Original Article

Indian Journal of Genetics and Molecular Research
Volume 4 Number 2, July - December 2015
DOI: https://dx.doi.org/10.21088/ijgmr.2319.4782.4215.3

Cytogenetic Evaluation of the Individuals with Down Syndrome in North Coastal Andhra Pradesh

V. Lakshmi Kalpana*, P. Gowri Sairam, V. Sowjanya**, A. Anuradha***, G. Sudhakar******

Abstract

Mental retardation (MR), defined by the World Health Organization(WHO) as an intelligence quotient (IQ) <70, is characterized by significantly limited cognitive functioning, coupled with limitations in adaptive skills. Down syndrome is the most common genetic cause of intellectual disability in the population and is due to a gene dosage effect of the presence of an additional chromosome 21(Vundinti etal.,2011) or a partial trisomy, mainly in the 21q22 region (Frias etal.,2002). The main aim of this study is to evaluate the Cytogenetic causes for the occurrence of the mental retardation. The present study was carried out with 50 MR cases and 50 age and sex matched healthy controls from North Coastal Andhra Pradesh. With prior informed consent, 5 ml of blood was collected into sterile heparinized tubes from 50 MR cases and 50 healthy controls for evaluation of the chromosomal abnormalities by using Leucocyte Blood Culture and G-banding techniques. Out of 100 mentally retarded people analyzed,76(76%) had normal karyotype and remaining 24(24%) were Down syndrome. Among them regular free trisomy constituted 18(18%) cases, Robertsonian translocations in 4(4%) cases and mosaicism was recorded in 2(2%) cases. The study confirmed the findings of earlier studies carried out in India and other countries. It emphasizes free trisomy 21 was found to the most frequent autosomal aberration of Down syndrome when compared with Robertsonian translocations and mosaics.

Keywords: Mental Retardation; Trisomy; Translocations; Mosaics.

Introduction

Mental retardation (MR), defined by the World Health Organization(WHO) as an intelligence quotient (IQ) <70, is characterized by significantly limited cognitive functioning, coupled with limitations in adaptive skills in two or more of the following areas: social skills, community living, communication, home living, health, self-direction, work, and leisure (Curry etal.,1997; Battaglia etal.,1999). MR is generally divided into mild (IQ of 50-70), moderate (IQ of 35-50), and severe (IQ of 20-35); those cases in which the IQ is below 20 are occasionally defined as profound.(Battaglia etal.,1999; Chiurazzi etal.,2000).

The incidence of mental retardation (IQ<70) is found to be 2-3% worldwide (Lewis, 2007). Its incidence in developing countries is about 2-3 times more as compared to developed countries. Males are found to be more affected than females. The risk of mental retardation is found to be higher in children with congenital structural defects (Decoufle et al., 2001). Despite extensive studies in area of mental retardation the overall prevalence of MR is still not known with certainty. It is approximately 1-3% (Munro, 1986; DeVries et al., 2005) and in India too it is estimated to be 2 – 3% of the population (Kaur et al., 2003).

The cause of mental retardation may be genetic (30%) or environmental, congenital (for example, fetal exposure to teratogenic agents, chromosome disorders), or acquired (for example, central nervous system infection, head trauma). Chromosomal aberrations account for 15% of mentally retarded individuals (Mulley et al., 1992). Monosomies and

Author's Affiliation: *Asst.Professor, **Research Scholar, ***Post doctoral fellow (DST), ****Professor, Deparment of Human Genetics,Andhra University,Visakhapatnam-530003.

Corresponding Author: V. Lakshmi Kalpana, Asst.Professor, Department of Human Genetics, Andhra University, Visakhapatnam-530003.
Email: vlakshmikalpana.gen@auvsp.edu.in

trisomies are reported to be more frequent than tetrasomies, pentasomies, double aneuploids, polyploidy, etc., whereas subtelomeric rearrangements account for 5% of mental retardation / malformation syndrome

(Archer et al., 2005). Several types of structural aberrations are also known to cause mental retardation, the common ones being deletion, duplication, inversion, translocation and is chromosome formation (Flint et al., 1995; Holinski-Feder et al., 2000; Walter et al., 2004).

Down syndrome is the most common genetic cause of intellectual disability in the population and is due to a gene dosage effect of the presence of an additional chromosome21(Vundinti etal.,2011) or a partial trisomy, mainly in the 21q22 region (Frias et al.,2002).

Down syndrome (DS) or trisomy 21, with its characteristic clinical features is the most frequently observed autosomal aneuploidy with an incidence of about 1 in 700 live births. The prevalence of DS in India is 0.88 per 1000 (1 out of 1139) to 1.09 per 1000 (1 out of 916) and three DS children are reported to be born every hour (Rajangam and Thomas,1992; Malini and Ramachandra, 2006). In general, over 95% of Down syndrome individuals possess free trisomy21 resulting from non-disjunctional error of chromosome 21 during gametogenesis in one of the parents. While about 2-4% result from a translocation of chromosome 21 on to a D or Ggroup chromosome, 1-2% are mosaics showing a normal cell line additionally, due to mosaicism (Nussbaum et al., 2001).

Mental retardation (MR) is among the more significant public health problems because of its prevalence is about 2 to 3 percent in the population, the few therapeutic options that are currently available, and the resulting life-long harm to the affected persons, their families, and society as a whole. Causes of MR are numerous and include genetic and environmental factors. In at least 30 to 50 percent of cases, physicians are unable to determine etiology despite thorough evaluation .Very few studies have been focused on the MR in North Coastal Andhra Pradesh. Therefore, the main aim of this study is to evaluate the Cytogenetic causes for the occurrence of the mental retardation.

Materials and Methods

The present study was carried out with 100 MR cases and 100 age and sex matched healthy controls from North Coastal Andhra Pradesh. The patients above 2 years from Labenshiff Mentally Handicapped, Visakapatnam; Asramdham Manovikas Kendram, Anakapalli; Behara Manovikas Kendram, Srikakulum were included in this study. The study was consented and approved by the institutional ethical committee for blood sample collection to evaluate the chromosomal abnormalities.

With prior informed consent, 5 ml of blood was collected into sterile heparinized tubes from 100 MR cases and 100 healthy controls and were transported to the Department of Human Genetics, Andhra University, Visakapatnam for Leucocyte Blood Culture and G-banding techniques

Results and Discussions

Down syndrome usually presents three cytogenetic variants:

- Regular trisomy 21 (T21)—with karyotype 47, XX,+21 or 47,XY,+21, present in ~95% of the cases.
- Robertsonian translocations (rob)—involves the rearrangement of chromosome 21 with another acrocentric chromosome (group D or G) (46,XX or 46,XY, rob(D or G;21)(q10; q10),+21 .
- Mosaicism—presence of two or more different cell lines in the same individual. In this case, one line with T21 and another normal line, represented by the formula 47,XX or XY,+21/ 46,XX or XY and corresponds to 1–3% of all cases.(Frias et al., 2002; Hulten et al.,2008)

Table 1: Frequency of chromosomal abnormalities in MR cases and controls

	Mr Cases		Controls	
Chromosomal Analysis	No.	%	No.	%
Normal	76	76	100	100
Abnormal	24	24	-	-

Table 1 represents the frequency of chromosomal abnormalities in MR people and controls. In MR people, the frequencies of normal and abnormal karyotypes were 76% and 24% respectively whereas in controls all were normal without any chromosomal abnormalities.

Table 2: Distribution of cytogenetic results of MR cases

Chromosomal Abnormality	No. of Mr Cases	%
Regular Trisomy	18	18
Translocations	4	4
Mosaics	2	2
Total	24	24

Table 2 demonstrates the distribution of cytogenetic results of mentally retarded people. The frequencies of regular trisomy, translocations and mosaics were 18%, 4% and 2% respectively. The frequency of regular trisomy was higher than the frequencies of translocations and mosaics in mentally retarded people of the present study. The total number of chromosomal abnormality was 24 (24%).

Down syndrome is the most common autosomal trisomy, and is the most common genetic cause of severe learning difficulties. Its incidence ranges from 1 in 600 to 1 in 1000 in live-born infants (Devlin and Morrison, 2004; Roizen and Patterson, 2003).

In agreement with previous reports (Thompson et al., 1993), there was considerable karyotype variability in individuals with DS, including cases of free trisomy and partial free trisomy of chromosome 21, as well as Robertsonian translocations between chromosomes 14 and 21 and between the two chromosomes 21. These observations emphasize the importance of cytogenetic confirmation in cases of DS. In addition to indicating the risks of recurrence of the syndrome, karyotyping can also be useful in the clinical follow-up of some disorders associated with DS. Certain diseases, such as Duodenal stenosis, Alzheimer's disease and Acute leukemia, seem to be more common in DS patients than in the general population (Fong and Brodeur, 1987). In the case of acute leukemia, for example, the incidence can be 14-30 times more frequent in DS patients (Iselius et al., 1990). By identifying each karyotype associated with DS, it is possible to inform the patient's family about the susceptibility to Acute leukemia, Duodenal stenosis and Alzheimer's disease and help them notify the associated symptoms. Treatment can then be introduced early, thereby increasing the patient's life expectancy.

Table 3: Frequency of chromosomal abnormalities among Down syndrome people in different countries

S. No	Reference	No. of Down Syndrome People	Regular Trisomy21	Translocations	Mosaics	Country
1	Cassiman Et al.,1975	88	81(92.1)	6(6.8)	1(1.1)	BELGIUM
2	Speed Et al., 1976	153	144(94.1)	2(1.3)	7(4.6)	SCOTLAND
3	English Et al.,1989	65	63(96.9)	1(1.5)	1(1.5)	ENGLAND
4	Stoll Et al.,1990	391	368(94.1)	14(3.6)	9(2.3)	FRANCE
5	Al-awadi Et al.,1991	1024	985(96.2)	24(2.3)	9(0.9)	KUWAIT
6	Mutton Et al.,1996	5737	5411(94.3)	220 (3.8)	66(1.2)	ENGLAND AND WALES
7	Mokhtar Et al.,2003	673	642(95.4)	18(2.7)	5(0.7)	EGYPT
8	Devlin,2004	208	197(94.7)	3(1.45)	8(3.85)	IRELAND
9	Azman	149	141(94.6)	1(0.7)	7(4.7)	MALAYSIA
10	Et al., 2007 Amayreh ET AL.,2009	80	74(92.5)	2(2.5)	3(3.8)	JORDAN
11	Present Study	24	18(18)	4(4)	2(2)	India

Table 3 explains the frequency of chromosomal abnormalities among Down syndrome people in different studies.The frequencies of regular trisomy, translocations and mosaics were found to be vary in different studies. The variation in frequencies between the present study and other studies may be attributed to the differences in the sample size taken or geographical variation.

Among all cases studied from table 3, the frequency of translocations and mosaicism was very much lower than the frequency of standard regular trisomy. This could be attributed to the high fertility rate and trends towards reproduction even at an advanced maternal age (El – Zanaty et al., 1998).

T21 is frequently the result of non disjunction in maternal meiosis (~90%). Most occur in meiosis I (MI). Errors of meiosis II (MII) only constitute 20% of maternal errors (Frias et al., 2011). Trisomies of paternal origin are less common (3% in MI and 5% in MII) (Vekemons et al., 2005) and, in ~4% of the cases the additional chromosome is the result of a post zygotic error (Girirajan et al., 2009).

It is important to mention that the percentage of mosaicism may be under diagnosed because the number of cells analyzed generally is insufficient to detect cell lines in low proportion. Mosaicism in Down syndrome may originate from a normal zygote or as a result of a trisomy due to post zygotic non-disjunction or trisomic rescue, respectively (Gonzalez et al.,1998).

It has been reported that Robertsonian tranlocation may arise as a sporadic event (de novo) or may be transmitted by a carrier parent (familial). One-quarter of Robertsonian translocations in Down syndrome are familial and three-quarters are de novo (Shaffer et al., 1992). In familial Robertsonian translocation Down Syndrome, one of the parents(almost always the mother) is a translocation heterozygote and has transmitted the translocation in an unbalanced state to the offspring. Males with Robertsonian translocation are occasionally associated with infertility due to oligospermia because the translocation may disrupt spermatogenesis (Guichaoua et al., 1992). The distinction between de nova and familial forms of translocation Down syndrome is crucial. For the de nova translocation, a recurrence risk of less than 1% applicable. In the case of familial Robertsonian translocation Down syndrome, the genetic risk for the female carrier to have a live born child with translocation Down syndrome is above 10% while the likelihood to detect translocation trisomy 21 at amniocentesis is about 15%. For the male carrier ,the risk to have a child with Down syndrome is small i.e., 1%. (Gardner et al.,1996)

The homologous translocation 21q21q is one of the most common chromosomal rearrangement in Down syndrome. Classically this rearrangement has been termed Robersonian translocation. Molecular studies have proved that most denovo arrangements (21q21q) are isochromosomes derived from single parental chromosome 21 and only a small proportion are consistent with true Robertsonian tranlocations (Shaffer et al.,1993). Molecular studies suggest that many of these de nova cases originate at an early post zygotic mitosis so the recurrence risk is low (Robinson et al.,1994). However a small number of recurrences in subsequently born sibling are otherwise recorded and parental mosaicisam can be the basis of such recurrence (Sachs et al.,1990).

Diagnosis of Down syndrome is usually suspected at birth because of characteristic phenotypic features but this can be difficult to ascertain and the diagnostic accuracy ranged from 100% in non disjunction and translocation to as low as 37% of mosaic Down syndrome(Devlin and Morrison,2004). This could be due to the fact that these patients may be phenotypically less severely affected than persons with non disjunction or translocation. Therefore, chromosomal analysis is needed to confirm the diagnosis, to determine the risk of recurrence and for genetic counseling.

Genetic diagnosis by cytogenetic screening thus proved to be crucial in counseling of parents, and special education and management of MR children. The karyotype of parents with chromosomally abnormal children could help to establish the inheritance or recurrence risk in the family, and proved significant in prevention and genetic counseling.

47, XY, +21(DS Male Trisomy)

Karyotype

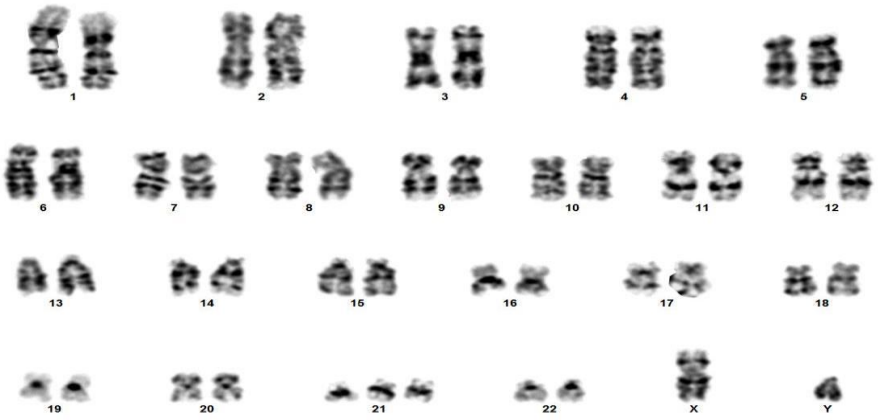

Fig. 1:

47, XY, +21(DS Male Trisomy)

Karyotype

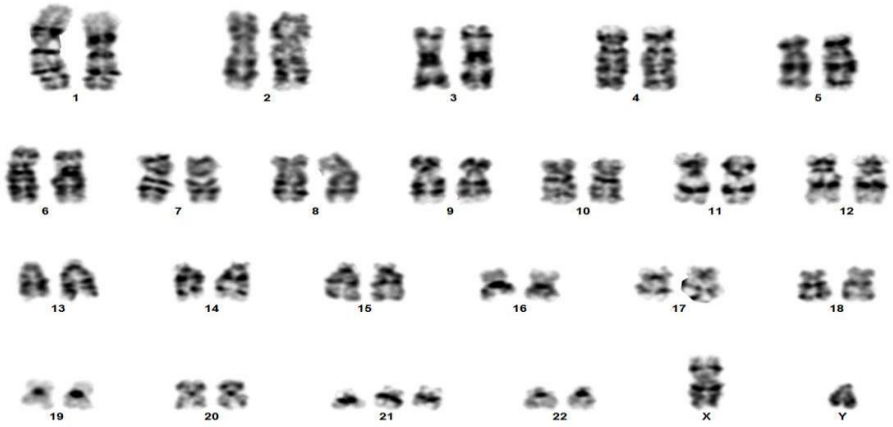

Fig. 2:

46, XX, t (21;21), +21(DS Female Robertsonian Translocation)

Karyotype

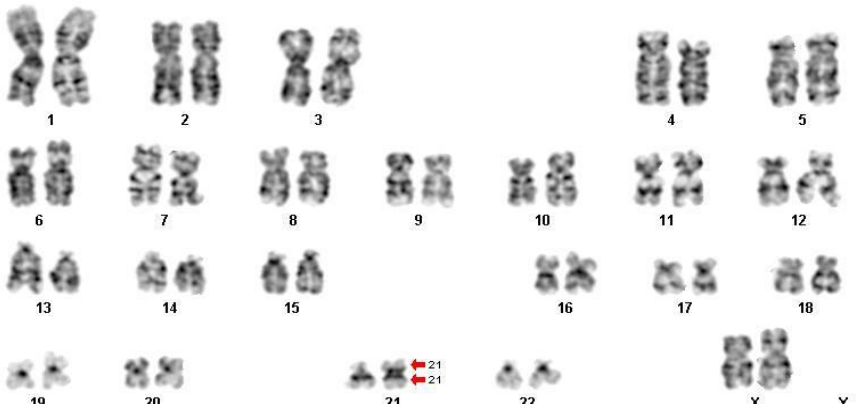

Fig. 3:

Metaphase

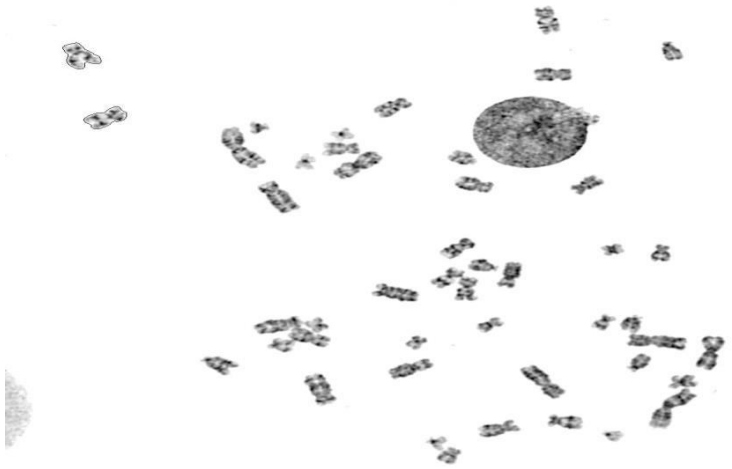

Fig. 4:

References

1. Curry, C.J., Stevenson, R.E., Aughton, D., Byrne, J., Carey, J.C., Cassidy, S., Cunniff, C.,Graham, Jr.J.M., Jones, M.C., Kaback, M.M., Moeschler, J., Schaefer, G.B., Schwartz, S., Tarleton, J. and Opitz, J. Evaluation of Mental Retardation: Recommendations of a Consensus Conference. American Journal of Medical Genetics. 1997; 72: 468-477.
2. Battaglia ,A., Bianchini, E and Carey ,JC.Diagnostic yield of the comprehensive assessment of developmental delay/mental retardation in an institute of child neuropsychiatry. Am J Med Genet. 1999; 82: 60–66.
3. Chiurazzi ,P., and Oostra, BA., Genetics of mental retardation .Curr Opin Pediatr. 2000; 12: 529– 535.
4. Lewis RA.Columbia University Pediatric Faculty Practice.2007;New York, Review provided by Vermid. From <http://www.nlm.nih.gov/medlineplus/ency/ article/001523.htm>Retrieved November 12.
5. Decoufle, P., Boyle, CA., Paulozzi, LJ and Lary, JM. Increased risk for developmental disabilities in children who have major birth defects: A populationbased study. Pediatrics. 2001; 108(3): 728-734.
6. Munro, JD.Epidmiology and extent of mental retardation. Psy Clinics North Am. 1986; 9: 591-624.
7. De Vries, B., Pfundt, R., Leisink, M and Koolen, D. Diagnostic genome profiling in mental retardation. Am J Hum Genet. 2005; 77: 606-616.
8. Kaur AK, Mahajan S, Singh JR . Cytogenetic profile of individuals with mental retardation. Int J Hum Genet. 2003; 3(1): 13–16.
9. Mulley, JC., Kerr ,B., Stevenson, R and Lubs, H. Nomenclature guidelines for X-linked mental retardation. Am J Med Genet.1992; 43: 83-89.
10. Archer ,HL., Gupta ,S., Enoch, S., Thompson, P., Rowbottom, A and Chua, I. Distinct phenotype associated with a cryptic subtelomeric deletion of 19p13.3-pter. Am J Med Genet. 2005; 136(1): 38-44.
11. Flint, J., Wilkie, AO., Buckle, VJ., Winter ,RM., Holland, AJ and McDermid, HE.The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat Genet. 1995; 9(2): 132-140.
12. Holinski-Feder, E., Reyniers, E., Uhrig, S., Golla, A., Wauters, J., Kroisel, P. Familial mental retardation syndrome ATR-16 due to an inherited cryptic subtelomeric translocation, t(3;16) (q29;p13.3). Am J Hum Genet. 2000; 66: 16-25.
13. Walter, S., Sandig ,K., Hinkel, GK., Mitulla, B., Ounap, K, amd Sims, G. Subtelomere FISH in 50 children with mental retardation and minor anomalies, identified by a checklist, detects 10 rearrangements including a de novo balanced translocation of chromosomes 17p13.3 and 20q13.33. Am J Med Genet. 2004; 128(4): 364-373.
14. Vundinti, BR., Ghosh, K.Incidence of Down syndrome: hypotheses and reality. Indian J Hum Genet. 2011; 17: 117-119.
15. Frias, S., Ramos, S., Molina, B., del Castillo, V and Mayén, D. Detection of mosaicism in lymphocytes of parents of free trisomy 21 offspring. Mutat Res. 2002; 520: 25-37.
16. Rajangam, S., Thomas, IM. Down syndrome and birth order. Man in India. 1992; 72: 239-242.
17. Malini, SS., Ramachandra, NB. Influence of advanced age of maternal grandmothers on Down syndrome. BMC Med Genet. 2006; 147: 4.
18. Nussbaum, RL., McInnes, RR and Willard, HF. Thompson and Thompson Genetics in Medicine. 2001; Philadelphia: WB Saunders Company.
19. Hultén, MA., Patel, SD., Tankimanova, M., Westgren. M., Papadogiannakis, N., Jonsson, AM. On the origin of trisomy 21 Down syndrome. Mol Cytogenet. 2008; 1: 21.
20. Devlin, L., and Morrison, PJ. Accuracy of the clinical diagnosis of Down syndrome. Ulster Med J. 2004; 73: 4-12.
21. Roizen, NJ., Patterson, D. Down's syndrome. Lancet. 2003; 361(9365): 1281–1289.
22. Thompson, M.W., McInnes, R.R. and Willard, H.F. Genética Médica.1993; 5th edn. Guanabara Koogan, Rio de Janeiro.
23. Fong, C., and Brodeur, G.M . Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet. Cytogenet. 1987; 28: 55-76.
24. Iselius, L., Jacobs, P and Morton, N. Leukaemia and transient leukaemia in Down syndrome. Hum. Genet. 1990; 85: 477-485.
25. Cassiman, JJ. Sex chromatin and cytogenetic survey of 10 417 adult males and 357 children institutionalized in Belgian institutions for mentally retarded patients. Humangenetik. 1975; 28: 43-8.
26. Speed, RM., Johnson, AW and Evans, HJ. Chromosome survey of total population of mentally subnormal in north-east Scotland. Journal of medical genetics. 1976; 13: 295-306.
27. English, CJ., Davison, EV., Bhate ,MS and Barrett, L. Chromosome studies of males in an institution for the mentally handicapped. Journal of Medical Genetics. 1989; 26: 379–81.
28. Stoll, C . epidemiology of Down syndrome in 118 265 consecutive births. American journal of medical genetics supplement. 1990; 7: 79-83.
29. AL-Awadi SA. cytogenetic profile of Down syndrome

in Kuwait: A decade of experience. Journal of the Egyptian Public Health Association. 1991; 16 (suppl): 259-69.

30. Mutton, D., Alberman ,E and Hook, EB.Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down syndrome cytogenetic register and the association of clinical cytogeneticists. Journal of Medical Genetics. 1996; 33: 387–94.
31. Mokhtar ,MM., Abd, El .,Aziz, AM and Nazmy, NA. Cytogenetic profi le of Down syndrome in Alexandria, Egypt.2003; Eastern Mediterr Health J [online]; 9: Nos 1/2. Available at: www.emro. who.int/publications/emhj/0901_2/cytogenetic. htm.
32. Azman, BZ., Ankathil ,R., Siti Mariam, I. Cytogenetic and clinical profile of Down syndrome in Northeast Malaysia. Singapore Med J Jun. 2007; 48(6): 550-554.
33. El-zanaty 1998. current fertility. In: Egypt demographic and health survey. Cairo , ministry of health, and Calverton, mary land, ORC / Macro,7.
34. Vekemans, M 2005. Trisomy Encyclopedia of Life Sciences (ELS). Chichester: John Wiley & Sons, Ltd.
35. Girirajan, S. Parental-age effects in Down syndrome. J Genet. 2009; 88: 1-7.
36. González-Herrera, L., Pinto-Escalante, D and Ceballos-Quintal, JM. Prevalencia de mosaicos en 100 individuos con diagnostic de síndrome de Down. Rev Biomed. 1998; 9: 214- 222.
37. González-Herrera, L., Pinto-Escalante, D and Ceballos-Quintal, JM Prevalencia de mosaicos en 100 individuos con diagnostic de síndrome de Down. Rev Biomed. 1998; 9: 214- 222.
38. Schaffer, LG., Jockson-Cook, CK., Stasiowski, BA., Spence ,JE and Brown JA.Parental origin determination in thirty de novo Robersonian tanslocations. American Journal of Medical Genetics. 1992; 47: 383-386.
39. Guichaoua, MR., Speed, RM., Luciani, JM., Delafontaine, D., and Chandley, AC .Infertility in human males with autosomal translocations II Meiotic studies in three reciprocal rearragements, one showing tertiary monosomy in 45-chromosome individual and his father. Cytogenetic Cell Gene. 1992; 60(2): 96-101.
40. Gardner, RJ and Sutherland, GR. Chromosomal abnormalities and genetic counseling. In: RJ Gardner, GR Sutherland (Eds): Elements of Medical Cytogenetic.1996; USA: Oxford University Press.
41. Schaffer, LG.Further characterization of 19 cases of rearrangement (21q21q) and delineation as isochrmosomes or Robertsonian translocation in Down syndrome. American journal of medical genetics. 1993; 47(8): 1218-22.
42. Robinson, WP. A somatic origin of homologous Robertsonian translocation and isochromosome. American journal of Human genetics. 1994; 47(8): 1218-22.
43. Sachs, ES et al. Trisomy 21 mosaicism in gonads with unexpectedly high recurrence risks. American journal of medical genetic supplement.1990; 7: 186-8.