Journal of the Anatomical Society of India 67 (2018) 133–138

Contents lists available at ScienceDirect

Journal of the Anatomical Society of India

journal homepage: www.elsevier.com/locate/jasi

Original Article

# Cytogenetic, epidemiological and clinical profile of children with Down syndrome in Karnataka

Krishnaveni Sharath[a,*], Asha K.R.[b], Lakshmi Prabha Subhash[b], Jayarama S. Kadandale[b]

[a] SreeBalaji Medical College, Chennai, Tamil Nadu, India
[b] Sri Siddharta Medical College, Tumkur, Karnataka, India

ARTICLE INFO

*Article history:*
Received 6 March 2018
Accepted 1 November 2018
Available online 3 November 2018

*Keywords:*
Down syndrome
Karyotyping
Maternal age
Robertsonian translocation
Genetic counseling

ABSTRACT

*Introduction:* Down syndrome is one of the best recognized and the most common chromosomal aneuploidy with high life expectancy than other chromosomal aneuploidies. The clinical features are quite distinguishing and easily identifiable, but a karyotype analysis is always better to confirm the diagnosis. It is also needed for calculating the risk of recurrence and for genetic counseling. This study was done to analyze the clinical features, cytogenetic and epidemiological profile of Down syndrome children in Tumkur and Bangalore region of Karnataka.
*Material and methods:* Karyotyping was done in 75 children with clinical features of Down syndrome by standard methods. Information about epidemiological & clinical features was documented. Informed written consent was taken from the parents. Comparison was made in the observed epidemiological profile, clinical features and the karyotype obtained.
*Results:* Among the 75 children with clinical features of Down syndrome, 59 had trisomy 21, 11 had translocation and 2 had mosaicism and 3 had a normal karyotype. The mean maternal age was 28.5 years. The prominent abnormalities noted were craniofacial features (71.8%). Characteristic limb abnormalities were also commonly observed (48.4). Congenital heart disease was diagnosed 56.1% cases analyzed.
*Discussion:* Efforts should be made to establish early diagnosis and proper screening. Confirmation of clinical diagnosis by Karyotyping is essential to determine the precise diagnosis, calculate recurrence risk and provide basis for genetic counseling.


## 1. Introduction

Down syndrome is one of the best recognized and the most common chromosomal aneuploidy with the incidence ranging-from 1 in 600 to 1 in 1000 live births.[1] The incidence in India is 0.88–1.09 per 1000. An estimated number of 21,400 infants are born every year in India with Down syndrome.[2,3]

The cardinal clinical features of Down syndrome include characteristic features like oblique palpebral fissure, flat nasal bridge, brachycephaly, and high arched palate, low set ears, protruding tongue, simian crease, Sandle gap, brachydactly, hypotonia, congenital heart disease, short stature and mental retardation. No single phenotype is pathognomonic but the combination of dysmorphism is usually recognizable.[4]

Diagnostic hypothesis of DS can be performed in pre and post natal period and confirmed by chromosome analysis. Cytogenetic investigation of individuals with clinical features of DS is fundamental to establish precise diagnosis which has implication in genetic counseling process. Cytogenetically there are three types of Down syndrome free trisomy 21, Robertsonian translocation or rarely mosaicism.[5]

Free trisomy 21 is characterised by the presence of 3 complete copies of chromosome 21, resulting due to nondisjunction during meiosis and seen in about 95% of cases. The cause of non disjunction error is not known, but attributed to increased maternal age. Advanced maternal age remains the only well documented risk factor for meiotic maternal non disjunction.[3]

Translocations are attributed to 3–4% of the cases, with Robertsonian translocation involving chromosome 14 and 21 being most common type. The chromosome 21 translocates to chromosomes 13, 14 or 15 group (D group) and chromosome 21 and 22 (G group).[5] Translocations occur in 10% of children born to

*Abbrevations:* DS, down syndrome; MA, maternal age; RT, Robertsonian translocation.

* Corresponding author at: Sree Balaji Medical College, BIHER, Chrompet, Chennai, India. Tel.: +91 9061745504.
*E-mail addresses:* drkrishna_veni@yahoo.com (K. Sharath), drashakeshavraj@gmail.com (A. K.R.).

https://doi.org/10.1016/j.jasi.2018.11.001
0003-2778/

mothers aged between 15–19 years.[1] Karyotype analysis for both parents is indicated in translocations as either of them may be carriers for balanced translocation involving with chromosome 21. There is an increased risk of aneuploid offspring in every conception with translocation carriers and recurrence risk depends on the chromosomes that are fused and the sex of the carrier parents.[6]

Mosaicism is characterised by cells containing 46 chromosomes and others with 47 chromosomes, is reported in 1% of Down syndrome patients. There may be 2 cell lines with 46 as well as 47, +21 chromosomes components. The origin of the extra 21 may be from zygote with 46 or 47 cell lines. In the former, non disjunction phenomenon leads to mosaic cell lines of 45/46/47 chromosomes, where the cell lines with 45 chromosomes - 21 become non viable. On the other hand, in cell lines with 47, anaphase lag of the extra 21 results in 46 and 47 cell lines. This is associated with increased maternal age as trisomy 21. In mosaicism, the typical clinical features of DS may be less prominent, depending on the percentage of normal to trisomy 21 cell lines.[3]

The risk of recurrence differs greatly between cases as free trisomy &mosaicism generally do not recur (approximate risk of <1% with maternal age less than 30).[7] For translocations, if both parents of children with translocation DS present with normal karyotype, the risk of recurrence is 2%–3%. However, if the mother is carrier of balanced translocation, the recurrence risk is up to 20% and if it is carried by the father the recurrence risk is up to 5%. On the other hand, if one of the parents is the carrier involving both the chromosomes 21, the recurrence risk is 100%. Familial recurrence of RT is seen in 1/4th of cases whereas in remaining cases it arises de novo. Thus, once diagnosed as a case of translocation DS, a karyotype analysis of both parents is recommended.[8]

This study was done to analyze the clinical features, cytogenetic and epidemiological profile of Down syndrome children in Tumkur and Bangalore region of Karnataka.

## 2. Materials & methods

Cytogenetic evaluation was carried out on 75 children referred to cytogenetic division of Sri Siddharta medical College, Tumkur with clinical diagnosis of Down syndrome. Information on age, parental age at birth, birth order, consanguinity and clinical features were documented. Informed written consent was taken from the parents. About 3 ml of intravenous blood was drawn and cultured in suitable culture media containing phytohaemoagglutin, growth media and antibiotic at 37° for 72 hours. Colchicine was added at 69th hour to arrest cell division at metaphase stage. Hypotonic treatment was given to the centrifuged pellet with KCl (0.75 ml) and left at room temperature for 15 minutes. The solution was centrifuged and treated with freshly prepared fixative of 3:1 methanol: acetic acid. This was further centrifuged and supernatant was removed. This was done till a clear pellet was obtained. Slides were prepared by dropping the pellet on clean slides. GTG banding was used to stain & identify the chromosomes under oil immersion lens using Carl Zeiss microscope.[9] Karyotyping was also advised in parents of children with translocation type of Down syndrome.

Statistical data was analyzed using chi square test for goodness of fit.

## 3. Results

Out of the 75 children with clinical features of Down syndrome, 59 had trisomy 21(78.7%), 11 had translocation (14.7%),2 had mosaicism (2.6%) and 3 had a normal karyotype(4%). One child showed trisomy 21 with chromosome 13–14 translocation (Figs. 1–3).

Out of the 75 children, 42 were males & 33 females. The Male: female ratio was 1.3:1. The average age at presentation was 2.6 years. The youngest patient was 2 day old infant and the oldest patient included in the study was a 17 year old adult. The mean maternal age at conception was 28.5, ranging from 18 years to 42 years and the mean paternal age was 31.6 years ranging from 21 to 49 years. 19 mothers were aged above 35 (25.3%) during the conception of these Down syndrome children. 12 couple with DS children had a history of consanguineous marriage (16%) (Fig. 4).

Among the clinical features listed in Table 1 it can be seen that the common craniofacial abnormalities comprised flat facies (82.7%), flat nasal bridge (85.3%), oblique palpebral fissure (64%), flat occiput with brachycephaly (68%), small mouth (78.7%), high arched palate & protruding tongue (75.4%). The frequency of low set or dysplastic ears was 48%. A total of 70% of cases had hypotonia. Characteristic limb abnormalities like Sandle gap (60%) and clinodactly (49.3%) was also commonly observed. Simian crease was observed in27 children (36%).

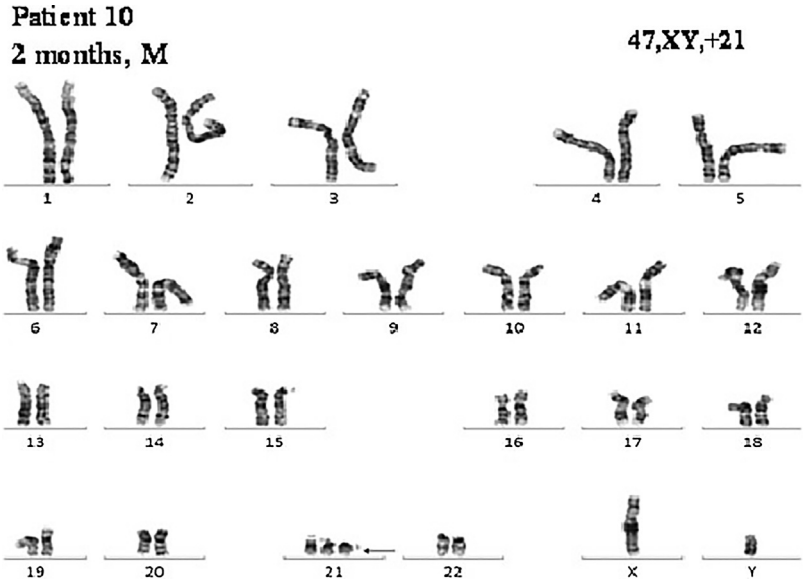


**Fig. 1.** Karyotyping showing trisomy 21 in a male child.

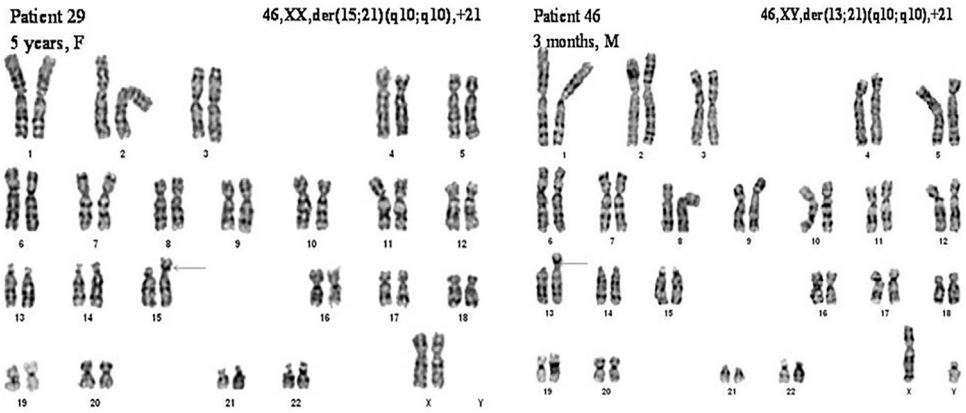


**Fig. 2.** Two karyotypes showing translocation type of DS.


**Fig. 3.** Karyotype with trisomy 21 and translocation.13–14.

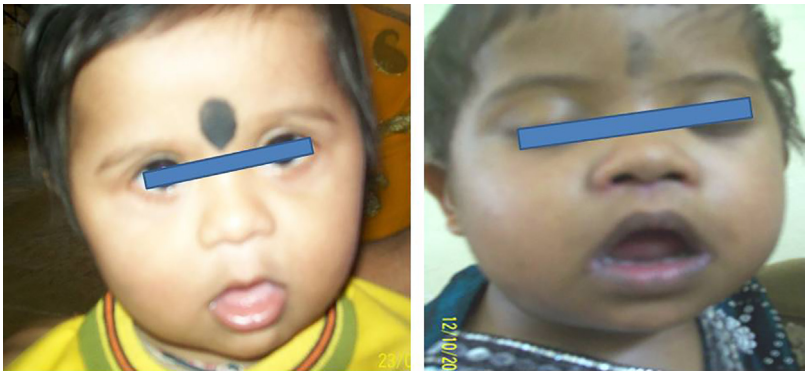

**Fig. 4.** Photograph showing typical craniofacial features of DS.

All the children were asked to go for cardiac evaluation (ECHO), thyroid profile (T3, T4 and TSH), ophthalmological, ENT and Psychiatric evaluation (for calculation of IQ). Congenital heart disease was observed in 23 (56.1%) children out of 41 who underwent ECHO. The cardiac abnormalities observed in DS children were atrial septal defect,[9] ventricular septal defect,[6] patent foramen ovale,[3] patent ductus arteriousus,[2] tetralogy of Fallot[1] and combined abnormalities.[2] Among 37 children who underwent thyroid profile 20 showed normal thyroid profile, 13 showed hypothyroidism and 4 hyperthyroidism (46%). Ophthalmological evaluation of DS children showed 6 children with refractive errors, 2 of them had congenital xerosis out of which 1 became blind, 3 children showed squint and 5 were operated for congenital cataract. There were2 children with biliary atresia for which they were operated. There were 3 children with umbilical hernia. The average IQ in 55 children evaluated was 46.5. On neurological evaluation it was seen that 6 children had seizure disorder.

The parents with translocation Down syndrome were asked to give their blood for karyotyping. Out of the 11 couple, only 9 couples underwent Karyotyping. Their karyotyping did not show any abnormalities.

## 4. Discussion

Despite the high detection rate of Down syndrome by various antenatal screening programmes, it is still the common genetic cause for mental retardation.[10]

**Table 1**
Common clinical features of DS as seen in our study.

| Clinical feature | No of children | Percentage (%) |
|---|---|---|
| Flat facies | 62 | 82.7 |
| Oblique palpebral fissure | 49 | 64 |
| Flat nasal bridge | 64 | 85.3 |
| High arched palate & protruding tongue | 57 | 76 |
| Low set or dysplastic ears | 36 | 48 |
| Flat occiput with brachycephaly | 51 | 68 |
| Small mouth | 59 | 78.7 |
| Simian crease | 27 | 36 |
| Sandle gap | 45 | 60 |
| Clinodactly | 37 | 49.3 |
| Speech & language delay | 39 (out of 45) | 86.7 |
| Hypotonia | 32 (out of 45) | 71.1 |
| Developmental delay | 34 (out of 45) | 75.6 |

All cases were diagnosed postnatal in the present study. An observation of free trisomy in 78.7%, translocation in 14.7%, mosaicism in 2.6% and normal karyotyping in 4% normal karyotyping is in accordance with other studies by Chandra et al (86.2%, 12.8% and 1%) and Jayasekara (88.8%, 8.6% and 2.6%).[11,12] The rare phenomenon of clinical DS with normal karyotype is attributed to undetected mosaicism or partial trisomy. The diagnosis is based on Jacobson's criteria in these cases. The present study differs significantly from Azman et al (94.6%, 4.7% and 0.7%) and Jaoud et al (96.2%, 3.2% and 0.6%).[13,14] it is difficult to suggest reasons for the discrepancy in the frequencies of cytogenetic abnormalities in various studies. It may be due to divergence in study periods, duration of study period, maternal age and the population studied.

The age at diagnosis ranged from a 2 day infant to a 17 year old adult with a mean of 2.6 years. This delay may reflect low awareness of the family as well as health care provider for early suspicion of affected newborns. Also since there was no facility for karyotyping in Tumkur region previously, genetic diagnosis could not be made. The delay in diagnosis causes postponement of intervention programmes and appropriate therapy.[15]

The sex ratio was 1.3:1. The higher male sex ratio may be inherent tendency of 'Y' chromosome belonging to the 'G' group chromosome to be closer to its other members, 21and 22, especially, the smallest acrocentric, the 21.[3] It may also be due to the fact that in Indian society the male children are more likely to be bought to hospital than female.[2]

Advanced maternal age is an important risk factor in the free trisomy due to imperfections in chromosomal segregation. In this study 25.3% of mothers were aged above 35 years when they conceived these DS children. Occurrence of DS independent of maternal age presents an evidence for other risk factors for this syndrome. Recently several studies have related the maternal risk factor for DS with genetic polymorphism involved in folate mechanism. Hypomethylation of the centromeric DNA as a result of abnormal folate metabolism leading to abnormal chromosomal segregation and studies point to the role of polymorphisms in some genes involved in homocysteine metabolism as risk factors for DS.[6] Due to cultural norms in India, because most pregnancies occur in younger women, approximately 80% of all babies with trisomy are born to women under the age of 35. It is very much evident in this study as 56 children with DS were born to mothers below 35 years of age.[16]

Maternal age also relates to the type of chromosome abnormality. Translocations occurs in 10% of the time in children born to mothers between 15–19 years of age.[17] In our study 7 translocation children were seen in mothers aged between 18–20 age group and 4 between 21–34 age group. Whenever chromosome analysis reveals a translocation, both parents should undergo karyotyping to check for balanced translocation. However none of the 9 cases examined of 11 of translocation had balanced translocation in parents.

The effect of paternal age has not been extensively studied. In our study the average paternal age was 31.56. Since the paternal & maternal age is correlated, it should be adjusted to maternal age. A study to assess the possible nonlinear trends between parental age and prevalence of Down syndrome in a large sample is advocated.[18]

It would also be of interest to know whether the incidence of DS has changed over time, since if radiation exposure of parents is a major factor in producing chromosomal aberrations, one would expect an increase in incidence rate with increasing exposure of the population to medical & other radiational exposure.[19] However, since the advent of better and safer antenatal techniques for diagnosis of DS, the rate of medical termination of such fetus may also have increased, thereby making the calculation of incidence difficult.[7] A 15 year study by Chengfei Deng in China showed that DS prenatal prevalence rate(PPR) during 1996 to 2011 was 1.99 per 10 000, presenting an increasing trend till 2003 but a decreasing tendency since then. The proportion of DS diagnosed prenatally increased from 7.55% during 1996 to 2002 to 47.70% during 2003 to 2011. During 2003 to 2011, the high termination rate (probably due to the one child policy in China) led to 55% reduction in the overall DS.[10] Also many of the DS pregnancies are aborted naturally. According to a study in England & Wales to know the natural history of DS pregnancy without medical intervention, the loss rates were approximately 50% for those fetuses ascertained at 15–17 completed wk, 43% at 18 wk, 31% at 19 wk, 25% at 20 wk, and then a levelling off at approximately 20%–25% for fetuses ascertained at 21–28 completed wk.[20] Therefore in a country like India where there is no standard registry for pregnancy it is difficult to get the exact incidence of DS.

Theoretically consanguineous marriages have higher risk of producing off springs with genetic damage than that of general population. Consanguineous marriages are common in Karnataka especially in regions of Mysore, Tumkur and Kolar with uncle – niece unions being very common. Among the 75 couples who had DS children only 12 couples (16%) were married among relatives. DS, being a chromosomal aneuploidy is not associated with consanguinity.[21]

Although DS has responsible no pathognomonic features, it manifests as combination of clinical features that make the clinical diagnosis possible. Craniofacial features are the most conspicuous for diagnosis. The knowledge of clinical manifestations of DS is important to make an early postnatal diagnosis. The diagnostic accuracy based on clinical features in neonatal period ranges from 73 to 100%. The comparison of clinical characteristics between our study and related literature is given in Table 2.

Frequencies of DS clinical features vary within the different studies and some of them described only specific characteristics. Bibliographic reviews on DS have shown large variabilities of these characteristics.[8] Comparison of frequencies observed for some clinical features were in accordance with the previous studies, whereas some features disclosed significant statistical difference. According to Devlin and Morrison's study the accuracy of DS clinical diagnosis varies according to the cytogenetic alteration involved (90% for trisomy, 100% for translocation and 37.5% for mosaicism.[24]

Several other factors may contribute to the phenotype variability in DS, such as allelic heterogenecity for chromosome 21 genes present in three copies, the individual's genetic constitution and environmental factors.[8] India is a country with large ethnic heterogenecity and great geographic area. The

**Table 2**
Comparison of clinical features of DS in our study and previous studies.

| Characteristics | Present study | Kava et al[22] | Azman et al[13] | Ranganathan et al[4] | Ahmed et al[23] | Bertelli et al[8] |
|---|---|---|---|---|---|---|
| Flat facies | 82.6 | 50.9 | 64.9 | 84.5 | 83 | 94.8 |
| Oblique palpebral fissure | 64 | 89.3 | 89.3 | 100 | 63 | – |
| Flat nasal bridge | 85.3 | 75.9% | – | 86.7 | 61 | 93.5 |
| High arched palate &protruding tongue | 76 | 79.9% | – | – | – | 35.5 |
| Low set or dysplastic ears | 48 | 66.9% | 56.1 | 68.9 | 45.7 | 40.3 |
| Simian crease | 36 | 33.2 | – | – | 64.7 | 83.9 |
| Sandle gap | 60 | 46.2 | – | – | 46.4 | 64.5 |
| Clinodactly | 49.3 | 36.1 | – | – | 24.7 | 46.7 |
| Congenital heart disease (out of 34) | 56.1 | 34.9 | 49 | – | 34.9 | – |

miscegenation of immigrant population has provided for gene propagation and contributed for the characteristics of Indian population.[25]

The incidence of congenital heart diseases in DS ranges from 45 to 60%. In our study it was 56.1% which is similar to study by Bertelli et al.[8] For knowing the difference of incidence of CHD in different karyotype pattern of DS, larger study with bigger sample size should be done. To compare the different cardiac defect also the sample size is small. The association between endocardial cushion defects and trisomy 21q suggests that genes located on chromosome 21 contribute to development of heart. Several genes have been proposed as candidates for CHD in down syndrome, the most common being COL6A1 gene.[26]

### *4.1. Genetic counseling*

The children included in this study were offered genetic counseling by a qualified genetic consultant used a multi specialty team for diagnosis of associated defects and treating them, thereby improving the quality of life.

A multi-specialty team is the mode to manage the affected DS children and their families. Each child with DS should have an access to an early intervention program as soon as possible. Programs for children aged < 3 years should be designed to comprehensively monitor & enrich their development by focusing on gross and fine motor skills, language, personal and social development. Many children & adults can enhance their quality of life through speech and physical therapies, regular medical checkups and treatment, nutritional & occupational therapy. Parents of DS children should be provided with referrals to support group and organizations that advocate for persons with DS and their families.,[93]

In free trisomy 21 homogenous or mosaic, the karyotype of parents is not required. The geneticist will assure the couple that the risk of recurrence is same as that of general population and it depends on age of mother. In translocation, the karyotype of parents is obligatory and confirms the character of anomaly. Familial recurrence of RT is seen in $1/4^{th}$ of cases whereas in remaining cases it arises de novo. Rare cases Trisomy 21 is by translocation of two chromosomes 21. If one parent is carrying this in balanced state, the risk of recurrence is 100%. The geneticist will inform the possibilities of antenatal diagnosis if desired.[14]

## 5. Conclusion

Craniofacial features were the commonly observed characteristic in DS children. The comparison between our data and related literature showed considerable variability of the phenotype features frequencies of DS among studies. In addition to environmental factors, this can also reflect individual and population characteristics.

This study has provided a basis for further epidemiological surveys of DS in Karnataka. Confirmation of clinical diagnosis by Karyotyping is essential to determine the precise diagnosis, calculate recurrence risk and provide basis for genetic counseling.

## Funding

The research titled “Cytogenetic, clinical and Epidemiological profile of children with Down syndrome in Karnataka” was not funded by any external resource.

## Conflict of interest

There is no conflict of interest among the authors in this study.

## Acknowledgement

The authors would like to thank Dr Meenakshi Bhat MD (Paed), MRCP, Genetic counselor for helping us with the study. We also acknowledge the technical staff of genetic lab, Sri Siddhartha Medical College and Center for Human genetics especially, Mrs Harshal for helping in the technical details in Karyotyping. We also thank the parents of the children for allowing us to withdraw blood and cooperating with our study.

## References

1. Regina Mary R, Gothandam KM. TLM. Increased frequency of down syndrome in young mothers of rural population. *J Ecobiotechnol*. 2010; Aug 23.
2. Verma Ic, Mathew S, Elango R, Shukla A. Cytogenetic studies in Down syndrome. *Indian Pediatr*. 1991;.
3. Margaret M, Amudha S, Tilak P, Devi R, Rajangam S. Cytogenetic Analysis in Down Syndrome. *Int J Hum Genet*. 2010;10(3):95–99.
4. Ranganathan Priya, Ann Agnes mathew SR. Cardinal Features: Down syndrome. *South Asian Anthropol*. 2005;5(1):97–101.
5. Biselli Joice, Goloni-Bertollo Eny, Ruiz Mariangela, Pavarino-Bertelli E. Cytogenetic profile of down syndrome cases seen by a general genetics outpatient service in Brazil. *Downs Syndr Res Pract*. 2009;(February).
6. Polipalli Sunilkumar, Karra Vijay Kumar, Jindal Ankur, et al. Cytogenetic analysis for suspected Chromosomal Abnormalities; A five year experience. *J Clin Diagn Res*. 2016;10(9).
7. Sheth F, Rao S, Desai M, Vin J, Sheth J. Cytogenetic analysis of Down syndrome in Gujarat. *Indian Pediatr*. 2007;44:774–777.
8. Pavarino Bertelli EC, Biselli JM, Bonfim D, Goloni-Bertollo EM. Clinical profile of children with Down syndrome treated in a genetics outpatient service in the southeast of Brazil. *Rev Assoc Med Bras*. 2009;55(5):547–552.
9. Ellaithi M, Nilsson T, Elagib A, Fadl-Elmula I, Gisselsson D. *A first cytogenetic study of down syndrome in Sudan*. [cited 2017 Dec 24];14(2).
10. Loane Maria, Morris Joan K, et al. Twenty-year trend in the prevalence of Down syndrome and other trisomies in Europe: Impact of maternal age and prenatal screening. *Eur J Hum Genet*. 2013;21:27–33.
11. Chandra N, Cyril Cyrus, Lakshminarayana Prema, et al. Cytogenetic evaluation of Down syndrome: A Review of 1020 Referral cases. *Int J Hum Genet*. 2010;10 (1-3)1–14 87–93.
12. Jayasekara R. Chromosomal anomalies in srilanka : A cytogenetic profile. *Ceylon J Med Sci*. 1988;.
13. Azman BZ, Ankathil R, et al. Cytogenetic and clinical profile of Down syndrome in Northeast Malaysia. *Singapore Med J*. 2007;48(6):550–554.

14. Jaouad IC, et al. Cytogenetic and epidemiological profiles of Down syndrome in a Moroccan population: A report of 852 cases. *Singapore Med J*. 2010;51 (2):133–136.
15. El-Gilany A-H, Yahia S, et al. Cytogenetic and comorbidity profile of down syndrome in Mansoura university children's hospital. *Egypt Indian J Hum Genet*. 2011;17(September (3))157–163 [cited 2017 Dec 24].
16. Kumar P, Gupta A, Fotra R, Upma Raina S, Sethi S, et al. Chromosomal anomalies in referred cases with suspected genetic disorders: First report from Jammu and Kashmir. *Int J Hum Genet*. 2010;.
17. Kothare S, Shetty N, Dave U. Maternal age and chromosomal profile in 160 down syndrome cases – Experience of a tertiary genetic centre from India. *I J H G*. 2002;2(1):49–53.
18. Dzurova D, Pikhart H. Down syndrome, paternal age and education: Comparison of California and the Czech Republic. *BMC Public Health*. 2005;5(1)69 Dec17 [cited 2017 Dec 24].
19. Cohen Bernice H, Abraham M, llilienfeld ATS. Some epidemilogical aspects of mongolism: A review. *AJPH*. 1963;53(2):223–236.
20. Hook EB, Mutton DE, Ide R, Alberman E, Bobrow M. The natural history of Down syndrome conceptuses diagnosed prenatally that are not electively terminated. *Am J Hum Genet*. 1995;57(October (4)):875–881.
21. Mouzan Mohammad IEl, Salloum Abdullah AAl, Herbish Abdullah SAl, Mansour M, Qurachi b AAAO. Consanguinity and major genetic disorders in Saudi children: A community-based cross-sectional study. *Saudi Genet J*. 2008;28(3):169–173.
22. Kava MP, Tullu MS, Muranjan MN, Girisha KM. Down syndrome: Clinical profile from India. *Arch Med Res*. 2004;35(January (1)):31–35.
23. Ahmed I, Ghafoor T, Samore NA, Chattha MN. Down syndrome: Clinical and cytogenetic analysis. *J Coll Phys Surg Pak*. 2005;15(7):426–429.
24. Devlin L, PJM. Accuracy of the clinical diagnosis of down syndrome. *Ulster Med J*. 2004;73(1):4–12.
25. Kaur A, Singh JR. Chromosomal abnormalities : Genetic disease burden in India. *Int J Hum Genet*. 2010;10(1-3):1–14.
26. Bromham NR, Woodhouse JM, Cregg M, Webb E, Fraser WI. Heart defects and ocular anomalies in children with Down's syndrome. *Br J Ophthalmol*. 2002;86 (December (12)):1367–1368.