>et al.</i> , 2010	1572	1400 (89.1)	111 (07.1)	29 (01.8)	32 (2.0)
India ³⁴	Chandra <i>et al.</i> , 2010	1020	855 (83.8)	51 (05.0)	110 (10.8)	04 (0.4)
India ³⁵	Poddar <i>et al.</i> , 2012	45	42 (93.3)	-	03 (06.7)	-
India ⁴	Jayalakshamma <i>et al.</i> , 2010	870	756 (86.9)	77 (08.8)	37 (04.3)	-
Pakistan ³⁶	Ahmed <i>et al.</i> , 2005	295	282 (95.6)	11 (03.7)	02 (00.7)	-
Europe						
France ³⁷	Stoll <i>et al.</i> , 1990	391	368 (94.1)	14 (03.6)	09 (02.3)	-
England and Wales ³⁸	Mutton <i>et al.</i> , 1996	5737	5411 (94.4)	220 (03.8)	66 (01.2)	40 (0.7)
Danemark ³⁹	Zhu <i>et al.</i> , 2013	987	932 (94.4)	29 (02.9)	26 (02.6)	-
Ireland ⁴⁰	Devlin and Morrison, 2004	208	197 (94.7)	03 (01.4)	08 (03.8)	-
Bosnia and Herzegovina ⁴¹	Mačkić-Đurović <i>et al.</i> , 2014	73	60 (82.1)	05 (06.9)	08 (11.0)	-
Kosovo ⁴²	Kolgeci <i>et al.</i> , 2013	305	285 (93.4)	17 (05.6)	03 (01.0)	-
America						
Brazil ⁴³	Trevisan <i>et al.</i> , 2014	644	598 (92.9)	26 (04.0)	20 (03.1)	-
Mexico ⁴⁴	Garduño-Zarazúa <i>et al.</i> , 2013	510	445 (87.3)	15 (02.9)	43 (08.4)	07 (1.4)
Chile ⁴⁵	Astete <i>et al.</i> , 1991	83	68 (81.9)	-	15 (18.1)	-
Australia						
Australia ⁴⁶	Staples <i>et al.</i> , 1991	635	596 (93.9)	26 (04.1)	13 (02.0)	-

DS = Down syndrome; T21 = Trisomy 21

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be that the ovaries of young women are biologically older than their chronological age, which may lead to increased incidence of nondisjunction.⁵⁴

Parent's consanguinity was observed in 22.7% of the effected children with DS. This result agrees with those of literature, where about 17% of patients were products of consanguineous marriages in Egypt.¹⁹ However, the effect of consanguinity on nondisjunction of chromosome 21 has not been clearly defined.⁵⁵⁻⁵⁷

In the current study, the frequency of nondisjunction (free trisomy 21), mosaicism, and translocation were 91%, 4.5%, and 4.5%, respectively. Our results are similar to a study performed in Tunisia by Chaabouni *et al.*,¹⁶ where the frequencies were 91.2%, 4.8%, and 4%, respectively, and another study in India by Verma *et al.*³² the frequencies were 91.6%, 4.1%, and 4.1%, respectively.

The frequency of nondisjunction in previous international studies in North Africa countries ranged from 91% to 96% [Table 3]. In Tunisia, we noted (91.2%),¹⁶ Libya (96%),¹⁷ Egypt (96.1%),¹⁹ and Morocco (96.2%).⁵ However, in Middle East, Asia, Australia, and America countries, the frequency ranged from 81% to 98%. The lowest frequencies were noted in Chile (81.9%),⁴⁵ India (83.8%),³⁴ Iraq (84.6%),²⁷ and Iran (88%).²⁹ While for European countries, the value of free trisomy 21 was around 94%, except in Bosnia and Herzegovina, where the frequency was lower than found in other European countries (82.1%).⁴¹

Previous studies have reported that the frequency of translocation DS varied from 0.67% to 8.8%, where the lowest frequency was noted in Iran, the UAE, and Malaysia,^{23,31,29} and the highest frequency was reported in India (8.8%).⁴ The frequencies around 4% were noted in Tunisia (4.1%),¹⁶ Saudi Arabia (4.1%),²⁰ India (4.1%),³² Australia (4.1%),⁴⁶ and Brazil (4%).⁴³

For mosaic DS, the frequency in previous studies varied from 0.6% to 18.1%. 0.6% was noted in Morocco⁵ and Libya,¹⁷ whereas 18.1% was noted in Chile.⁴⁵ Our frequency (4.5%) is similar to those found in Tunisia,¹⁶ Malaysia,³¹ and India.⁴ However, it is higher than other reports in Egypt (0.8%),¹⁹ Oman (2.79%),²² Jordan (3.8%),²⁸ China (3.5%),³⁰ France (2.3%),³⁷ Denmark (2.6%),³⁹ and Brazil (3.1%).⁴³ In contrast, it is lower than that reported in Iraq (12.8%),²⁷ Iran (11.3%),²⁹ India (10.8%),³⁴ Bosnia (11%),⁴¹ Mexico (8.4%),⁴⁴ and Chile (18.1%).⁴⁵

Among all studied cases here and in previous studies, the frequency of translocation and mosaicism was very much lower than the frequency of free trisomy 21. This could be attributed to the high fertility rate and trends toward reproduction even at an advanced maternal age.⁵⁸

For nondisjunction trisomy 21, the most common error is maternal nondisjunction in the first meiotic division, with meiosis I error occurring 3 times as frequently as meiosis II errors. Most mosaic cases result from a trisomic zygote with the mitotic loss of chromosome 21. The DS cases with unbalanced translocation usually are *de novo* and nearly 25% result from familial transmission.³¹

Various studies have reported the frequency of free trisomy 21 associated with structural and/or numerical anomalies of other chromosomes (nonclassical type of DS) to be 0.15%–2.4%. 0.15% was noted in Oman,²² 0.67% in Libya,¹⁷ 0.7% in England and Wales,³⁸ 1.2% in Egypt,⁵⁸ and 2.4% in Turkey.¹⁰ Whereas, in our study, we did not find this type of DS.

CONCLUSION

In this study, cytogenetic analysis by karyotyping was done for all cases that have clinical features of DS to confirm the clinical diagnosis and to determine the frequency of different types of DS. Our results suggest that free trisomy 21 karyotype is more frequent in DS cases than translocation and mosaic karyotypes. These results were comparable to many international studies in the world. Of the various factors analyzed during the present study, advanced maternal age, and higher parity were the major influencing factors contributing to Down's syndrome. These should be considered as important factors for genetic counseling and to make aware the affected families about the recurrence risk and the options available.

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Conflicts of interest

There are no conflicts of interest.

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