A Retrospective Study in Cases with Sex Chromosome Anomaly in Samsun and Around

Samsun ve Çevresinde Cinsiyet Kromozomu Anomalili Olgularda Retrospektif Çalışma

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ABSTRACT Objective: Chromosomes have a major role in the etiology of disorders associated with sexual development. In this study, we investigated the frequency of chromosome anomalies in patients with sex anomaly. **Material and Methods:** Cytogenetic analysis was performed using Giemsa banding and karyotyping was based on the International System for Human Cytogenetic Nomenclature. X chromatin analysis was made from buccal epithelial cells. **Results:** We studied 153 patients [101(66%) female and 52 (34%) male] who were referred to the Cytogenetic Laboratory of Ondokuz Mayıs University Faculty of Medicine, Medical Biology Department and Medical Genetic Section in 2000-2005. Among the 153 patients, 62 (40.5%) were referred for primary amenorrhea, 32 (20.9%) hypogonadism, 19 (12.5%) late puberty, 11 (7.1%) ambiguous genitalia, 1 (0.7%) undescended testes and 28 (18.3%) for other diagnoses. One hundred and thirty one (85.5%) cases had normal karyotype. Chromosome abnormalities were observed in 22 (14.5%) cases. The most frequent chromosome abnormality was Turner syndrome in 9 (5.9 %) cases. The rest were as follows; 2 (1.3%) Klinefelter syndrome, 6 (4%) mosaic Turner syndrome, 2 (1.3%) testicular feminization syndrome, 1 (0.7%) mos 46,XX[59]/46,XY[41] and 2 (1.3%) other abnormalities. In cases where mosaicism was detected, metaphases up to 100 were analyzed. In mosaic cases, 2 (1.3%) had Turner syndrome variant. The genotype of these variants were mos 45,X[35]/46,X,i(X)(q10)[65] and mos 45,X[40]/46,X,i(X)(q10)[60]. Cases of monosomy 45,X were negative for X chromatin. Turner syndrome variant mos 45,X[35]/46,X,i(X)(q10)[65] had 20% and mos 45,X[40]/46,X,i(X)(q10)[60] had 25% X chromatin. In klinefelter syndrome cases, double X chromatin was observed. **Conclusion:** Karyotypes of the patients with diagnosed genital anomalies was investigated and the relationship between genotype and phenotype was assessed. The results of this study suggested that chromosomal analyses were necessary for the clinical management of sex anomaly patients.

Key Words: Sex chromosome aberrations; cytogenetics

ÖZET Amaç: Cinsiyet gelişimi ile ilişkili hastalıkların etiyolojisinde kromozomlar önemli bir rol oynar. Bu çalışmada, cinsiyet anomalisine sahip olgularda kromozom anomalisi görülme frekansı incelenmiştir. **Gereç ve Yöntemler:** Sitogenetik analiz için Giemsa Tripsin Bantlama yöntemi uygulandı ve Uluslararası İnsan Sitogenetik Terminoloji Sistemi (International System for Cytogenetic Nomenclature-ISCN)'ne göre karyotipleme gerçekleştirildi. Bukkal epiteliyal hücreler kullanılarak X kromatin analizi yapıldı. **Bulgular:** 2000-2005 yılları arasında Ondokuz Mayıs Üniversitesi Tıp Fakültesi Tıbbi Biyoloji Anabilim Dalı Tıbbi Genetik Bilim Dalı Sitogenetik laboratuvarına klinikten sevk edilen 153 [101 (%66) kadın ve 52 (%34) erkek] hasta çalışıldı. Yüz elli üç hastanın 62 (%40,5)'si primer amenore, 32 (%20,9)'si hipogonadizm, 19 (%12,5)'u geç puberte, 11 (%7,1)'i kuşkulu genital organ, 1 (%0,7)'i inmemiş testis ve 28 (%18.3)'i de diğer ön tanılarla laboratuvarımıza gönderildi. 131 (%85,5) olgu normal karyotipe sahipti. Kromozom anomalisi 22 (%14,5) olguda görüldü. En sık görülen kromozom anomalisi 9 (%5,9) olgu ile Turner sendromu idi. Diğer anomaliler ise; 2 (%1,3) olguda Klinefelter sendromu, 6 (%4) olguda mozaik Turner sendromu, 2 (%1,3) olguda testiküler feminizasyon sendromu, 1 (%0.7) olguda mozaik mos 46,XX[59]/46,XY[41] kromozom kuruluşu ve 2 (%1.3) olguda diğer anomaliler şeklindeydi. Mozaisizm saptanan olgularda 100'er metafaz analiz edildi. Mozaik TS olgularından 2 (%1,3)'si Turner sendromu varyantıydı. Bu varyantların genotipleri ve X-kromatin oranları sırasıyla, 45,X[35]/46,X,i(X)(q10)[65] için %20 ve 45,X[40]/46,X,i(X)(q10)[60] için %25 olarak saptandı. Monozomi 45'li olgular, X kromatini açısından negatifti. Klinefelter sendromu olgularında çift X kromatin gözlendi. **Sonuç:** Genital anomali saptanan olguların karyotipi incelenerek genotip-fenotip arasındaki ilişki değerlendirildi. Bu çalışmanın önemi, cinsiyet anomalili hastaların klinik takibinde kromozom analizinin gerekli olduğunu vurgulamış olmasıdır.

Anahtar Kelimeler: Cinsiyet kromozomu aberasyonları; sitogenetik

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Sex chromosomes play a crucial role in the etiology of normal sexual development. Sexual development disorders are associated with aberrant sex chromosome karyotypes.[1] The human embryo is a bisexual organism with primordial gonads until 7 weeks after fertilization. Depending upon the genetic gender of the embryo (46,XY or 46,XX), either testicles or ovaries will form.[2,3] Once the gonads begin to differentiate as testes or ovaries, they secrete factors, anti-Mullerian hormone and testosterone from the testes, which determine the following sexual development of the embryo.[4] Sex specific genes present on X and Y chromosomes and autosomal genes also play a role in sex determination.[5]

Sex chromosome anomalies are common and these anomalies produce syndromes that include congenital and developmental anomalies. The numerical chromosome aberrations in these patients arise by non-disjunction either during meiotic divisions occurring in germ-cell development or in early embryonic mitotic cell divisions.[6] These anomalies are sometimes detected prenatally with amniocentesis. Sex chromosome anomalies are often hard to recognize at birth and may not be diagnosed until puberty.[7]

In this study, we investigated the frequency of chromosome anomalies in patients with sex chromosome anomaly between 2000 and 2005 in Samsun and around.

MATERIAL AND METHODS

We studied 153 patients who were referred to the Ondokuz Mayıs University Faculty of Medicine Samsun, Turkey between 2000-2005. Detailed pedigree analysis and clinical reports were reviewed in all subjects. Patients gave informed consent after the procedures were explained. Cytogenetic analysis was performed using phytohemagglutinin-stimulated peripheral blood lymphocyte cultures.[8] Metaphase chromosomes were banded by Giemsa banding (GTG) technique and 25 metaphase plaques were analyzed for each case.[9,10] In cases where mosaicism was detected, metaphases up to 100 were analyzed. Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2005). In some cases, the culturing was repeated twice. X chromatin analysis was made from buccal epithelial cells. Buccal mucosa smear was taken and 100 cells were counted for each subject and the incidence of sex chromatin was calculated. Buccal mucosal cells were stained with Schiff and Fast Green and were scored for X-chromatin.[11]

RESULTS

We studied 153 patients (101 female and 52 male). Age and sex distribution of cases were shown in Table 1. Patients were referred for primary amenorrhea, hypogonadism, late puberty, ambiguous genitalia, undescended testes and other diagnoses (Table 2).

A hundered and thirty one (85.5%) cases had normal karyotype. Chromosome abnormalities were observed in 22 (14.5%) cases. The most frequent chromosome abnormalities in decreasing order were Turner syndrome (TS), mosaic TS, Klinefelter syndrome (KS), testicular feminization syndrome (TFS), mosaic 46,XX/46,XY and other aberrations (Table 3). Karyotype details and the association between karyotype and phenotype of the cases were listed in Table 4.

TABLE 1: Age and sex distribution of the cases (n= 153).

Number of cases	153
Mean age ± SD	20.77 ± 7.33
Age range	2-53
Sex	
Female	101 (66%)
Male	52 (34%)

TABLE 2: Referral causes of the cases.

Referral cause	Number and percent of cases
Primary amenorrhea	62 (40.5%)
Hypogonadism	32 (20.9%)
Late puberty	19 (12.5%)
Ambiguous genitalia	11 (7.1%)
Undescended testes	1 (0.7%)
Other diagnosis	28 (18.3%)

TABLE 3: The karyotype results of the cases (n= 153).

Karyotype	Number and percent of cases
46,XX	131 (85.5%)
46,XY	
45,X	9 (5.9%)
Mosaic TS	6 (4%)
47,XXY	2 (1.3%)
TFS	2 (1.3%)
46,XX/46,XY	1(0.7%)
Others	2 (1.3%)

TS: Turner syndrome.

Out of 153 sex chromosome anomaly cases, only 9 (5.9%) cases showed 45,X; while 6 (%4) cases showed mosaic Turner syndrome genotype. In mosaic cases, 2 (1.3%) who had TS variant had been referred with late puberty/primary amenorrhea. These variant genotypes were mos 45,X[35]/46,X,i(X)(q10)[65] and mos 45,X[40]/46,X,i(X)(q10)[60]. Cases of monosomy X were negative for sex chromatin. TS variant mos 45,X[35]/46,X,i(X)(q10)[65] had 20%, mos 45,X[40]/46,X,i(X)(q10)[60] had 25% sex chromatin. Double Barr bodies were observed in KS case.

CONCLUSION

TS is the most common sex chromosome disorder in females, occurring in approximately one in 2000-3000 live births.[12] TS is a well defined disorder characterized by X chromosome numerical and/or structural abnormalities. Today, we know that nearly 50% of patients have such chromosomal constitution, while the rest have other X chromosome defects, including different forms of mosaicism.[13] In our study, TS was present in 9 (5.9%) and mosaic TS in 6 (4%) of the 153 cases (Table 3). In mosaic cases, 2 (1.3%) who had TS variant hed been referred with late puberty/primary amenorrhea. The genotypes of these variants were mos 45,X[35]/46,X,i(X)(q10)[65] and mos 45,X[40]/46,X,i(X)(q10)[60]. Cases of monosomy X were negative for sex chromatin. TS variant mos 45,X[35]/46,X,i(X)(q10)[65] had 20% and mos 45,X[40]/46,X,i(X)(q10)[60] had 25% sex chromatin.

Different karyotypes as follows have also been reported 46,X,i(Xq); 45,X/46,XX/46,X,r(Y); 45,X/47,XXX, 46,XdelXq; 46,XdelXp, 45,X/46,X,r(?), 45,X/46,X, dic(Y), 45,X/46,XX, 45,X/46,X,r(X).[14-19]

KS is the most common genetic cause of human male infertility with a reported prevalence of 0.1-0.2% in the general population and of up to 3.1% in the infertile male population.[20,21] This syndrome is characterized by the presence of one or more extra X chromosomes and only a minority are diagnosed before puberty.[20] 47,XXY is the most prevalent type; about 20% have 46,XY/47,XXY mosaicism, higher grade sex chromosomal aneuploidies (48,XXXY, 48,XXYY, 49,XXXXY) or structurally abnormal X chromosomes.[20-23] In our study, KS was observed in 2 (1.3%) cases who were pure 47, XXY and there were no variants. Double Barr bodies were analyzed in these cases.

TABLE 4: Association between karyotype and phenotype of the cases.

Referral cause	Total number of cases	Karyotype	Number of chromosome abnormalities
Primary amenorrhea /Late puberty		45,X	6
	81	Mos TS	5
		46,XY female	1
Hypogonadism		47,XXY	2
	32	45,X	3
		46,XY female	1
Ambiguous genitalia		Mos TS	1
	11	46,XX/46,XY	1

TS: Turner syndrome.

TFS is the most common cause of male pseudohermaphroditism.[24] In the study by Ganguly et al, 280 adolescent girls were investigated and overall 80 (29%) cases had some chromosomal anomaly. Among the sex chromosome anomaly cases, 34% were TFS, 51% were pure line, mosaic and other variants of TS.[25]

In our study, TFS was present in 2 (1.3%) of the 153 cases. TFS cases had been referred with primary amenorrhea and other diagnoses. Similar findings have been reported earlier.[26,27]

Primary amenorrhea occurs in 1-3% of women in the reproductive age group.[28] In our retrospective study 153 patients were investigated. Regarding the association between karyotype and phenotype (Table 4); of the 81 cases with primary amenorrhea/late puberty, 6 had 45,X, 5 had 46,XX/45,X mosaic TS and 1 had TFS. Among the 32 cases with hypogonadism, TS was found in 3, KS in 2 cases and TFS in 1 case. Eleven cases were referred as ambiguous genitalia and 1 case was mosaic TS, 1 case was mos 45,X[59]/46,XY.[41]

In the study by Etem et al, 62 cases with primary amenorrhea were investigated. Chromosomal aberrations were seen in 11 (17.7%) cases, 7 (11.29%) cases were TS, 4 (6.44%) cases were TFS.[29] Solak et al made the cytogenetic analysis of 23 cases with primary amenorrhea and reported that 2 cases had TFS.[30] Numerous chromosomal abnormalities have been associated with hypogonadism and ambiguous genitalia.[1] Kaurs et al investigated 156 sex anomaly cases and reported that 40 (25.6%) cases showed abnormal karyotypes.[31]

We investigated the frequency of sex chromosome anomalies in patients who were referred to the Cytogenetic Laboratory of Ondokuz Mayıs University Faculty of Medicine between 2000-2005.

Chromosomes play an important role in the etiology of disorders associated with sexual development. An accurate diagnosis of sex chromosome anomaly could provide an appropriate counseling and/or treatment protocol with hormone replacement therapy or removal of unwanted gonads. This could help to achieve normal development and protect the person from gonadal malignancy. Chromosomal analysis is necessary for appropriate clinical management of these patients.

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