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# Cytogenetic Analysis of 1400 Referral Cases: Manipal Experience

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**ABSTRACT** Karyotypes were examined in 1400 cases, suspected of having chromosomal abnormalities. A total of 343 (24.5 per cent) had abnormal karyotypes including 43 (3.07%) polymorphic variants; 14.28% of children exhibited chromosome abnormalities including 12.07% of Down syndrome, 2.21% of congenital anomalies including global developmental delay, 0.5% with intersex disorders. Chromosomal abnormalities were observed in individuals with pubertal failures including short stature and amenorrhea in females (3.35%), and were recorded in 0.43% males. Cases of reproductive failures (3.64%) included recurrent miscarriages, bad obstetric history and infertility. Of these 2.28% were instances of polymorphic variants. Fifty seven patients who diagnosed of various symptoms of cancer were studied and found to have 56% structural variations including Philadelphia chromosome. Cytogenetic analysis is found to be useful in providing genetic counseling.

## INTRODUCTION

A routine chromosome analysis being used as a starting point for the diagnosis of cytogenetic investigation of congenital malformations and developmental delay or mental retardation in children, reproductive delays and failures in adults. Based on family history and clinical phenotypes, subsequent workup for genetic diagnosis can follow DNA based techniques. There is an accelerating demographic switch to non-communicable diseases/ or syndromes in our population and they are important causes of morbidity and mortality. The prevalence rate of genetic disorders is high (Verma and Bijarnia 2002) due to high birth rate and consanguinity (Bittles 2002). Accurate diagnosis of the affected member is of paramount importance for genetic counseling (Phadke 2004). Chromosomal disorders account for 11.3% in population suspected to have genetic disease (Verma et al. 2001) and the chromosome abnormalities amount to 7.5% (Iravathy et al. 2006). Most of the chromosomal abnormal fetuses are eliminate by miscarriages, still the frequency of abnormalities in live borns is about 0.6% (Conor and Ferguson-Smith 1991). The aim of the present cytogenetic evaluation was to determine the incidence of chromosomal abnormalities in suspected patients referred by clinicians from various hospitals in and around Manipal including Kasturba Hospital to Manipal Life Sciences Centre of Manipal University. Cytogenetic analysis was carried out in 1400 patients and the abnormalities have been recorded

## MATERIALS AND METHODS

Fourteen hundred patients during the period, 2003 to 2008 were examined by clinicians and detailed clinical and family histories were recorded and were subjected to cytogenetic evaluation. Blood and in some cases bone marrow samples were collected from the patients into heparinized test tubes. Analysis was done on cultured blood lymphocytes stimulated with phytohemagglutinin (PHA) using standardized protocol according Moorhead et al. (1960). The karyotype of each patient was determined as per standardized techniques namely G (giemsa) banding (Seabright et al. 1971). Wherever found necessary C (centromere) banding (Sumner 1971), R (reverse) banding (Dutrillaux 1973) and Q (quinacrine) banding (Casperson et al. 1970) techniques were applied to confirm the structural abnormalities. Chromosomal analysis was done under 100x, magnification. Fifty well spread metaphases were captured and analyzed using Ikaros automated karyotyping software. Whenever translocations and unusual karyotypes were found, blood samples were collected from their parents and chromosome were studied, to have data on inheritance. Karyotype analysis was as per to ISCN (2005) standards, karyotypes were prepared

## RESULTS AND DISCUSSION

According to WHO report (2005) on diseases in developing world, genetic and congenital disorders are the second most common cause of infant and childhood death, occurring with a birth prevalence of 2.5-6%. In our study, a total of 343 (24.5 per cent) cases were found to have abnormal karyotypes (Figs. 1-3) including 43 (3.07%) exhibiting polymorphic variants; 200 (14.28%) of children with chromosome abnormalities including 169 (12.09%) cases of Down syndrome, 31(2.21%) cases of congenital anomalies also with global developmental delay, and 7 (0.5%) of intersex disorders. Abnormal karyotypes from pubertal failures including short stature and amenorrhea in females (3.35%), in males (0.43%) were noted. In adults, chromosomal abnormalities were seen in cases of reproductive failures (3.64%) including recurrent miscarriages, bad obstetric history and infertility. Fifty seven patients with diagnosis of various symptoms of cancer were studied and found to have 56% structural variations including Philadelphia chromosome.

Down syndrome is the commonest identifiable cause of learning disability and the most common anomaly in the group of trisomies, and also the most frequent (57.29%) of all chromosome abnormalities (Table 2). Nearly 58% of Down syndrome children were less than one year old, indicating that the diagnosis was precocious in most cases, among them 53.84% were males. One hundred eighty six suspected patients of Down syndrome were referred for cytogenetic confirmation during the period of study. We found 169 children with abnormalities. The standard trisomy 21 was found in 90.69%, translocations in 4.65% and mosacism in 4.65% of these cases. These results are compared with the earlier reports by Papp et al. (1977), that is trisomy 21 in 91.7%, translocations in 3.9% and mosaicism in 4.4%. Of 186 children, clinically indicated as Down syndrome, 17 had normal karyotype. In our study one female child with trisomy 13 and two male children with trisomy 18 and one with mosaicism for trisomy 18 were observed. Although trisomy 13 and trisomy 18 are generally considered to be lethal, long-term survival of patients has been reported (Rasmussen et al. 2003).

Developmental delay is among the commonest problem encountered in community pediatric practice. Laboratory investigations are not a

**Table 1: 1400 Referral cases suspected for chromosome abnormalities (24.5%)**

| *Referral diagnosis* | *Number of cases* |
|---|---|
| Children with suspected for down syndrome (186), Children with developmental delay, failure to thrive, multiple congenital anomalies, hypertonia, dysmorphic facies, mental retardation including, fragile X syndrome (3), Allagile syndrome (1) Achondroplasia (1), Polycystic kidney disease(1), cerebral palsy (1), hepatosplenomeghaly (1), brachycephaly (1), Bloom syndrome (2) Oral Facial Digital syndrome (1), Respiratory distress syndrome (2), progeria (1), coloboma iris (1), bilateral analptholmia (2), ligamental laxicity (1), denysdrash syndrome(1) | 438 (169+31) |
| Children with Ambiguous external genitalia and Intersex Disorders | 37(7) |
| Suspected cases of Turner syndrome - short stature (27 patients), primary amenorrhea (62 patients) , secondary amenorrhea (11), Premature ovarian failure/syndrome (4) | |
| Suspected cases of Klinefelter syndrome 14(6) | 104(47) |
| Couples with H/o Repeated spontaneous abortions and H/o Bad Obstetric history (213 females+198 males), and Primary and secondary(42) Infertility (14) | 467(51) |
| Cancer and leukemia patients | 57(32) |
| Cases including parents of Down syndrome and genetic counseling | 282 (0) |
| Total | 1400 (343) |

**Table 2: Chromosomal abnormalities in children with autosomal syndromes and congenital anomalies**

| *Karyotype (number of cases)* | | | |
|---|---|---|---|
| 47,XY+21 | (86) | 47,XX+21 | (71) |
| 46,XY/47,XY+21 | (2) | 46,XX/47,XX+21 | (2) |
| 47,XY+mar | (1) | 46,XX,dup(18)(q21-q23) | (1) |
| 46,XY dup (21)(q11q-12) | (1) | 46,XX,dup(21)(q11-q12) | (1) |
| 47,XY+18 | (2) | 47,XX+13 | (1) |
| | | 46,XX/47XX+18 | (1) |

substitute for the evaluation of a child with global developmental delay, but are a useful adjunct in determining etiology (McDonald et al. 2006). Global developmental delay is a common cause of referral in pediatrics and its prevalence is around 2-3% in the general population (Kabra and Gulati 2003). Developmental delay is a subset of the developmental disabilities defined as neurocognitive impairments as well as significant limitations in adaptive living skills (social, communication, work, leisure, daily living). In our study, we investigated 211 children with global developmental delay and/ or dysmorphisms for chromosome analysis, and 11.84% children had chromosome abnormalities. Precise genotype-phenotype correlations have not been possible because of constitutional chromosomal analysis. The rest 88.16% of children the confirmation of single probe findings/ or molecular analysis (Rauch et al. 2006) should be strongly considered and appropriate family studies need to be carried out (Table 3).

Turner's syndrome affects about 1/2500 female infants born alive. The syndrome results from total or partial absence of one of the two X chromosomes normally present in females. Considerable cytogenetic data on short stature and primary/ secondary amenorrhea are available in literature (Ten et al. 1990; Zhao et al. 2008). We evaluated the results of 65 females, who were suspected for Turners' Syndrome, of which 27 were diagnosed as short stature, 62 with primary amenorrhea, 11 with secondary amenorrhea and 4 cases were premature ovarian failure. The age of these patients' ranged from 5 to 36 years at the time of evaluation. Chromosome abnormalities were recorded from 47 (45.2%) patients'. Mosaicism was prevalent (48.9%), and the karyotype 45, X was found in only 36.17% of the patients. Sex reversal namely testicular feminization syndrome was observed in 14.9%, and all these patients had normal female phenotype with 46, XY complement. Ambiguous genitalia are a congenital physical abnormality where the outer genitals do not have the typical appearance of either sex. This results in social and psychological impact on an individual and requires medical, psychological and social attention so that appropriate sex-of-rearing can be assigned. Physical examination of external genitalia, internal gonads and cytogenetic analysis should be performed since ambiguity can result from chromosomal abnormality. For appropriate and effective management and counseling of patients, cytogenetic confirmation and knowledge of the development of the genital tract and of the interaction between genetic sex and environment (Forest 1992) is mandatory. The localization of specific genes involved in the process of sex determination and sexual differentiation have made it possible to determine the mutations and other molecular events for diagnosis and treatment (Migeon and Wisniewski 2003). We have carried out cytogenetic analysis of 37 children with intersex disorder, and seven (18.9%) were found to have abnormalities including sex reversals, which correlates with earlier study (Rajasekhar et al. 1999) on sexual ambiguity

Repeated pregnancy loss carries immeasurable cost as well as costly to the health care system and the selective chromosome analysis would result in a more efficient care. Miscarriage is a common clinical problem with approximately 10-15% in general population, and 50% of these

**Table 3: Chromosome abnormalities noted in familial cases**

| | *Karyotype* | |
|---|---|---|
| *Child* | *Maternal* | *Paternal* |
| 47,XY+ inv dup (21) (q11q12) | 47,XX+ inv dup (21) (q11q12) | 46,XY |
| 47,XY+21(15Ps+) | 46,XX | 46,XY,(15Ps+) |
| 46,XY,der(6) ins(6;4)(p24;p13p16)mat | 46,XX,ins(6;4)(p24;p13p16) | 46,XY |

**Table 4: Karyotypes in 57 cancer patients and abnormalities 32 (56.1%)**

| *Karyotype (number of cases)* | | | |
|---|---|---|---|
| 46,XY | (16) | 46,XX | (9) |
| 46,XY,t(9;22) (q34;q11.2) | (21) | 46,XX,t(9;22)(q34;q11.2) | (7) |
| 46,XY,t(9;22)/ | | 46,XX,t(8;21) (q22;q22) | (1) |
| 54,XY+7,+9,+10,+13,+14,+15,+19,+21 | (1) | 46,XX /45,XX-7 | (1) |
| | | 46,XX, del (20) (p12-p13) | (1) |

attributable to detectable chromosome abnormalities (Ogasawara 2000). The incidence of chromosome abnormalities in reported cases ranged from none (Rowely et al. 1963; Khudr 1974). In couples with recurrent pregnancy loss, an initial workup should include a chromosome analysis of the male and female partner. The most common chromosome abnormality is a translocation. Other chromosome abnormalities include chromosome inversions, X-chromosome inactivation, sex chromosome mosaicism and ring chromosomes. Single gene defects might also be responsible for multiple miscarriages, but will not be detected by a karyotype. Cytogenetic investigations as shown in tables 1, 5, 6 were undertaken on 279 couples with subfertility and infertility. Chromosome abnormalities (10. 9%) including 6.8% of heteromorpic variants of acrocentric association and premature centromere divisions reported by Anuradha et al. (2002) and Lakshimi et al. (2004), were also observed in our study (Table 6). Common cytogenetic polymorphisms detected by G-banding are considered as heteromorphisms and include heterochromatic regions of chromosomes 1, 9, 16 and Y and also prominent acrocentric short arms, satellites and stalks. The individual blocks of heterochromatin show specific reac-tions to staining and have different frequencies of mutation (Verma 1988). Duplication, deletions and inversions of these

**Table 5: Suspected for sex chromosome abnormalities (73 cases)**

| *Karyotypes of Turner syndrome and variants (45)* | | *Karyotypes of Klinefelter syndrome and variants (10)* | |
|---|---|---|---|
| 45,X | (17) | 47,XXY | (7) |
| 45,X/46,XX | (8) | 46,XY/47,XXY | (2) |
| 45,X/47,XXX | (1) | 46,XY/47,XY+mar(X) | (1) |
| 46,X,i(X)(q10) | (2) | | |
| 45,X/46,X,i(X)(q10) | (4) | *Karyotypes in cases with primary amenorrhea, short stature and Intersex disorders (20)* | |
| 45,X/46,XX/46,X,i(X)(q10) | (1) | | |
| 45,X/46,XX/46,X,t(X;X)(q24;q24) | (1) | | |
| 46,X,del (X)(pter→p11.2) | (1) | | |
| 45,X/46,del(X)(p11.2-p22.2) | (1) | 46,XY female | (18) |
| 45,X/46,del(X)(qter→q13.2) | (1) | 46,XX male | (1) |
| 45,X/46,X,r(X)(p22q25) | (2) | 46,XX;46,XY | (1) |
| 45,X/46,XX,(22pstk+) | (1) | | |
| 46,X+mar(X) | (1) | | |
| 47,XXX | (2) | | |
| 46,XX/47,XXX | (1) | | |

**Table 6: Chromosomal structural abnormalities and polymorphic variants**

| *Structural abnormalities(16) (number of cases)* | | *Polymorphic variants(43) (number of cases)* | |
|---|---|---|---|
| 46,XY,del (5)(pter→p13) | (2) | 46,X,inv(Y)(p11q12.1) | (2) |
| 46,XX,del(5)( pter→p13) | (1) | 46,XY,inv(9qh)(p12q13) | (1) |
| 46,XY,del(18)(q22-q23) | (1) | 46,XXinv(9qh)(p12q13) | (3) |
| 46,XX dup(18) (q22) | (1) | 46, XX(13ps+) | (4) |
| 46,XX,dup (14)(q31) | (1) | 46,XY(13ps+) | (1) |
| 46,XX.t(10;14) (q24-q32) | (1) | 46,XX (14ps+) | (2) |
| 47,XX,t(2;12)(p13;q24)+21 | (1) | 46,XY(15ps+) | (3) |
| 46,XX t(10:22)(q23:q13) | (1) | 46,XX(15ps+) | (1) |
| 46,XY/46,XY t(7:14) (q11;p13) | (1) | 46,XX(22ps+ve) | (6) |
| 46XX/46,XX t(12:14)(q23;q13) | (1) | 46XY47,XY)+ marker (X) | (1) |
| 46,XY/46,XY,t(14;7)(p13;q11) | (1) | 46,XX,(1qh+) | (1) |
| 46,XX/46,XX,t(16;22(q12-21;q13.2) | (1) | 46,XX(9qh+) | (4) |
| 46,XY,inv(8)(p12q21) | (1) | 46,XY(9qh+) | (5) |
| 46,XX,inv(7)(p12p22) | (1) | 46,X,(Yqh+) | (6) |
| 46,XY,inv(7)(p12:p22) | (1) | 46,XX,D/D.D/G,G/G associations | (1) |
| 46,XY with instances polyploidy | (1) | | |
| 46,XX with instances of polyploidy | (2) | | |

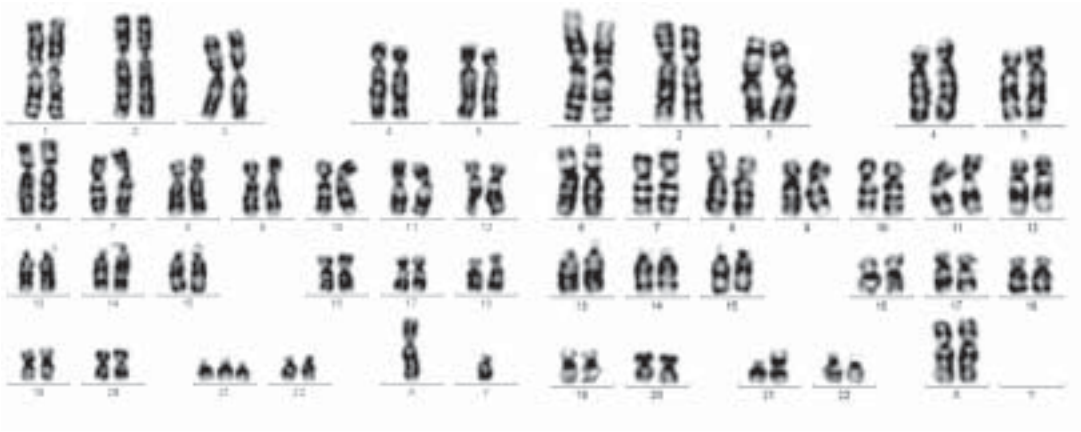

**Fig. 1. Karyotypes showing 47, XY+21 and 46,XX, rob(21;21)(q10;q10) chromosome constitutions in Down syndrome children**

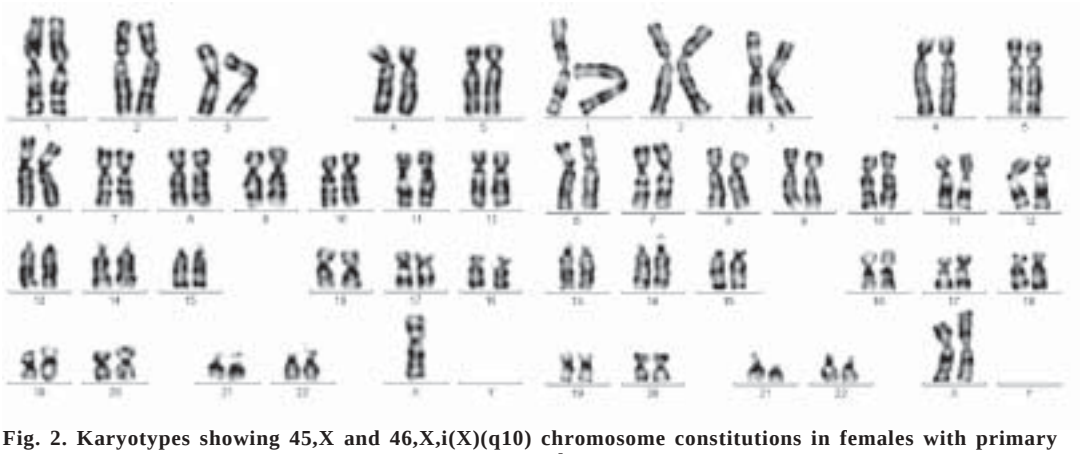

**Fig. 2. Karyotypes showing 45,X and 46,X,i(X)(q10) chromosome constitutions in females with primary amenorrhea**

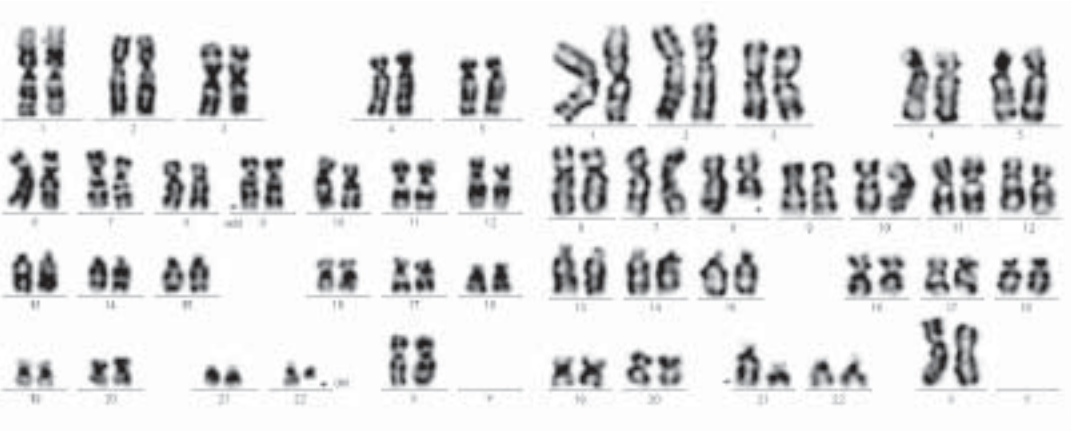

**Fig. 3. Karyotypes showing 46,XX,t(9;22) (q34;q11.2) and 46,XX,t(8;21) (q22;q22) chromosome constitutions in Chronic myeloid and Acute lymphoid Leukemia patients**

regions are caused by pairing abnormalities of homologous chromo-somes in prophase of meiosis I. Polymorphic variants on chromosomes are considered 'normal', as heterochromatin has no coding potential and nucleolar organizing regions (NOR) contain genes coding for rRNA. Polymorphic variants of chromosomes probably play a significant role in infertility and genes for fertility and viability are now thought to reside in heterochromatin (Madon 2005), but may not have an impact on phenotype (Sahin et al. 2006). The t(9;22)(q34;q11.2) results in the formation of a Philadelphia chromosome (Ph) and generates an active chimeric BCR-ABL tyrosine kinase (Michael et al. 2000; Pratibha and Amre 2002). This chromosomal anomaly is most commonly associated with CML and ALL. Chromosome analysis of leukemia has revealed specific changes in disease. The role of conventional cytogenetics has been shown to be of use for diagnosis and monitoring of disease response to therapy. In the present study as shown in table 4, 32(56.14%) patients were confirmed to have an abnormality namely Ph+ positive in 87.5%, and rest (12.5%), including one patient of AML with dyspoiesis had 46,XX, t(8;21)(q22;q22). One patient with CML was with Ph+ and hyper-diploidy and one patient was observed to have monosomy 7.

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## REFERENCES

Anuradha N, Satyanarayana M, Manjunatha KR 2002. Satellite Associations in Recurrent Aborters. *Int J Hum Genet,* 2(1): 61-64.

Bittles H 2002. The Impact of Consanguinity on the Indian Population. *Indian J of Hum Genet,* 8(2): 45-51.

Caspersson T, Zech L, Johansson C, Modest EJ 1970. Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma,* 30: 215–227.

Connor JM, Ferguson-Smith MA 1991. *Essential Medical Genetics*. 3rd Edition. London: Blackwell Scientific Publications.

Dutrillaux B 1973. Nouveau systeme de marquage Chromosomeiqe les bandes T. *Chromosoma,* 41: 395- 402.

Forest MG 1992. Etiopathogenesis, classification, investigation and diagnosis in intersex. *Indian J Pediat,* 59: 475-485.

Iravathy GK, Vimarsh R, Sohani V, Geeta C 2006. Cytogenetics and genetic counseling of patients in North India. *JK Science,* 8(1): 28-30

Kabra M, Gulati S 2003. Mental retardation. *The Indian J Pediatr,* 70(2): 153-158.

Khudr G 1974. Cytogenetics of habitual abortion: a review. *Obstet Gynecol Surv,* 29: 299-310

Lakshmi KV, Satyanarayana M, Prabhaker S and Sarvani R 2004. Chromosomal Instability in recurrent spontaneous aborters. *Int J Hum Genet,* 4(1): 31-36.

Madon PF, Athalye AS, Parikh FR 2005. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online,* 11(6): 726-32.

McDonald L, Rennie A, Tolmie J, Galloway P, McWilliam R 2006. Investigation of global developmental delay. *Archives of Disease in Childhood,* 91: 701-705.

Michael WN, Vieira DS, Mendiola R, Schultheis B, Goldman JM, Melo JV 2000. *BCR-ABL* Tyrosine Kinase Activity Regulates the Expression of Multiple Genes Implicated in the Pathogenesis of Chronic Myeloid Leukemia. *Cancer Research,* 60: 2049-2055.

Migeon, CJ, Wisniewski, AB 2003. Human sex differentiation and its abnormalities. *Best Practice and Research Clinical Obstetrics and Gynaecology,* 17: 1-18.

Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford D 1960. Chromosome preparations of leucocytes cultured from human peripheral blood. *Exp Cell Res,* 20: 613-616.

Ogasawara M, Aoki K, Okada S, Suzumori K 2000. Embryonic karyotype of abortuses in relation of previous miscarriage. *Fertil Steril,* 73: 300-304.

Padke SR 2004. Genetic counseling. *Indian J Pediatr,* 71(12): 1079-1082.

Papp Z, Osztovics M, Schuler D, Mehes K, Czeizel E, Horvath L, Szemere G, Laszlo J 1977. Down's Syndrome: Chromosome Analysis of 362 Cases in Hungary. *Hum Hered,* 27: 305-309.

Pratibha S, Amare K 2002. Chronic Myeloid Leukemia: Cytogenetics and Molecular Genetics. *Ind J of Hum Genet,* 8(1): 4-10.

Rajasekhar M, Kumar KSD, Sadhanani MD, Jyothi A 1999. Sexual ambiguity: Clinical and cytogenetic evaluation of 105 cases. *Medical Science Research,* 27: 205-207

Rasmussen SA, Wong LY, Yang Q, May KM, Friedman JM 2003. Population-based analyses of mortality in trisomy 13 and trisomy 18. *Pediatrics,* 111: 777-784.

Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, Becker C, Zenker M, Huffmeier U, Thiel C, Ruschendorf F, Nurnberg P, Reis A, Trautmann U 2006. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. *Am J Med Genet A,* 140(19): 2063-2074.

Rowley PT, Marshall R, Ellis JRA 1963. Genetic and cytological study of repeated spontaneous abortion. *Ann Hum Genet,* 27: 78-100.

Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O and

Zeyneloglu HB 2008. Chromosome heteromorphism an impact on infertility. *J Assist Reprod Genet*, 25:191–195.

Seabright MA 1971. Rapid banding technique for human chromosomes. *Lancet*, 2: 971-972.

Sumner AT 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res*, 75: 304–306.

Ten SK, Chin YM, Noor JP, Hassan K 1990. Cytogenetic studies in women with primary amenorrhea. *Singapore Med J*, 31: 355-359

Verma IC 2001. Burden of genetic disorders in India. *Indian J Padiatr*, 68(1): 25.

Verma RS 1988. Heteromorphisms of Hetero-chromatin. In: RS Verma (Ed.): *Heterochromatin Molecular and Structural Aspect*s: New York: Cambridge University Press, New York, pp. 276-291

Zhao X, Shen GM, Feny Q, Sun XG, Luo Y 2008. Cytogenetic studies of 131 patients with primary amenorrhea (including three novel abnormal karyotypes). *Yi Chuan*, 30(8): 996-1002.

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