Cytogenetic analysis of 4216 patients referred for suspected chromosomal abnormalities in Southeast Turkey

M. Balkan[1], H. Akbas[1], H. Isi[1], D. Oral[1], A. Turkyılmaz[1], S. Kalkanli[1], S. Simsek[1], M. Fidanboy[1], M.N. Alp[1], A. Gedik[2] and T. Budak[1]

[1]Department of Medical Biology and Genetic, Medical Faculty,
Dicle University, Diyarbair, Turkey
[2]Department of Urology, Medical Faculty,
Dicle University, Diyarbair, Turkey

Corresponding author: M. Balkan
E-mail: balkanmah@gmail.com; mahbal@dicle.edu.tr

ABSTRACT. We reviewed cytogenetic studies performed on 4216 patients who were referred to the Cytogenetics Unit at Dicle University Hospital, Diyarbair, Southeast Turkey, between 2000 and 2009. The cases were grouped according to the reason of referral for cytogenetic analysis. The frequencies of the different types of numerical and structural abnormalities were determined, and the relative frequency of cases with abnormal karyotypes was calculated in each group. The most common reason for requesting cytogenetic testing was referral for Down syndrome and for repeated abortions. The highest frequencies of abnormal karyotypes were found among cases that were referred due to suspicion of Down syndrome (84.8%). Among the chromosomal abnormalities, sexual chromosomal abnormalities were found in 239 cases (17.6%), and Klinefelter syndrome was the most frequent sex chromosomal abnormality. Autosomal abnormalities were found in 1119 cases (82.4%), and Down syndrome was the most frequent autosomal chromosomal

abnormality. In conclusion, the high rate of chromosomal abnormalities (32.2%) found in this population demonstrates the importance of cytogenetic evaluation in patients who show clinical abnormalities. This is the first report on cytogenetic testing in the southeast region of Turkey. This type of study provides a basis for determining the risks of recurrence and for deciding on clinical treatment and genetic counseling.

Key words: Cytogenetic; Chromosomal abnormalities; Genetic counseling; Turkey

INTRODUCTION

Chromosome analysis is an important component to the diagnosis and evaluation of genetic disorders including congenital anomalies, developmental delay, and intellectual disability (Méhes and Bajnoczky, 1981; Milia et al., 1984; Butler and Hamill, 1995; Al Husain and Zaki, 1999; Kim et al., 1999; Duarte et al., 2004; Goud et al., 2005; Solak et al., 2007). Approximately 1000 chromosome syndromes that make a major contribution to human morbidity and mortality have been reported so far (Goud et al., 2005). Chromosomal abnormalities affect at least 7.5% of all conceptions. Most of these abnormalities are spontaneously aborted and the frequency in live births is 0.6% (Duarte et al., 2004). The increased awareness of the importance of chromosomal abnormalities in some diseases such as a cause of intellectual disability or dysmorphism, infertility and so on has generated an increased demand of cytogenetic studies (Al Husain and Zaki, 1999). This has led to an increased recognition of many chromosomal disorders that otherwise would have been missed. In addition, it has noticed by Al Husain and Zaki (1999) that some clinicians refer cases for cytogenetic study before exhausting other less expensive and time-consuming tests that may lead to the final diagnosis. In some instances, the patients were referred just to exclude the possibility of having an associated chromosomal abnormality (Al Husain and Zaki, 1999).

In the present study, we determined the commonest causes of requesting cytogenetic study at the Dicle University Medical Faculty Hospital, Diyarbair. In addition, we calculated the frequency of chromosomal abnormalities among individuals who showed such pathological features suggesting the presumable presence of some chromosomal abnormalities and compared these figures with those reported in previous similar studies. This study is the first report from the Southeast region of Turkey with tables showing a review of the literature and summarizing overall incidences. We hope that awareness of these frequencies will help clinicians working in Diyarbair and other country to determine the priority of requesting cytogenetic study in individual cases. It should also help to recognize the commonest presentations of the prevalent chromosomal abnormalities in the area, thus allowing proper genetic counseling to be offered.

MATERIAL AND METHODS

The cytogenetic findings from 5688 cases obtained between 2002 and 2009 were reviewed. The samples were referred to various medical sites, but the majority (80%) were

referred to the Departments of Dicle University Hospital in the city of Diyarbakir, Southeast Turkey. All samples were analyzed in the Medical Biology and Genetic Department Laboratory at Dicle University for cytogenetic analyses. The laboratory provides a postnatal and prenatal service to departments of different hospitals in Diyarbair and its surrounding province in Southeast Turkey. The laboratory appraisal of the cases was the responsibility of the Department of Human Genetics. 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.

The age of the patients ranged from birth to 50 years with a mean of 14.3 (SD = 12.45) years. Of the 4216 patients, 48.3% were females and 51.1% were males. They presented disorders such as congenital anomalies, intellectual disability, amenorrhea, etc., together with a clinical suspicion of some chromosomal abnormalities. Clinical features and hypothesized diagnosis are reported in Table 1. For routine cytogenetic analysis, 0.3-mL peripheral blood samples were collected from the patients into heparinized test tubes, and then was incubated in complete lymphocyte culture medium in incubator at 37°C for 72 h. Metaphases are harvested by adding colcemid for 60 min followed by hypotonic KCl treatment for 5 min and fixation using standard 3:1 methanol-acetic fixative (all reagents were from Gibco Life Technologies Ltd., Paisley, UK).

The karyotype of each patient was determined by G-banding using trypsin and Giemsa (GTG) (Seabright, 1971) and C-banding using barium (Sumner, 1972) and Giemsa (CBG) (Salamanca and Armendares, 1974) when necessary. At least 30 cells were routinely analyzed; in cases of mosaicism, 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 an inversion or unusual karyotypes, their parents or other family members were also tested. Translocations not detected by conventional light microscopy were submitted to the fluorescence *in situ* hybridization (FISH) method using whole chromosome painting (WCP) libraries (cytocell for WCP) and α-satellite DNA probes (Samonte et al., 1996), and a minimum of 100 metaphases for each patient were examined. The karyotypic descriptions were reported according to the International System for Human Cytogenetic Nomenclature recommendations (ISCN, 1995).

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.

Those patients who were identified as having chromosomal abnormalities received post-cytogenetic genetic counseling in our Department. Our genetic counseling center was established in 1994 to serve patients. The genetic counselors in our department are professionals who have completed a master's program in medical genetics and counseling skills.

All individuals were informed of the nature of the study, and the signatures of the couples for their informed consent were obtained. All patients interviewed clearly understood that the research was independent of their care, which would not be affected in any way because their participation in the study. None patient declined to participate.

RESULTS

We found that two groups of people referred for examination (couples with repeated abortions and patients with Down syndrome) accounted for more than 50% of the cases (Table 1). The next most common referrals were for intellectual disability, dysmorphic features, congenital anomalies, developmental delay, and Turner and Klinefelter's syndromes (Table 1).

The highest frequencies of abnormal karyotypes found among cases were referred due to suspicion of Down syndrome (53.2), following by Klinefelter's syndrome (25.3%) and Turner syndrome (19.6%) (Table 1). The other groups showed abnormal karyotypes followed by cases with amenorrhea, male infertility, ambiguous genitalia, intellectual disability, dysmorphic features, congenital anomalies and developmental delay, and repeated abortions.

Abnormal chromosomes were found in 16.1% of the cases (Table 1), with 80.0% of these being numerical abnormalities; the remaining 20% were structural variants (Table 2). Of the 734 numerical abnormalities, 543 cases were in the form of trisomies, 111 cases had Klinefelter's syndromes and 80 cases had X monosomies. The frequencies of the different forms of abnormal karyotype are shown in Table 2.

Table 1. Distribution of chromosomal abnormalities according to the reason for referral for cytogenetic study.

Reason for referral	Total		Abnormal	
	N	%	N	%
Down's syndrome	1048	18.4	557	53.2
Klinefelter's syndrome	364	6.4	92	25.3
Turner's syndrome	486	8.5	95	19.6
Primary or secondary amenorrhea	342	6.0	56	16.4
Male infertility	134	2.4	21	15.7
Ambiguous genitalia	162	2.9	22	13.6
Intellectual disability/dysmorphic features/ congenital anomalies/developmental delay, and so forth	568	10.0	30	5.3
Repeated abortions	1892	33.3	44	2.3
Obesity	22	0.4	0	
Miscellaneous	330	5.8	0	
Consanguineous marriages	340	6.0	0	
Total	5688		917	16.1

Table 2. Distribution of numerical and structural chromosomal abnormalities in 1358 cases.

Numerical	No. of cases
Trisomy 21 (including mosaic)	535
Trisomy 18	4
Trisomy 13	2
Trisomy 8	2
Klinefelter's syndrome (including mosaic)	111
Monosomy X	80
Total	734 (80.2%)
Structural	No. of cases
Isochromosome X	33
Unbalanced translocation	24
Markers	4
Balanced translocation	36
Inversions	52
Addition	2
Others	6
46,XX male	10
46,XY female	16
Total	183 (20.0%)

The largest group of referrals was for repeated abortions; both husband and wife were examined in 1892 cases representing 946 couples (Table 1). Fourty-four males and sixteen females had chromosomal abnormalities. These include 12 inversions, 9 balanced reciprocal translocations, 17 Robertsonian translocation, and 1 addition, 1 deletion and 4 markers (Table 3).

Table 3. Chromosomal abnormalities in cases referred for suspicion of repeated abortions.

Results	No. of cases	Abortions
45,XX,der(13;14)(q10;q10)	2	3
45,XY,der(13;14)(q10;q10)	3	2
45,XX,der(14;21)(q10;q10)	3	3
45,XY,der(14;21)(q10;q10)	4	3
45,XX,der(13;21)(q10;q10)	3	3
45,XY,der(13;15)(q10;q10)	2	3
46,XX,add(6)(p12)/46,XX	1	3
46,XY,del 13p	1	2
46,XX,t(3;5)(q13;q22)	1	2
46,XY,t(3;18)(p11;q11)	1	2
46,XY,t(3;7)(p11;q24)	1	6
46,XY,t(4;6)(p25;q31)	1	2
46,XX,t(18;22)(p11.1;22)	1	2
46,XY,t(5;10)(q24;p15.3)	1	3
46,XX,t(4;10)(q25;q26)	1	6
46,XX,t(8;9)(q22;p24)	1	4
46,XX,t(13;14)(q13;q34)	1	2
46,XX, inv,(9)(p13;q13)	2	2
46,XY, inv,(9)(p13;q13)	2	5
46,XX, inv(9)(p12;q13)	2	5
46,XY, inv,(9)(p12;q13)	3	3
46,XX, inv,(9)(p13;q12)	2	4
46,XX, inv(8)(p12;q23)	1	4
47,XX,+mar(22).	1	5
47,XY,+mar(22).	1	4
47,XY,+mar(18).	2	3
Total	44	

A total of 1048 cases were referred for suspected Down syndrome. Of these, 499 had trisomy 21, 32 cases had trisomy 21 with an inversion of chromosome 9 and 24 cases had Robertsonian translocation (Table 4).

Of the 568 cases referred for intellectual disability, dysmorphic features, congenital anomalies, developmental delay, and so forth, 30 cases (13.6.1%) had chromosomal abnormalities. These abnormalities include 11 trisomies, 5 isochromosomes, 4 inversions, 3 balanced translocations, 2 Klinefelter's syndromes, 2 monosomies, and one deletion (Table 5).

Table 4. Chromosomal abnormalities in cases referred for suspicion of Down syndrome.

Results	No. of cases
47,XX,+21	229
47,XY,+21	265
47,XX,inv(9)(p13;q13),+21	10
47,XY,inv(9)(p13;q13),+21	8
47,XX,inv(9)(p12;q13),+21	6
47,XY,inv(9)(p12;q13),+21	8
46,XY,der(14;21)(q10;q10)	10
46,XX,der(14;21)(q10;q10)	12
mos 47,XX,+21/46,XX	2
mos 47,XY,+21/46,XY	3
46,XX, der(21;21)(q10;q10)	1
46,XY, der(21;21)(q10;q10)	1
46,XY,t(9;10)(p24::q25)	2
Total	557

Table 5. Chromosomal abnormalities in cases referred for suspicion of intellectual disability, dysmorphic features, congenital anomalies, developmental delay, and so forth.

Results	No. of cases
47,XX,+21	2
47,XY,+21	2
47,XX,+13	2
47,XX,+18	2
47,XY,+18	3
47,XY,+8	1
47,XXY	2
47,XY,i(18)p	2
46,X,i(Xq)/45,X	2
45,X	2
45,X/47,XY, i(Yq)/47,X,i(Yq),i(Yq)	2
46,XX,inv(9)(p13;q13)	2
46,XY,inv(9)(p13;q13)	2
46, XY,t(16;22)(p11;q13)	1
46, XY,t(16;22)(p11;q13)	1
46,XY,t(9;10)(p24;q25)	1
46,XY,del(Yq)(1.2)	1
Total	30

Of the 486 cases referred for Turner's syndrome, 95 (19.6%) were found to have abnormal chromosomes (Table 6). Of these, 64 cases had monosomy X (including mosaic), the remaining were variant Turner syndrome and inversion of chromosome 9. Of the 364 cases referred for Klinefelter's syndrome; 92 cases (25.7%) had 47,XXY (including mosaic). Table 6 shows the number of cases in other diagnostic groups and the number of abnormal cases that were detected in each group.

Table 6. Chromosomal abnormalities in cases referred for suspicion of Klinefelter's syndrome, Turner's syndrome, primary or secondary amenorrhea, ambiguous genitalia, and male infertility.

Results	No. of cases
Klinefelter's syndrome	
47,XXY	86
47,XXY/46,XY	6
Total	92
Turner's syndrome	
45,X	58
45,X/46XX	6
45,X/46,X,i(X)(q10)/46,XX	4
45,X/46,X,i(X)(q10)	4
46,X,i(X)(q10)	6
46,XX,dup(X)(q21.3q24)	1
46,XX,inv(9)(p13;q13)	16
Total	95
Primary or secondary amenorrhea	
45,X	18
46,XY	10
46,X,i(X)	6
45,X/46,X,i(X)	4
46,XX,inv(9)(p13;q12)	4
46,XX,inv(9)(p13;q13)	14
Total	56
Ambiguous genitalia	
46,XX male	10
46,XY female	7
45,X	2
46,XX,inv(9)(p13;q13)	2
46,XY,t(3;4)(p25;q31)	1
Total	22
Male infertility	
47,XXY	17
46,XY,t(5;6)(q35;q21)	1
46,XY,t(3;7)(q36;q24)	1
46,XY,t(7;9)(p13;q12)	1
46,XY,t(3;18)	1
Total	21

DISCUSSION

In this study, we evaluated the pattern of referral of cases for cytogenetic study in Southeast Turkey, and we compared the distribution of referrals for our study and similar studies performed in Turkey by Solak et al. (2007) and in Saudi Arabia by Al Husain and Zaki (1999). These studies were chosen for comparison because they apply a similar methodology to our study and cases were grouped into almost the same types of referrals (Table 7). We found that there are statistically significant higher frequencies of two groups of people referred for examination (patients with Down syndrome and Klinefelter's syndrome) (Table 1). These variations may be explained by social and economical influences. For example, the parents of children with Down syndrome in Turkey need support to exist, such as the welfare of the handicapped and free and special education/training.

Table 7. Comparison of the current study with the ones of Solak et al. (2007) and Al Husain and Zaki (2007).

	Current study				Solak et al. (2007)				Al Husain and Zaki (2007)			
	Total referrals		Abnormal karyotype		Total referrals		Abnormal karyotype		Total referrals		Abnormal karyotype	
	N	%	N	%	N	%	N	%	N	%	N	%
Down's syndrome	1048	18.4	557	53.2	116	5.5	72	62.1	140	7.0	120	85.7
Klinefelter's syndrome	364	6.4	92	25.3	-	-	-	-	38	1.9	12	31.6
Turner's syndrome	486	8.5	95	19.6	48	2.3	6	12.5	94	4.7	10	10.6
Amenorrhea	342	6.0	56	16.4	46	2.2	6	13.0	62	3.1	8	12.9
Male infertility	134	2.4	21	15.7	212	10.0	18	8.5	38	1.9	12	31.6
Ambiguous genitalia	162	2.9	22	13.6	160	7.6	6	3.7	90	4.5	4	4.4
Intellectual disability/ Dysmorphic features/ Congenital anomalies/ Developmental delay	568	10.0	30	5.3	230	10.9	12	5.2	628	31.4	58	9.2
Repeated abortions	1892	33.3	44	2.3	564	26.7	12	2.1	546	27.3	20	3.6

Of the 5688 cases evaluated, 917 cases (16.1%) showed chromosomal abnormalities (Table 1). The frequency of chromosomal anomalies was considerably higher than that related to an unselected population (0.5-0.6%) (Hamerton et al., 1975; Hook and Hamerton, 1977; Patil et al., 1977), and it was also higher than the 6.3% by Solak et al. (2007), while it was similar to 14.3% by Butler and Hamill (1995) and the 13.4% by Al Husain and Zaki (1999), and smaller than the 21.6% found by Milia et al. (1984), the 27.2% found by Verma and Dosik (1980), 28.6% by Santos et al. (2000), and 29.3% by Duarte et al. (2004). The differences in the frequencies of the chromosomal abnormalities among these studies could explain increased interest in genetic diseases by physicians and reflect variations in criteria for inclusion of the patients and the cytogenetic methods used and discordance of classification criteria (Kim et al., 1999). In addition, the high frequency of abnormal cytogenetic findings in our group might have been due to the inclusion of the cases with inversion of chromosome 9 as abnormal variants, and the high frequency of Down syndrome in the chromosomal anomalies.

The highest frequencies of abnormal karyotypes were found among cases who were referred due to suspicion of Down syndrome (53.2%), following by Klinefelter's syndrome (25.3%) and Turner syndrome (19.6%) (Table 1). This reflects the ease of diagnosis in these syndromes.

Chromosomal abnormalities are one of the most important causes of male infertility (Balkan et al., 2008; Akgül et al., 2009). The overall incidence of a chromosomal factor in in-

fertile males ranges between 2 to 8%, with a mean value of 5% (Akgül et al., 2009). This value increases to about 15% in azoospermic males, largely due to cases with 47,XXY aneuploidy (Akgül et al., 2009). Among patients with male infertility in our study, the incidence of a chromosomal abnormality was 45.7% and Klinefelter's syndrome was the most common type of karyotype abnormality (Table 6). All of those with Klinefelter's syndrome had azoospermia, but translocation carriers had oligospermia.

The clinical findings in the patients with ambiguous genitalia were genital ambiguity, except two cases who had 45,X, and their phenotypes also included short stature.

Cases with intellectual disability, dysmorphic features, congenital anomalies, and developmental delay came next seventhly in frequency among abnormal karyotypes (Table 1). Retrospective clinical examination of those patients with developmental delay showed that some of them have some subtle dysmorphic features that were missed by the referring physician (Al Husain and Zaki, 1999). This shows the need to stress thorough clinical search for subtle dysmorphic features in cases of intellectual disability. Those features may give the clue to diagnosis and early requisition of cytogenetic study before exhausting other routine investigations.

The prevalence of carriers of chromosomal abnormalities among cases with repeated abortions was 4.6% per couple in our study. This value is reported as 7.4% (Yuce et al., 2007), 4.2% (Solak et al., 2007), 9.8% (Tunç et al., 2007), and 9.8% (Duzcan et al., 2003) in similar studies in Turkey, and 8.3% (Milia et al., 1984), 7.2% (Chandley, 1990), 8.1% (Méhes and Bajnoczky, 1981), and 7.4% (Al Husain and Zaki, 1999) in other countries.

Among sexual chromosomal abnormalities, the most frequent were Klinefelter's syndrome (25.3%) and Turner's syndrome (19.6%). Among cases with Klinefelter's syndrome, the classic karyotype (47,XXY) (93.5%) was more common than somatic mosaicism (46,XY/47,XXY) (6.5%), and 65% of the cases showed the classic, well-defined phenotype, whereas the others had various types of sexual behavior problems (such as reported by Duarte et al., 2004).

Among autosomal chromosomal abnormalities, the most frequent was Down's syndrome (88.6%). Nearly 70% of the Down's syndrome cases were children less than one year old. This increase in the frequency of the cases of Down syndrome in our study might be the result that a low percentage of mothers (55%) have gone for follow-ups at a health institution to have prenatal diagnosis during pregnancy. In addition to legal factors, religious factors also play a 76% role in parental decisions regarding abnormal prenatal test results. For example, interruption of pregnancy after 120 days (about the 16th week) is forbidden by Islamic Law unless continuation of pregnancy has a confirmed risk to the mother's life (Awwad et al., 2008). The high rate of birth [the range of existing children: 5 (1-12)] and low educational level in mothers indicate that more should be done for training these couples.

Males accounted for 53.1% of the Down's cases. In a study, it is observed a similar gender ratio as 54.6% males by Duarte et al., 2004. There was considerable karyotypic variability in individuals with Down's syndrome between our study and those studies previously reported (Al Husain and Zaki, 1999; Duarte et al., 2004). These observations emphasize the importance of cytogenetic confirmation in cases of Down's syndrome. Eventually, karyotyping can help indicating the risks of recurrence of the syndrome, and can also be useful in the clinical follow-up of some disorders associated with Down's syndrome.

It is reported that most fetuses with trisomy 18 are spontaneously aborted (Giaccardi et al., 1991). When the pregnancy is brought to term, the post-natal lifetime is limited to one or two months in 80% of the cases, except when there is somatic mosaicism. Our patients were

with nonmosaic trisomy 18 and all cases died within six months. Only two cases were identified as having Patau's syndrome. This syndrome is well known for its low life expectancy and the well-defined features that allow an early diagnosis in the first days of life, except in cases of mosaicism (Duarte et al., 2004). Taylor (1968) reported the mean lifetime of children with trisomy 13 to be 89.2 days, although there can be exceptions. Our patients died within two months and mosaicism was not observed in this individual.

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 (Amudha et al., 2005). While the frequency of consanguineous marriage was 20% on average in Turkey, our study population have a high rate of consanguineous marriages (39.8%). No chromosomal abnormality was seen in the groups of people referred for consanguineous marriages in our study (Table 1).

In conclusion, a high rate of chromosomal abnormalities (16.1%) found in our referred population demonstrates the importance of cytogenetic evaluation in patients who are clinically abnormal. Although there are limitations in our study, in particular that these data are from a single clinical service and therefore do not represent population prevalence, the present study is the first report from the Southeast region of Turkey with tables showing a review of the literature and summarizing overall incidences. We hope that the information obtained by such studies will provide a basis for determining the risks of recurrence and for deciding clinical treatment and genetic counseling.

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