Pattern of chromosomal abnormalities in cytogenetic study: A seven years experience

Saequa Habib[1], Sultana Gulshana Banu[1] , SM Shahedul Islam [1]

[1] Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[1,2,3]Associate Professor, Department of Pathology, Bangabandhu Sheikh Mujib Medical University
Corresponding Author
Saequa Habib

Abstract : Cytogenetic analysis is a vital tool for counseling, that deals with the human issues related to the incidence or risk of a hereditary condition in an exceedingly family and helps to grasp the diagnosing, prognosis and accessible management. This retrospective study was tired the Department of Pathology of Bangabandhu ruler Mujib Medical University, Dhaka, Bangladesh, to spot the frequency and patterns of body aberrations among patients over a amount of seven years (2011-2017). Total 6069 cases were analyzed with a range of clinical disorders. Chromosome analysis was performed on white blood cell culture according to standard methods. Chromosomal abnormalities were found in 1635(26.9%) cases together with polymorphic variants. the very best frequencies of abnormal karyotypes found among referred cases were Down syndrome 1072(65.6%), followed by Disorders of Sex Development 503(30.7%). Pure trisomy 21 was found in 976(91.1%) cases. Among the Disorders of Sex Development, the foremost frequent was the classic Turner syndrome (45, X) (159cases). Autosomal abnormalities were found in 1100(67.3%) cases, 530(32.4%) cases had sex body abnormalities and 05 (0.3%) cases had both autosomal and sex chromosomal abnormalities. Among the referred cases with infertility or subfertility, 21 cases had structural chromosomal abnormalities including 9 inversions, 4 translocations, 7 deletions, and 1 combination of deletion and inversion.We've got reviewed the incidence and distribution of chromosomal abnormalities. This data will enrich the information on the importance of body analysis, so will enhance awareness among clinicians for correct management and counseling.

Introduction

Genetic disorders are far more common worldwide. The lifetime frequency of genetic diseases is 670 per 1000. Chromosome abnormalities account for 50% of all spontaneous miscarriages and are present in 0.5–1.0% of all newborn infants [1]. The development of a reliable technique for chromosome analysis in 1956 led to the discovery that several previously described conditions were due to an abnormality in chromosome numbers. To date, at least 20,000 chromosomal abnormalities have been registered on laboratory databases [2]. Most of these are very rare in single basis, but they make a major contribution to human morbidity and mortality in total. Chromosome abnormalities account for a large proportion of spontaneous pregnancy loss and childhood disability, and contribute to malignancy throughout life as a consequence of acquired translocations and other aberrations. Chromosomal disorders arise from numerical or structural alterations in the autosomes and sex chromosomes. Numerical abnormalities include aneuploidy and polyploidy. In aneuploidy, a single extra chromosome is present or absent, usually as a result of non-disjunction in the first or second meiotic division. In polyploidy, more than two complete haploid sets are present instead of normal diploid complement. There are different structural abnormalities such as translocations, inversions, insertions, rings, and deletions. Translocations can be balanced or unbalanced. Carriers of balanced translocations are at risk of having children with unbalanced rearrangements; these children are usually physically and mentally handicapped [2].

Cytogenetics is a dynamic field of study which analyzes the number and structure of chromosomes. Karyotype is the term used to describe a photomicrograph of an individual's chromosome, arranged in a standard manner. In medical genetics, cytogenetic analysis is becoming an essential source of diagnostic information and evaluation of specific birth defects, genetic disorders, developmental delay, intellectual disabilities, and even cancers [3]. Increased awareness about chromosomal abnormalities among physicians has resulted in an increase in the identification of many chromosomal disorders. There is an accelerating demographic switch to non-communicable diseases and / or syndromes and they are important causes of morbidity and mortality. This study was done to evaluate the cytogenetic findings in patients referred for suspected chromosomal abnormalities. The frequency of the different types of numerical and structural abnormalities was determined and compared our results with those reported elsewhere.

Methods

The present study was conducted retrospectively over a period of seven years (2011-2017) in the Department of Pathology of Bangabandhu Sheikh MujibMedical University, Dhaka, Bangladesh, to identify the frequency and pattern of chromosomal aberrations among patients referred by a wide variety of specialties. Total 6069cases were analyzed. Before cytogenetic analysis, a detailed medical history of all cases was obtained. Patients presented with multiple congenital anomalies, intellectual disabilities, mongoloid features, mental retardation and/or developmental delay, ambiguous genitalia, primary amenorrhea, blood disorders, and short stature were included in the study.

For cytogenetic analysis, as per our protocol, a 5 ml peripheral blood sample was collected from all patients and stored in heparinized test tubes. Chromosomal analysis was performed on cultured lymphocytes in culture medium in an incubator at 37°C for 72 hours. Metaphase harvesting was done by adding colcemid for 45 minutes following hypotonic potassium chloride (KCl) treatment for 10 minutes and later fixation by using 3:1 methanol-acetic acid mixture. Chromosomal analysis was performed according to the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN, 2013) [4]. G-banding with trypsin and Giemsa (GTG) [5] was done to determine the karyotypes, numerical as well as structural abnormalities were reported on at least 100 well-

spread and well-banded metaphases after examination. Well banded metaphases were photographed and analyzed by Cytovision software at 400-550 band resolution. The relative frequency of each diagnostic group including their variants was calculated. The distribution of autosomal and sex chromosomal abnormalities and their karyotypes were described as well.

Results and Discussion

A total of 6069 referred cases with suspected chromosomal abnormalities were analyzed for cytogenetic evaluation. Their ages ranged from newborn to 56 years. A total of 1635(26.9%) cases were found to be having chromosomal abnormalities. Polymorphic variants were considered as chromosomal abnormalities in this study, referred mostly to infertility. The frequencies of chromosomal abnormalities found in other studies were 17.5%, 24.5%, 29.66%, and 12.23% [6, 7, 8, 9]. Distribution of the cases based on clinical indications is shown in Table 1. The highest frequencies of abnormal karyotypes found among referred cases were due to suspicion of Down syndrome (DS),1072(65.6%), followed by Disorder of Sex Development(DSD)503(30.7%).The commonest diagnosis DS was also reported in the studies done in Korea(40.9%) ,Manipal India (12.09%),Malaysia (68.42%), and Maharashtra India (47.95%)[6,7,8,9] .

Table 1. Chromosomal aberrations detected in the study population by karyotyping

Chromosomal aberrations	n	%
Down syndrome	1072	65.6
Disorders of sex development	503	30.7
Edward syndrome	04	0.2
Patau syndrome	01	0.1
Cri du Chat syndrome	01	0.1
Structural abnormalities in infertility/repeated abortions	21	1.3
Miscellaneous	33	2.0
Total	1635	100

Abnormal chromosomes were found in 1635(26.9%) of the cases, of which 1100(67.3%) were autosomal abnormalities and 530 (32.4%) were sex chromosomal abnormalities with 05(0.3%) cases having both autosomal and sex chromosomal abnormalities (Table 2). Kim et al. and Yik et al. found autosomal abnormalities 73% and 88.28% ,respectively along with sex chromosomal abnormalities 26.9% and 11.72% cases, respectively[6,8].

Table 2. Distribution of autosomal and sex chromosomal abnormalities

Abnormalities	n	%
Autosomal abnormalities		
Down syndrome	1072	65.6
Edward syndrome	04	0.2
Patau syndrome	01	0.1
Cri du Chat syndrome	01	0.1
Others	22	1.3
Total	1100	
Sex chromosomal abnormalities		
Turner syndrome	298	18.2
Other DSD	153	9.4
Klinefelter syndrome	52	3.2
Others	27	1.6
Total	530	
Both abnormalities	05	0.3
Total	1635	

Down syndrome (DS), observed in 1072 (97.4%) cases, was the most common autosomal abnormality with the highest frequency (65.6%) among the total abnormal results. Pure trisomy was found in 976(91.1%) cases .Thirty eight cases had mosaicism (3.5%) and 58 cases (5.4%) were translocation DS (shown in Fig. 1).In translocation DS extra chromosome 21 was attached to the chromosome 14, 21, 13, 22 or 15 (Table 3). Other studies also showed similar findings (Table 4).

Table 3. Distribution of variants of Down syndrome

Variants of DS	n (%)	Karyotype
Classical or pure trisomy	976(91.1)	47,XY,+21 or 47,XX,+21
Mosaic DS	38(3.5)	46,XY/47,XY,+21 or 46,XX/47,XX,+21
Translocation DS	58(5.4)	46,XX(Y),+t(13/21) 46,XX(Y),+t(14/21) 46,XX(Y),+t(15/21) 46,XX(Y),+t(21/21) 46,XX(Y),+t(21/22)
	1072(100)	

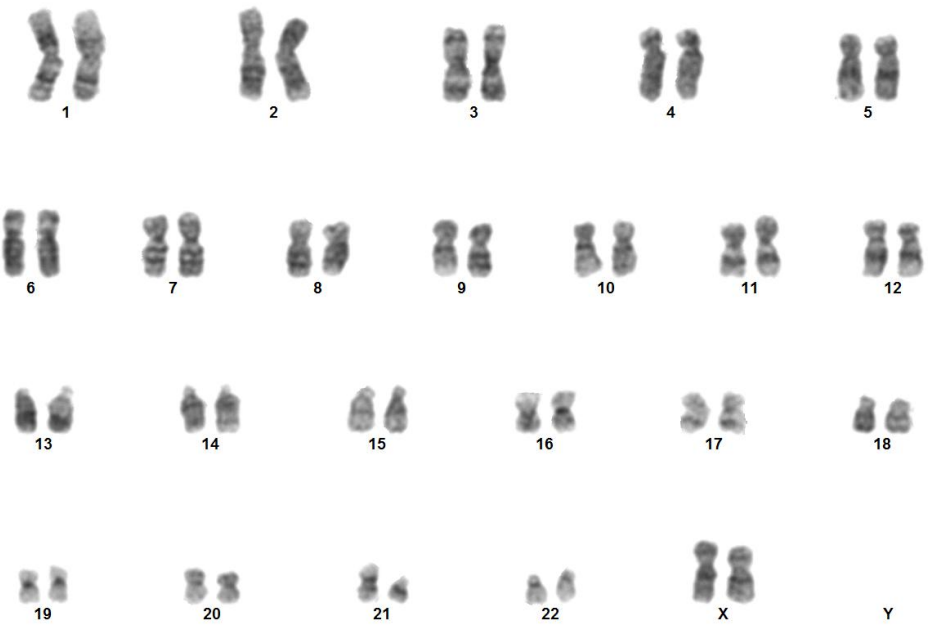

Fig. 1 Photomicrograph of a karyotype 46, XX, +t (21/21) of female child of Translocation DS (Giemsa stain, X100)

Table 4. Distribution of variants of DS in other studies including the present study

Study done by	n (%)		
	Classical or pure trisomy	Mosaic DS	Translocation DS
Kim et al. [6]	273(92.5%)	7(2.4%)	15(5.1%)
Rajasekhar et al. [7]	157(90.69%)	4(4.65%)	4(4.65%)
Yik MYet al. [8]	282 (94.31%)	7(2.34%)	9(3.01%)
Pal et al.[9]	152(92.68%)	1(0.61%)	11(6.71%)
Balkan et al .[10]	499(89.1%)	5(0.89%)	24(4.28%)
Thillainathan et al. [11]	560 (84.2%)	72 (10.8%)	33 (5.0%)
Polipalli et al. [12]	258 (85.2%)	32 (10.6%)	12 (3.9%)
Present study	976(91.1%)	38(3.5%)	58(5.4%)

The incidence of Edward syndrome (Trisomy 18) increases with maternal age and it occurs due to non-disjunction. The overall incidence of trisomy 18 is around 1 in 6000 live births; the majority of conceptions are lost spontaneously with only about 2.5% surviving to term [13]. In this study, only 4 (0.2%) cases of Edward syndrome were reported and one case of Patau syndrome was identified (shown in Fig. 2, 3). Patients with both syndromes have short life expectancies as a result of several life-threatening medical problems.

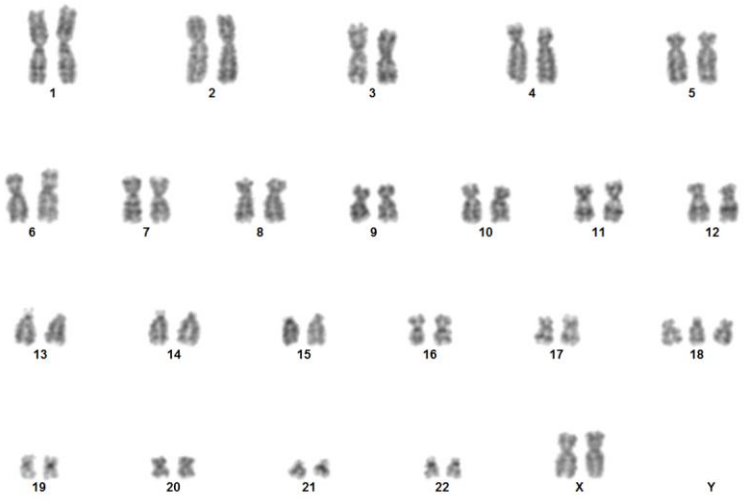

Fig. 2 Photomicrograph showing Trisomy 18 (ES), Giemsa stain, X100

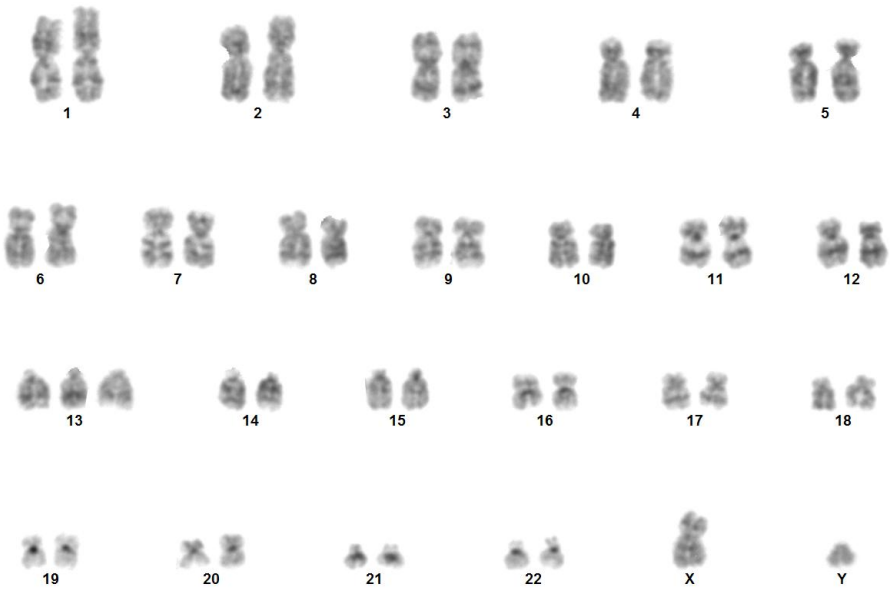

Fig. 3 Photomicrograph showing Trisomy 13 (PS), Giemsa stain, X100

Turner syndrome was the most common DSD among diagnosed cases. In 298 Turner patients, the most frequent result was the classical karyotype (45, X) (159cases). The other Turner showed mosaicism(127 cases) with isochromosome X (i) shown in Figure 4, triple X, ring chromosome X (r) shown in Figure 5 ,deletion X and a marker chromosome component in karyotyping. The most frequent sex chromosome abnormality in our male group was Klinefelter syndrome (50 cases). Mosaic Klinefelter was found in five cases .Mixed gonadal dysgenesis was observed in 14 cases. 46, XY DSD was more common (110 cases) than 46, XX DSD (29 cases). The details of sex chromosome aberrations are summarized in Table 5.

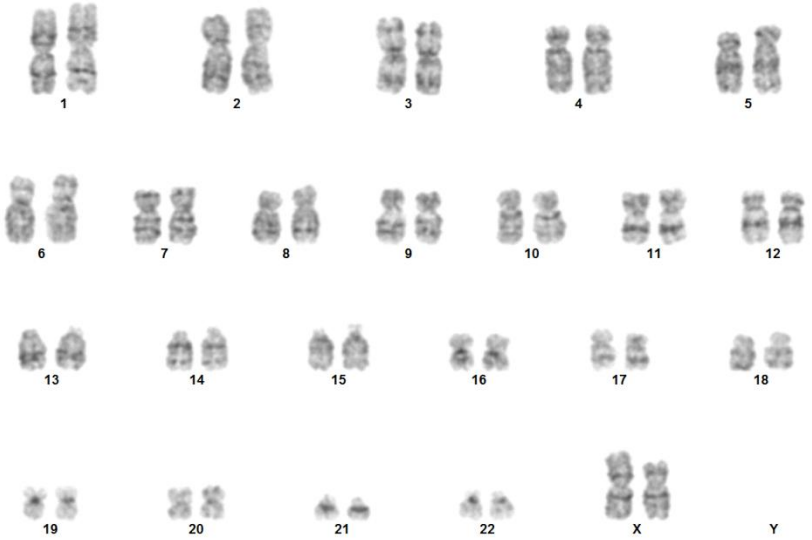

Fig. 4 Photomicrograph of a karyotype 46, X iso (Xq) of female child of TS showing isochromosome X (Giemsa stain, X100).

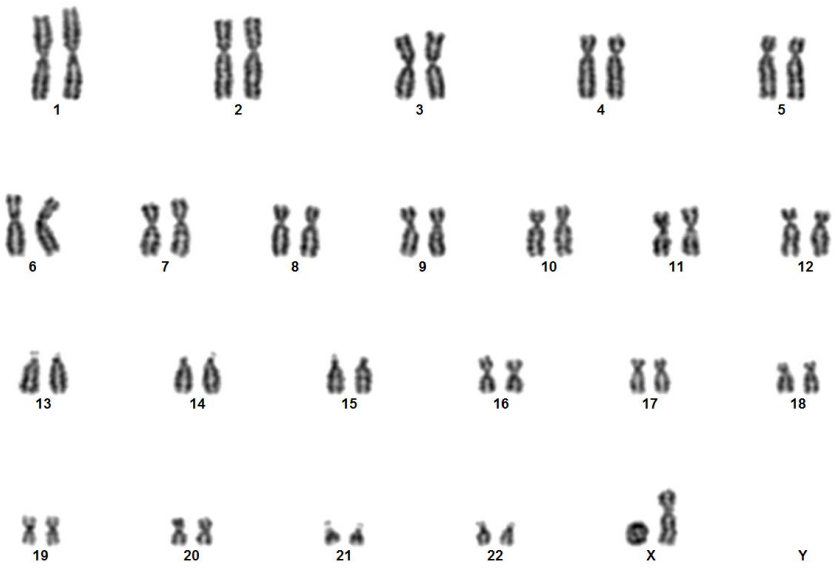

Fig. 5 Photomicrograph showing TS with ring X chromosome, Giemsa stain, X100

Table5. Distribution of disorder of sex development according to Chicago consensus nomenclature [14]

Types	n (%)	Karyotype
Sex Chromosome DSD		
Turner syndrome	298(59.2)	Classic 45,X (n=159)
		Mosaic[45,X/46,XX; 45,X/46,Xiso(Xq);45,X/46,Xr(X);45,X/47,XXX;45,X/46,Xdel(Xq);45,X/46,X,+mar;46,XX/46,Xdel(Xq); 45,X/46,XX/46,Xiso(Xq);46,Xiso(Xq)/47,Xiso(Xq)iso(Xq)] (n=127)
		Other variants [46,Xiso(Xq);46,Xdel(Xq); 46,Xdel(Xp)] (n=12)
Klinefelter syndrome	52(10.3)	Classic 47,XXY (n=45)
		Mosaic 46,XY/47,XXY(n= 05)
		Other variant 48,XXYY(n=02)
Mixed gonadal dysgenesis	14(2.8)	45,X/46,XY; 46XX/47,XXY
46,XY DSD	110(21.9)	46,XY
46,XX DSD	29(5.8)	46,XX
	503(100)	

Turner syndrome affects about 1/2500 female infants born alive. The syndrome results from the total or partial absence of one of the two X chromosomes normally present in females. Cytogenetic data on short stature and primary/ secondary amenorrhea are available in the literature [15, 16]. Duarte et al. [17] and Rajasekhar et al. [7] reported higher proportion of mosaic TS than the classic TS. Although the present findings found more classical monosomy than the mosaic karyotype of Turner syndrome, which is similar to the findings found by Balkan et al. [10]. Distribution of variants of TS in other studies including the present study is shown in Table 6.

Table 6. Distribution of variants of TS in other studies including the present study

Study done by	n(%) of variants of TS		
	Classical	Mosaic	Others
Kim et al .[6]	32(28)	58(50.8)	24(21)
Balkan et al .[10]	78(71.5)	18(16.5)	13(11.9)
Rajasekhar et al. [7]	17(36.17)	23(48.9)	1(2.2)
Thillainathan et al .[11]	27 (54.0%)	20(40%)	3(6%)
Polipalli et al.[12]	13 (25.4%)	38(74.5)	
Yik et al .[8]	13(36.1)	23(63.8)	
Pal et al .[9]	6(16.67)	10(27.78)	4(11.11)
Present study	159(53.36)	127(42.62)	12(4.02)

Klinefelter syndrome occurs due to non-disjunction and the additional X chromosome is equally likely to be maternally or paternally derived. The incidence of 1 in 600 live born males. There is no increased early pregnancy loss associated with this karyotype [13]. Fifty two (10.3%) cases were diagnosed as Klinefelter syndrome in this study .Among these 45 cases with 47, XXY karyotype showing primary features of this syndrome ,5(0.99%) were with mosaic karyotype and 2 cases with 48,XXYY (showing in Fig. 6). This finding is similar to the study done in Malaysia [8] which was 5.17%. But Kim et al. [6] found a relative frequency of 30.41%

KS. with Klinefelter syndrome individuals live near normal lives. The disorder is usually more apparent after marriage as between 95% to 99% of XXY males are infertile [8].

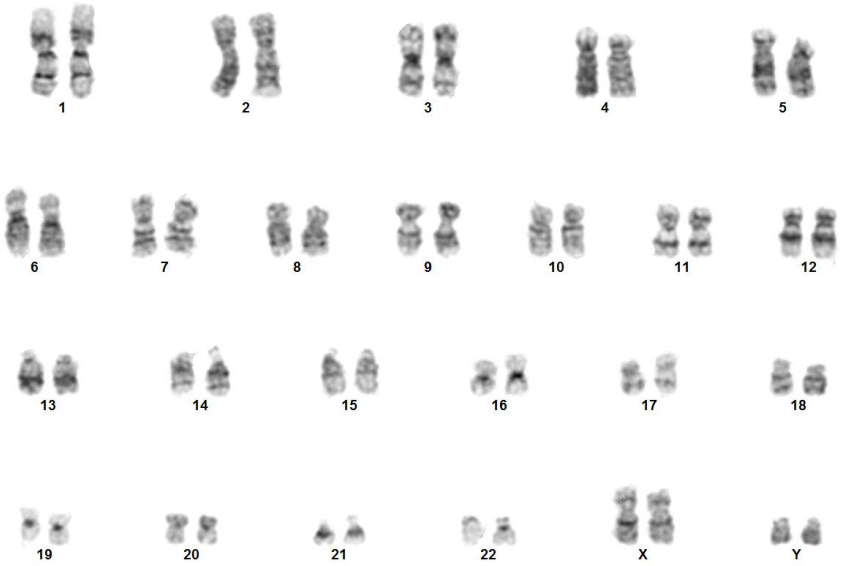

Fig. 6 Photomicrograph of a karyotype 48, XXYY (Giemsa stain, X100)

The condition of imperfect sexual differentiation into either male or female is called DSD. Sexual ambiguity develops due to several disorders of gonadal differentiation. Approximately 1 in 4500 live born infants shows genital ambiguity and needs immediate assignment of sex [18]. The development of external or internal genital tracts is very much under the strict control of genetic cascade and hormonal pathways [19]. Defects in the genetic or hormonal pathways including gene mutations or chromosome aberrations, in appropriate hormone levels or end organ unresponsiveness may result in genital ambiguity [20].In the present study 21.9% cases of 46, XY DSD was found. Rajasekhar et al. [7] and Polipalli et al. [12] reported 14.9% and 9.1% of XY females, respectively. The reason for the higher frequency in this study might be the inclusion of both children and adults and it was similar to the study done by Sangeetha et al. [21] which was 33.3%. An understating of sex determination and differentiation is essential to take appropriate investigation including cytogenetic analysis for proper diagnosis, treatment, and counseling.

Total 2011cases of referrals were for infertility or subfertility with a history of repeated spontaneous abortions (RSA). Both husband and wife were examined in 1612 cases representing 806 couples .Among these ,21 cases had structural chromosomal abnormalities of which thirteen were males and eight were females. The abnormalities included 9 inversions, 4 translocations, 7deletions (shown in Fig. 7), and 1combination of deletion and inversion (Table 7).The incidence of structural abnormalities was 1.3% including inversion of chromosome 9(shown in Fig. 8) which might be considered as polymorphic variants. Numerical anomalies like TS and KS were also found in cases referred to infertility.

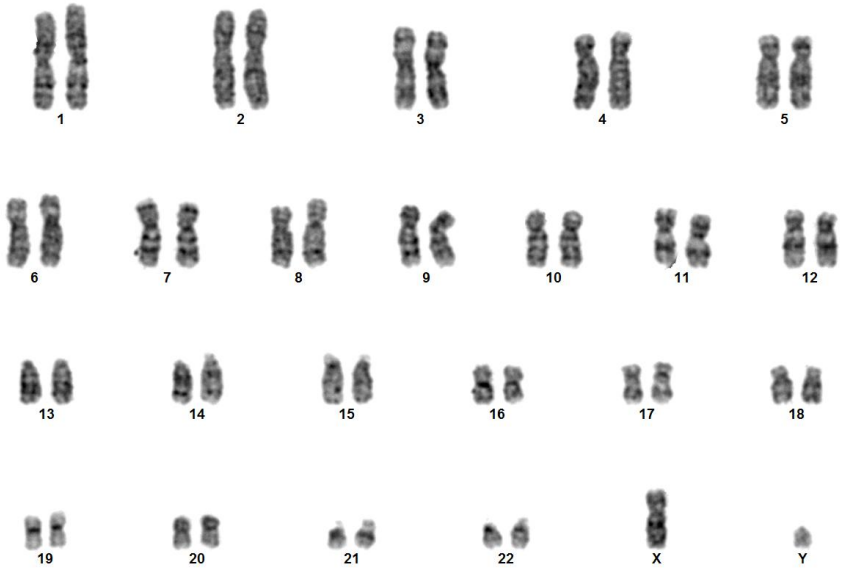

Fig. 7 Photomicrograph showing deletion on Y chromosome, Giemsa stain, X100

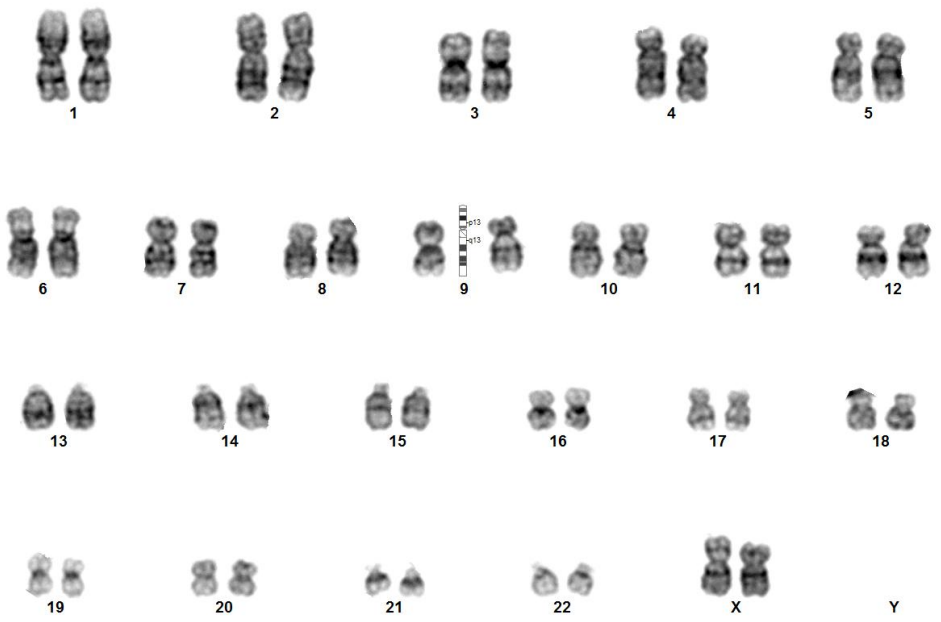

Fig. 8 Photomicrograph showing pericentric inversion of chromosome 9 in a female, Giemsa stain, X100

Table 7. Chromosomal structural abnormalities found in subfertility or infertility.

Karyotypes	No. of cases (n=21)
46, X inv(Y)	6
45,XX,rob(14q;15q)	1
46,X del(Y)	6
46,XX, der (12) (del14qter:12q), inv(9)	1
45,XX, t(14;15)	1
45,XX,rob(14;21)(q10;q10)	1
46,XX,del(10pter)	1
45,XX,t(15q;22q)	1
46,XX,inv(9)/46,XX	1
46,XY/46,Xinv(Y)	2

Repeated pregnancy loss needs more efficient care. Miscarriage is a common clinical problem with approximately 10-15% in the general population [22]. In couples with recurrent pregnancy loss, an initial workup includes a chromosome analysis of the male and female partner. The studies done by Balkan et al. [10] and Rajasekhar et al. [7] showed structural abnormalities in 2.3% and 2.8% cases, respectively which are similar to the present study. Although structural abnormalities were found more (7.3%) in recurrent pregnancy loss in the study done by Pal et al. [9]. Chromosome abnormalities (10. 9%) including 6.8% of heteromorpic variants of acrocentric association and premature centromere divisions were reported by Anuradha et al. [23] and Lakshimi et al.,2004 [24].

Total 33 cases were diagnosed with numerical, structural, and both numerical and structural abnormalities in the autosome or sex chromosome or in combination. These cases were referred for miscellaneous causes like intellectual disability or mental retardation, dysmorphic features, congenital anomalies, developmental delay, obesity, and others (Table 8).Double aneuploidy was found in 5 children. Among them, 3 male children had Down syndrome with inversion of Y chromosome, which might be representing the normal variation in Y-chromosome. Triple X syndrome was detected in 5 cases including two mosaic cases. One case of tetrasomy 18(iso 18p) was diagnosed, not confirmed by FISH. Three cases showed prominent satellites on chromosome 15, a normal variation.

Table 8. Abnormal karyotypes in miscellaneous referral causes

Karyotype	No. of cases
Both autosomal and sex chromosomal abnormalities	
48,XYY,+21	1
48,XXY,+21	1
47,Xinv(Y),+21	3
Sex chromosomal abnormalities	
47,XYY/46,XY	2
47,XXX	3
47,XXX/46,XX	2
47,XYY	4

46,Xdel(Yq)	2
Autosomal abnormalities	
47,XY,+der(21):t(3;21)	2
Tetrasomy 18p	1
46,XX,der(2):t(2qter;3q)	1
46,XX,15(p+)	1
46,XX,der(18):del8(qter):t(8q;18q)	1
46,XY,del(18q)	1
47,XY,+der(21): t(1/21)	1
45,XX,rob(14:21)(q10:q10)	1
45,XX,t(14;15)	1
46,XX inv 2(p,q)	1
46,XX, t(1qter/18pter)	1
Prominent satellite of one chromosome 15	3
Total	33

Out of 6069 cases, 4434 karyotypes were normal (46, XX or 46, XY). These patients were suspected for chromosomal aberrations according to their clinical features such as RSA, infertility, short stature including suspected TS, epilepsy, cerebral palsy, microcephaly, suspected DS or mental retardation. Single gene defects are also responsible for multiple miscarriages, but cannot be detected by karyotyping. Chromosomal abnormalities are one of the most important causes of male infertility [25, 10], which mostly include microdeletion in Y chromosome. Fluorescence in situ hybridization and other complementary molecular approaches might detect the actual abnormalities in these normal karyotypes. Moreover some referred cases need proper clinical examination.

Conclusion

The largest group of referrals was couples with repeated abortions or infertility, followed by patients with Down syndrome. The other referrals were for primary or secondary amenorrhea, Turner syndrome, Klinefelter syndrome, disorders of sex development, ambiguous genitalia, intellectual disability or mental retardation, dysmorphic features, congenital anomalies, developmental delay, obesity and miscellaneous.

Chromosomal analysis is needed for genetic counseling and appropriate management. We studied the frequency of chromosomal abnormalities in patients referred for various causes. We hope that knowledge of these frequencies will help clinicians to determine the priority of requesting cytogenetic studies in individual cases, thus allowing proper genetic counseling to be offered.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Statement of data availability

https://doi.org/10.17605/OSF.IO/C7HZ8

References

1. Kumar V, Abbas AK, Aster JC (eds): Genetic Disorders, in Robbins and Cotran Pathologic Basis of Disease, vol 1, pp 141-188(Elsevier, 2021).
2. Turnpenny P, Ellard S (eds): Chromosome Disorders, in Emerys Elements of Medical Genetics, pp 273-292 (Churchill livingstone, 2012).
3. Phadke SR: Genetic counseling. Indian J Paediatr71 (2):151–56(2004).
4. Simons A, Shaffer LG, Hastings RJ: Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 Edition. Cytogenet Genome Res 141(1):1–6(2013).
5. Seabright MA: Rapid banding technique for human chromosomes. Lancet Lond Eng l2 (7731):971–72(1971).
6. Kim SS , Jung SC , Kim HJ , Moon HR , Lee JS : Chromosome abnormalities in a referred population for suspected chromosomal aberrations: a report of 4117 cases. J Korean Med Sci 14(4):373-6(1999 Aug).
7. Rajasekhar M, Murugesan R, Rekharao, Shetty H, Jyothirao , et al.: Cytogenetic Analysis of 1400 Referral Cases: Manipal Experience. IntJ Hum Genet 10(1-3): 49-55 (2010).
8. Yik MY, Zakaria Z, Ramli SF, Isa NM, Razali RA, et al: Comprehensive cytogenetic analysis of cases referred for suspected chromosomal abnormalities: A Five-year study at Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia, Penang. Malaysia.J.of Biomed. &Clin. Sci. 2 (1): 27-32 (June 2017).
9. Pal AK, Ambulkar PS, Waghmare JE, Shende MR, Jain M ,et al: Cytogenetic Analysis for Suspected Chromosomal Anomalies- A Retrospective Study. J Clin Diagn Res 14(5): GC01-GC06 (2020 May).
10. Balkan M, Akbas H, Isi H, Oral D, Turkyilmaz A, et al. Cytogenetic analysis of 4216 patients referred for suspected chromosomal abnormalities in Southeast Turkey. Genet Mol Res 9 (2): 1094-1103 (2010).
11. Thillainathan S, Sirisena ND, Kariyawasam K WJC, Jayasekara RW, et al : Cytogenetic analysis of chromosomal abnormalities in Sri Lankan children. World J Pediatr, Online First, November 2014. www.wjpch.com doi: 10.1007/s12519-014-0526-5
12. Polipalli SK , Karra VK , Jindal A , Puppala M, Singh P, et al: Cytogenetic Analysis for Suspected Chromosomal Abnormalities; A Five Years Experience. J Clin Diagn Res 10(9): GC01–GC05 (2016 Sep).
13. Kingston HM. ABC of clinical genetics [Internet] London: BMJ books; 2002 [cited 2016 May 10]. Available from: http://elibrary.kiu.ac.ug:8080/xmlui/handle/123456789/2615.
14. Hughes LA: Disorders of sex development: a new definition and classification. Best Practice & Research Clinical Endocrinology & Metabolism 22(1):119-134(2008).

15. Ten SK, Chin YM, Noor JP, Hassan K: Cytogenetic studies in women with primary amenorrhea. Singapore Med J 31: 355-359(1990).
16. Zhao X, Shen GM, Feny Q, Sun XG, Luo Y: Cytogenetic studies of 131 patients with primary amenorrhea (including three novel abnormal karyotypes). Yi Chuan, 30(8): 996-1002(2008).
17. Duarte AC, Cunha E, Roth JM, Ferreira FLS, Garcias GL, et al: Cytogenetics of genetic counseling patients in Pelotas, Rio Grande do Sul, Brazil. Genet Mol Res GMR (3):303-08(2004).
18. Swain A, Lovell-Badge R: Mammalian sex determination: A molecular drama. Genes and Development 13:735-67(1999).
19. Yatsenko SA, Witchel SF: Genetic approach to ambiguous genitalia and disorders of sex development: What clinicians need to know? Semin Perinatol 41(4):232-43(2017).
20. Ogilvy-Stuart AL, Brain CE: Early assessment of ambiguous genitalia. Arch of Dise in Childh 89:401-07(2004).
21. Sangeetha R, Balachandar V, Velmurugan P, Manikantan P, et al: Cytogenetic study on sexual ambiguity in humans. Int J Hum Genet 10(1-3):81–6(2010).
22. Ogasawara M, Aoki K, Okada S, and Suzumori K: Embryonic karyotype of abortuses in relation to the number of previous miscarriages .Fertility and Sterility 73(2):73(2):300-4(2000 Feb).
23. Anuradha N, Satyanarayana M, Manjunatha KR: Satellite Associations in Recurrent Aborters. Int J Hum Genet 2(1): 61-64(2002).
24. Lakshmi KV, Satyanarayana M, Prabhaker S and Sarvani R: Chromosomal Instability in recurrent spontaneous aborters. Int J Hum Genet 4(1): 31-36(2004).
25. Akgul, M, Ozkinay F, Ercal D, Cogulu O, Dogan O, et al: Cytogenetic abnormalities in 179 cases with male infertility in western region of Turkey: Report and review. J Assist Reprod Genet 26: 119-122(2009).