Cytogenetic Findings at Down Syndrome and their Correlation with Clinical Findings

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Abstract

Down syndrome is a genetic state characterized by trisomy of chromosome 21. In the retrospective study for 12 years period (1991-2002) we have conducted correlation between cytogenetics analyses and clinical findings in our centre at 96 male and 83 female patients. Down syndrome was confirmed by cytogenetics analyses in 84(87,5%) male patients and excluded in 12(12,5%) male patients. Down syndrome was confirmed by cytogenetics analyses in 71(85,5%) female patients and excluded in 12(14,5%) female patients. Most common karyotype is free trisomy found in 139 (89,7%) examinees, than follows translocation form determined in 9 (5,8%), and mosaicism determined in 7 (4,5%) examinees. Our results indicate that cytogenetics analyses are necessary to confirm diagnosis of Down syndrome.

KEY WORDS: Down syndrome, cytogenetics findings, clinical findings

Introduction

Down syndrome is the first described syndrome with mental retardation as one of the characteristics. English doctor, Langdon Down (1), extensively described disease as "mongoloid idiotism". Lejeune (2) and Jacobs (3) demonstrated chromosomal abnormality - extra chromosome in the G group. By that example direct relation between anomaly of humane karyotype and phenotype was discovered. Polani et al. (4) discovered translocation form of syndrome and Clark mosaic form (5). Down syndrome is the most common trisomy in human pathology with 1:800 live births. Around 6000 children per year are born with Down syndrome. It estimates that 80 % of all pregnancy with trisomy 21 ends up with spontaneous miscarriage or dead infant; approximately 2% of miscarriages and 1% of dead infants can be related with trisomy 21 (6). Only one well-documented risk factor of trisomy 21 is parent's age, especially mother's age. Risk of children being born with Down syndrome is 1:1400 at women giving birth at age between 20 and 24, 1:900 at age of 30, 1:385 at age of 35, 1:106 at age of 40 and at women giving birth at age of 45 risk factor is 1:30 (7). Higher levels of Down syndrome are observed in male as compared to female (1,5:1). It refers only to free trisomy 21 (7). There are several prenatal diagnostic tests that can be performed to determine the occurrence of Down syndrome. These tests include amniocentesis, chorionic villus sampling (CVS), and percutaneous umbilical blood sampling (8,9). Even so, despite all preventive measures there is still large percentage of Down syndrome unrecognized prenatal (10). A newborn baby with Down syndrome often has physical features that can be recognized in the delivery room. These may include a flat facial profile, an upward slant to the eye, a short neck, and abnormally shaped ears, white spots on the iris of the eye and a single, deep transverse crease on the palm of the hand. Congenital heart disease is the determine factor of survival. Down syndrome children with leukemia have significant mortality rate. Therefore it is recommended to follow up children development in early age due to possible development of acute leukemia, especially acute leukemia M7 (11,12). Since acute leukemia associated with Down syndrome have special biological and clinical characteristics early diagnose of Down syndrome in patients with normal and nearly normal phenotype is fundamental for clinical diagnosis and observing (13). Average lifetime of people with Down syndrome is 50 years and some of them live over 70 years. Individuals with Down syndrome develop Alzheimer disease at an earlier age then individuals from general population. Cytogenetics analyses explain etiopathogenesis of Down syndrome. Without it, clinical diagnosis is uncertain. Cytogenetics analyses and molecular studies (7,14) show that dup21(q22.1-22.2) is sufficient to cause Down syndrome. It is Down syndrome critical region (Figure 1 and 2). The gene DSCR1, identified from this region (21q221-q22.2), is highly expressed in the brain and heart and is a candidate for involvement in pathogenesis of mental retardation and cardiac defects in Down syndrome (7). Basic goal of this retrospective study is to evaluate cytogenetics findings in patients with suspect clinical diagnosis of Down syndrome and establishes correlation between cytogenetics analyses and clinical findings.

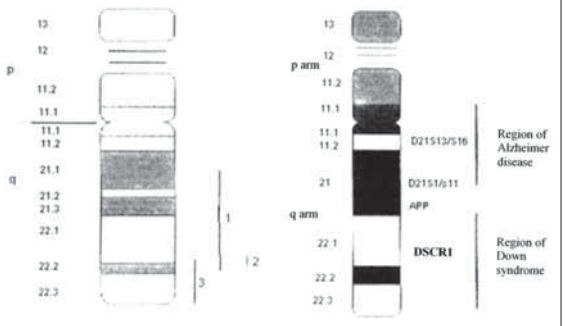


FIGURE 1. Phenotype map of trisomy 21. Segments 1,2,3 are associated with phenotype characteristics: MR, VCC and facial characteristics. Closeness of region for Alzheimer disease explains often association between Down syndrome and Alzheimer disease

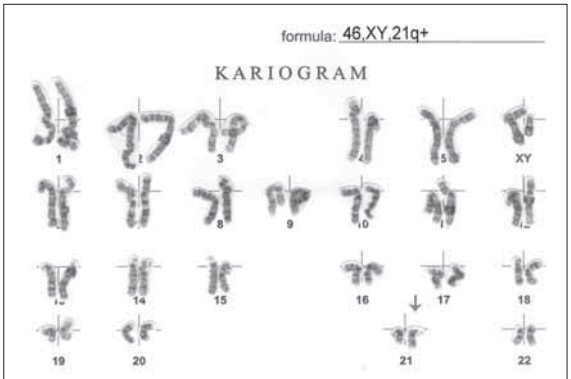


FIGURE 2. Karyogram of the person with partial trisomy 21.

Subjects and Methodes

The research was conducted as retrospective study for 12 years period (1991-2002) and included patients sent to

"Centre for Human Genetics" of Medical Faculty in Sarajevo from different medical canters within Federation of Bosnia and Herzegovina with diagnosis suspect Down syndrome. Down syndrome is diagnosed by karyotype. Several techniques are used in the laboratory: technique of standards, metaphase and promometaphase bending technique. Prometaphase (high resolution) technique insures better quality of cytogenetic analysis (15).

RESULTS

In "Centre for Human Genetics" in Sarajevo, in period 1991-2002, there were 96 male patients and 83 female patients with diagnosis suspect Down syndrome and anomalies multiplies. Cytogenetics analyses confirmed Down syndrome in 84(87,5%) male patients and excluded Down syndrome in 12(12,5%) male patients (Table 1). The results of research of correlation between cytogenetics analyses of male patients and diagnosis suspect Down syndrome in twelve years period (1991-2002) were tested by $\chi 2$ test. Demonstration/excluding Down syndrome is on the level of significance $p<0,05$. Cytogenetics analyses confirmed Down syndrome in 71(85,5%) female patients, and excluded syndrome in 12(14,5%) female patients (Table 2). The results of research of correlation between cytogenetics findings of female patients and diagnosis suspect Down syndrome in twelve years period (1991-2002) were tested by $\chi 2$ test. Demonstration/excluding Down syndrome is on the level of significance $p<0,05$. Cytogenetics findings of all examinees with diagnosis suspect Down syndrome are shown by Table 3. The research results of correlation between cytogenetics findings and clinical diagnosis of suspect Down syndrome, anomalies multiplies, brother/sister of proband and stigmata degenerative for twelve years period (1991-2002) were tested by $\chi 2$ test which proves significant difference in demonstrating/excluding Down syndrome at level $p<0,05$. Age and sex structure of examinees with confirmed diagnosis have been also analyzed (Table 4). The highest number of patients with Down syndrome was from 0-4 age group (144). There were 76(52,8%) male and 68(47,2%) female patients.

Age group 0-4:
$X \pm \sigma = 1,38\ 0,83$

CLINICAL DIAGNOSIS	CYTOGENETICS FINDINGS											
	47,XY,+21		46, XY t(13q;21q)		46, XY t(21q;21q)		47,XY,21/ 46, XY		46, XY		TOTAL	
	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%
DOWN SYNDROME	76	80,9	1	1,0	3	3,2	3	3,2	11	11,7	94	100,0
ANOMALIES MULTIPLIES	1	100,0	-	-	-	-	-	-	-	-	1	100,0
BROTHER/SISTER OF PROBAND	-	-	-	-	-	-	-	-	1	100,	1	100,0
ALL	77	80,3	1	1,0	3	3,1	3	3,1	12	12,5	96	100,0

TABLE 1. Cytogenetics findings of male patients with diagnosis suspect Down syndrome (1991-2002)

CLINICAL DIAGNOSIS	CYTOGENETICS FINDINGS											
	47,XX,+21		46,XX, t(13q;21q)		46,XX, t (14q;21q)		47,XX,+21 /46,XX		46,XX		Svega	
	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%
DOWN SYNDROME	61	75,3	1	1,3	4	4,9	4	4,9	11	13,6	81	100,0
BROTHER/SISTER OF PROBAND	-	-	-	-	-	-	-	-	1	100,0	1	100,0
STIGMATA DEGENERATIVE	1	100,0	-	-	-	-	-	-	-	-	1	100,0
ALL	62	74,7	1	1,2	4	4,8	4	4,8	12	14,5	83	100,0

TABLE 2. Cytogenetics findings of female patients with diagnosis suspect Down syndrome (1991-2002)

CLINICAL DIAGNOSIS	CYTOGENETICS FINDINGS					
	DEMONSTRATED DOWN SYNDROME		EXCLUDED DOWN SYNDROME		TOTAL	
	N⁰	%	N⁰	%	N⁰	%
DOWN SYNDROME	153	87,4	22	12,6	175	100,0
ANOMALIES MULTIPLIES	1	100,0	-	-	1	100,0
BROTHER/SISTER OF PROBAND	-	-	2	100,0	2	100,0
STIGMATA DEGENERATIVE	1	100,0	-	-	1	100,0
ALL EXAMINEES	155	86,6	24	13,4	179	100,0

TABLE 3. Cytogenetics findings of all patients with diagnosis suspect Down syndrome (1991-2002)

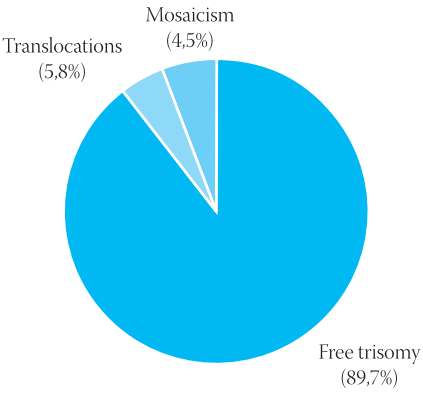
AGE GROUP	AGE AND SEX GROUP					
	MALE		FEMALE		TOTAL	
	N⁰	%	N⁰	%	N⁰	%
0-4	76	52,8	68	47,2	144	100,0
5-9	6	66,7	3	33,3	9	100,0
10-14	2	100,0	-	-	2	100,0
All	84	54,2	71	45,8	155	100,0

TABLE 4. Age and sex structure of confirmed Down syndrome patients (1991-2002)

Age group 5-9:
X ± σ=6,85 1,24

Age group 10-14:
X ± σ=12 2,82

Cytogenetics forms of Down syndrome are shown by Graph 1. Most common type is free trisomy (Figure 3) found at 139(89,7%) patients, afterwards is translocation type (Figure 4) determined at 9(5,8%) patients and mosaicism (Figure5) at 7(4,5%) patients. The research results of frequency of cytogenetics types of Down syndrome in twelve years period (1991-2002) tested by χ^2 and they showed statistic significant level of p<0,05. Maternal age of Down syndrome children has been considered and the results are shown by Graphs 2,3,4. Children with free trisomy 21 were delivered by mothers from age group 35-39 (38,8%, p< 0,001), than group 30-34 (25,2%, p<0,001) and age group 25-29 (17,3%, p<0,001). Children with mosaicism were delivered by mothers from age group 25-29 (42,8%, p<0,001) and age group 35-39 (28,6%, p=0,238). Children with translocation were delivered by mothers from age group 25-29 (33,3%, p<0,001) and age group 30-34 (33,3%, p<0,001) and age group 20-24 (22,2%, p=0,506). The results of cytogenetics findings that forms of Down syndrome (trisomy, mosaicism and translocation) compared with age of mothers for twelve years period (1991-2002) were tested with multiple regression tests. These tests have confirmed frequency of certain type of Down syndrome in certain maternal age groups.



GRAPH 1. Cytogenetics forms of Down syndrome (1992-2002)

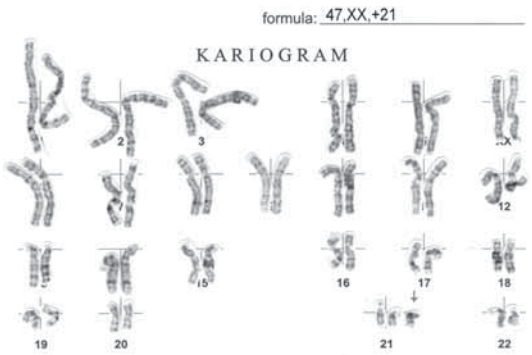


FIGURE 3. Down syndrome. Free trisomy 21

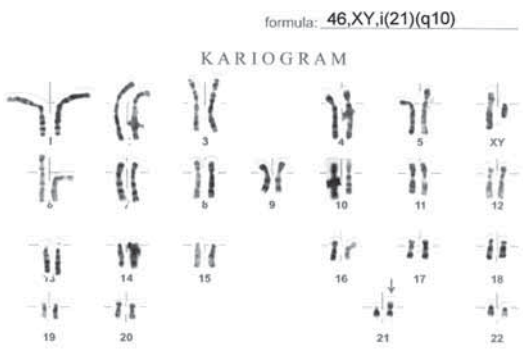
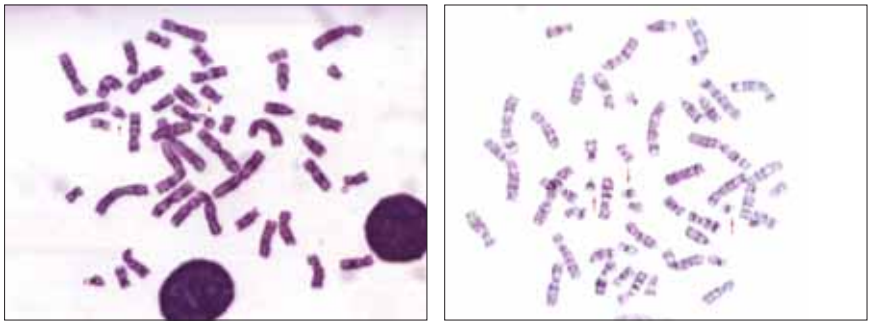
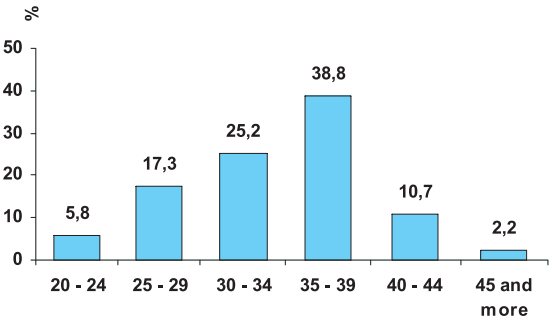


FIGURE 4. Down syndrome. Translocation form with isochromosome 21 [46, XY,i(21)(q10)]

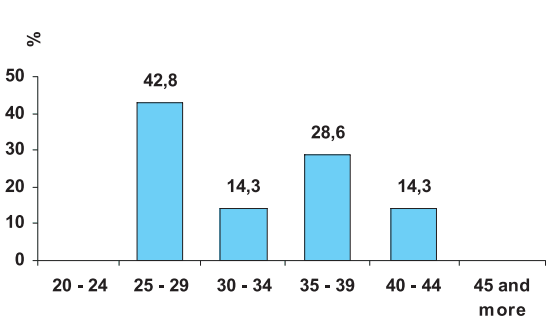


a) normal cell line b) cell line with trisomy 21

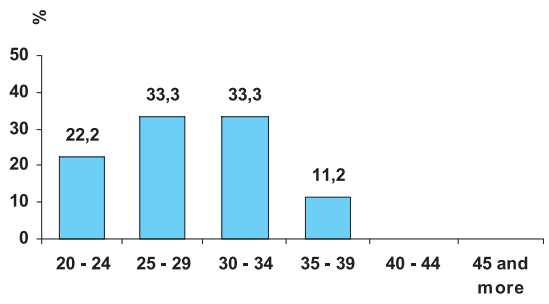
FIGURE 5. Down syndrome. Mosaic type: 46XX/47,XX,+21



GRAPH 2. Maternal age of Down syndrome children with free trisomy (1991-2002)



GRAPH 3. Maternal age of Down syndrome children with mosaicism (1991-2002)



GRAPH 4. Maternal age of Down syndrome children with translocation (1991-2002)

Discussion

A specification of the genotype of individul with Down syndrome always is trisomy 21 chromosome. Without this trisomy Down syndrome can not be diagnosed. It is a leading cause of genotype change, which, actually, conditions phenotype. The basic symptom of the phenotype is a mental retardation without which Down syndrome can not be diagnosed. According to the karyotype there are tree different types of Down syndrome: free, translocation and mosaicism. The most frequent karyotype is free trisomy. It is demonstrated at 94% of ceses (7). In our sample frequency is 89,0%. Free trisomy 21 is a result of meiotic nondisjunctions in one of the parents. The cause of this is the age of the parents. It is result of mother's (80%) or father's (20%) meiotic I or II nondisjunctions. It usually happens during the first maternal meiotic division. Possibility for failures to happen during the second maternal meioses division is rare. Possibility nondisjunctions during the first and the second father's meiosis is almost the same. Translocation type is found in 2,4% (7). In our sample frequency is 5,8%. The most usual type of translocation is centric fusion. It often includes a chromosome 14 (14/21 translocation), 21 (21/21 translocation) or 22 (22/21 translocation). Due to the centric fusion that combines two acrocentric chromosomes number of chromosomes is falsely normal (46), while the genome is increased by one 21 chromosome. Translocations could be resulted from 'de novo' mutation or inherited from one of the parents. If one of the parents is a passive carrier of Roberts translocation then the risk for offspring is 2-100%. The risk of the second child being born with Down syndrome is 100% if one of the parents is a carrier of translocation 21/21. If translocation is sporadic, new mutation, then the risk for offspring is about 1% or maybe higher due to possible mosaicism. Mosaicism could be found in 3,3% (7) of children with Down syndrome. In our sample we found 4,5% of this type of Down syndrome. Mosaicism takes place after fertilization. There are two cell lines in mosaicism: one with free trisomy and the other one with normal karyotype. Mosaicism is connected with large phenotype variability from a normal phenotype to classic trisomy 21. There is a family type of Down syndrome, which occurs in several family members in different generations. One of the causes of a family type is cryptic subtelomeric translocation of chromosome 21q. If it is suspected FISH technology ought to combined with routine cytogenetics analyses (16,17). Children with Down syndrome show singes of the brain damage right after the birth and since their mental development is slowed down and reduced they are prone to cognitive deterioration as well as early dementia development. This is usually resulting of disrupted metabolism of glucoses and oxide-reductive processes. There are hypotheses that use of nutritional factors prenatal, in early childhood or later on might prevent or slow down dementia development in Down syndrome population (18).

Conclusion

During the retrospective 12 year study (1991-2002) evaluation of patients cytogenetics findings was done, whom cytogenetics analysis were done in Center for Human Genetics of Medical Faculty in Sarajevo because of diagnosis suspect Down syndrome. Down syndrome diagnosis was confirmed by cytogenetics analyses in 84(87,5%) male patients and found negative in 12(12,5%) male patients. Down syndrome diagnosis was confirmed by cytogenetics analyses in 71(85,5%) female patients and found negative in 12 (14,5%) female patients. The age of greatest number of patients with Down syndrome was 0-4 (144). There were 76(52,8%) male and 68(47,2 %) female patients. The most frequent karyotype is free trisomy found at 139(89,7%) patients, followed by translocation type found at 9(5,8%) and mosaicism found at 7(4,5%) patients. Children with free trisomy 21 were delivered by mothers belonging to following age groups: age group 35-39 (38,8%, p< 0,001), than age group 30-34 (25,2%, p<0,001) and age group 25-29 (17,3%, p<0,001). Children with mosaicism were mostly born by mothers belonging to age group 25-29 (42,8%, p<0,001) and age group 35-39 (28,6%, p=0,238). Children with translocation were mostly born by mothers belonging to age group 25-29 (33,3%, p<0,001), age group 30-34 (33,3%, p<0,001) and age group 20-24 (22,2%, p=0,506). Cytogenetics analysis is need for monitoring of patients and genetic information. Clinical program of detection of complications in risic populations with Down syndrome might help to decrease mortality in children.

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