International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-1, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Biochemical and Genetic Evaluation of Down's Syndrome Family: Carrier and Trait Detection

Dutta AK*, Maharana J

Department of Biotechnology & Microbiology, St. Thomas College, Bhilai (CG) - 490006.

Manuscript No: IJPRS/V3/I1/00018, Received On: 09/01/2014, Accepted On: 18/01/2014

ABSTRACT

All the life forms are manifestation of genetic codes and the intricate relations between several human diseases and genetics is known since long. Genetic defects are manifested at either chromosomal level or phenotypic level. Down syndrome (trisomy 21) is the most commonly recognized genetic cause of mental retardation. The risk of trisomy 21 is directly related to maternal age. All forms of prenatal testing for Down syndrome must be voluntary. Women younger than 35 years should be offered maternal serum screening at 16 to 18 weeks of gestation. Down's syndrome is one of the commonest genetic abnormality viable with life its incidence evoked significant concern and research into its etiological factors need not be emphasized "the study deal particularly with pedigree analysis of Down's cases with special attention to associated spontaneous pregnancy loss in Down-baby's mother".

Selected cases of known Down syndrome & their chromosomal abnormalities constitute an important part of genetic problems and are one of the most specific causes for genetic referral. 20 Patients has been subjected for pedigree analysis and genetic counseling to understand the risk factor and phenotypic etiology in the generation. The present study is based on the etiological factors known for Down syndrome, but has its own significance on the basis of genetic counseling, pedigree analysis, maternal age, cytogenetic analysis. This piece of work is an attempt to understand the phenomenon and genetic involvement of the genetic disorder with reference to Down's syndrome.

KEYWORDS

Down's Sample, Counseling, Pedigree, Lymphocyte Culture, GTG Banding, Karyotyping

INTRODUCTION

Clinical Genetics is one of the most rapidly advancing fields in medicine. Diagnosis- a chromosomal disorder is based on clinical suspicion and cytogenetic studies.[1,2] Down's syndrome or trisomy 21 is a type of chromosomal genetic disorder caused by the presence of all or part of an extra 21st chromosome. The condition is characterized by a combination of major and minor differences in body structure.

***Address for Correspondence:**
Dr. Amit Kumar Dutta
Lecturer, Department of Biotechnology & Microbiology
St. Thomas College, Risali, Bhilai (CG)- 490006.
E-Mail Id: adjnchrc@yahoo.com

Down syndrome is associated with some impairment of cognitive ability and physical growth as well as facial appearance, usually identified at birth. Many of the common physical features of Down syndrome also appear in people with a standard set of chromosomes, directly related to maternal age. Women younger than 35 years should be offered maternal serum screening at 16 to 18 weeks of gestation.[1,4]

Down syndrome is usually caused by an error in cell division called nondisjunction. It is not known why this occurs. However, it is known that the error occurs at conception and is not

related to anything the mother did during pregnancy. It has been known for some time that the incidence of Down syndrome increases with advancing maternal age. However, 80% of children with Down syndrome are born to women under 35 years of age.

The medical consequences of the extra genetic material in Down syndrome are highly variable and may affect the function of any organ system or bodily process. The health aspects of Down syndrome encompass anticipating and preventing effects of the condition, recognizing complications of the disorder, managing individual symptoms, and assisting the individual and his/her family in coping and thriving with any related disability or illnesses.[8] The most common manifestations of Down syndrome are the characteristic facial features, cognitive impairment, congenital heart disease, hearing deficits, short stature, thyroid disorders, and Alzheimer's disease. Other less common serious illnesses include leukemia, immune deficiencies and epilepsy.

MATERIALS AND METHOD

The present study is based on the etiological factors known for Down syndrome, but has its own significance on the basis of genetic counseling, pedigree analysis, maternal age, cytogenetic analysis, hepatic profile and on the basis of hemoglobin electrophoresis.

Collection of Sample

Cytogenic Analysis

5-10 ml of venous blood was drawn with a sterile disposable needle (1.10 x 38mm) and syringe (10 ml) aseptically in to a sterile Bisou bottle containing 30 units of Heparin (1000 IU/MI) the blood is allowed to settle by gravity sedimentation.

Setting up of Culture

Culture tubes were thawed and the temperature was adjusted to 37^{0}C 0.5 ml of the plasma. Lymphocytes were inoculated in to each tube. After completion of culture, each tube was copped aseptically and tightly labeled and incubated for 70 hrs. The PH of the medium was approximately 7.1 and changes could be followed by noting the color of the phenol red indicator in the medium. All aseptic operations were carried out in a sterile culture room.

Harvesting of Culture

At 70 hrs. 0.5ml of 0.02% aqueous colchicine solution was added as the pretreatment agent. After 72 hr. incubation, the cell suspensions were poured in to labeled centrifuge tubes and were centrifuge for 10 mins at 500 rpm. The supernatant were expelled out hypotonic solution (0.56% KCl) was added to the cells at the bottom of tube with were suspended by the gentle washing, flushing. The tubes were kept at 37^{0}c for 30 min. The cell suspension was centrifuged again carefully at 900-1000 rpm, for 30 min. The supernatant was removed and 4 ml of fixative was added. The tubes were allowed to stand at room temperature for 30 min, centrifuge again, the fixative was removed and 0.2-0.5 ml of fresh fixative was added. The cells were gently flushed with a pasteur pipette. To avoid cell loss care was taken to see that cell suspension did not reach a higher level than the steam of the pipette.[9,10]

Pre-treatment

Pre-treatment agents are used for bringing about a scattering of chromosome with clarification of construction region and for obtaining a higher percentage of metaphase plates. The most popular pretreatment agent used in human chromosome analysis in colchicines. It prevents formation of spindles, resulting in accumulation of metaphor in rapidly dividing cells. Increased contraction of chromosome and accentuating the spitting of chromatids.

0.02% colchicine was usually used 20 mg of colchine was dissolved in 100 ml of double distilled water & stored in brown bottle in dark room at 40^{0}C.

Hypotonic Treatment

It is carried out to swell up cells and subsequently disperse chromosome to be effective, the solution must be of low osmotic pressure and ionic strength. Double distilled

water was used as the hypotonic agent the treatment was for 30 min at 37^0C.

Fixation

Fixatives are used to preserve the form of cells and their contents as closely as possible living state and render them resistant to further changes. A mixture of glacial acetic acid and methanol (1:3) was used as fixative.

After the hypo tonic treatment fixation was carried out for 30 mins. A second change was given after centrifugation.

Preparation of Slides

The air drying technique of Rothfels and Siminovitech (1958) was employed. Clean, grease free slides were chilled. The cell suspension was gently flushed and 2 to 3 drops allowed falling from a height of about 6 inches and even more if needed. The excess fixative was allowed to run off by filting the slides which were gently heated to help in the spreading of chromosomes. Slides were examined under ordinary light microscope with condenser lowered and illumination reduced. Depending on the number of cells obtained, the suspension was concentrated by centrifugation and reduction of fixative. A minimum of 4 slides were prepared from each culture suspension. The reminder was stored at -10^0C. One slide each culture was sustained in Giemsa to study blastoid transformation, the remaining slides stored to study banding.

Staining

Giemsa is most extensively used in human chromosome preparations.

RESULTS AND DISCUSSION

Pedigree Analysis

Out of 20 subjects the pedigree analysis of 9 subjects could not be analyzed due to some specified reasons that is at the time of family data collection, the demography making, the parents are not ready to disclosed required data and more over the aggressive behavior of the patient have not allow to complete the information.[6] (Fig 1)

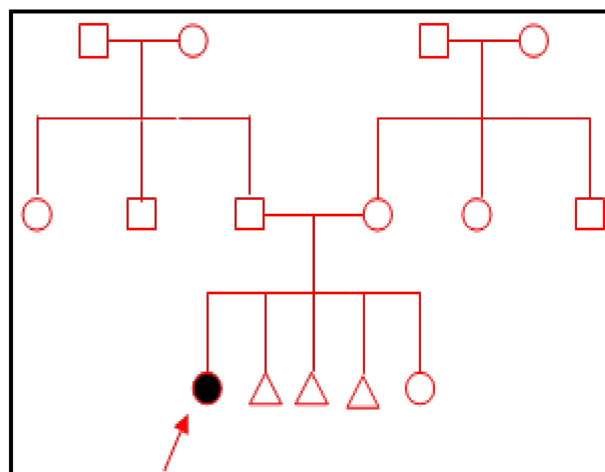


Figure 1: Showing the Pedigree Analysis of one patient

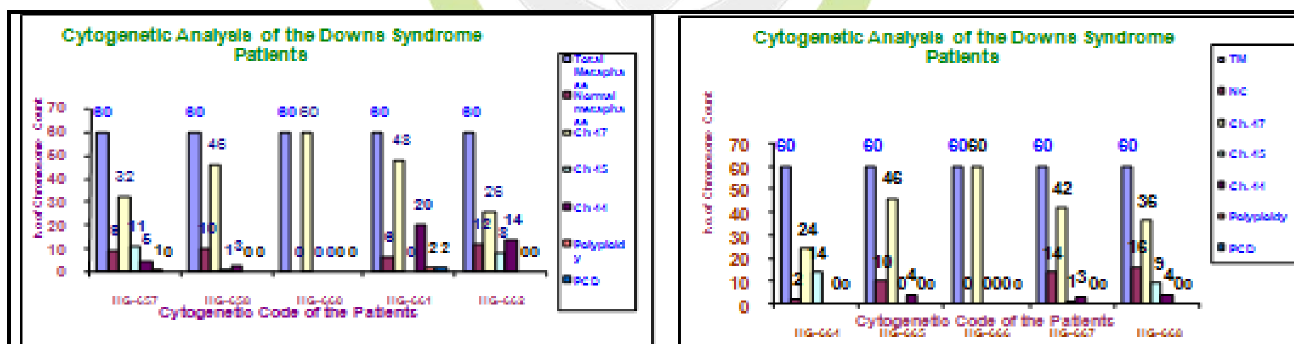
Dermatoglyphics Result

Intellectual ability and mental retardation was studied by one of the anthropometry parameters Dermatoglyphics of the subjects and their 'atd' (angular triradius) angle one noted down and compared with normal values. Out of 20 cases, 18 were found to have raised 'atd' angle and the average 'atd' angle was found to be 51.53°, Out of these 18 cases six were found to have 'atd' angle in the range of 45°-55°(33%), five in the range of (27%), one of the range of 55°-65° (5%), one in the range of 65°-75°(5%), five in the range of 75°-85°(27%) and one in the range of 95°-105°(5%). (Table 1) The intellectual ability was found to be more retarded and the retardedness increased with the increase of the 'atd' angle. Simian crease was found in 6 cases, Out of 16 cases, and one patient shows bilateral simian crease. (Fig 2a, b, c)

Figure 2 a,b,c: showing the atd angle of the Down's patient

Table 1: Showing the atd Angle of Down Syndrome Patients

Cyto Code	Right Hand	Left Hand	Average
HG-655	45°	48°	46.5°
HG-656	98°	105°	101.5°
HG-657	33°	33°, 45°	36°
HG-658	42°	49°	45.5°
HG-659	72°	80°	76°
HG-660	42°, 62°	38°, 62°	51°
HG-661	72°	80°	76°
HG-662	42°	53°	47.5°
HG-663	45°, 85°	94°	65°
HG-664	43°, 82°	94°	78.25°
HG-665	35°, 59°	39°, 63°	51°
HG-666	40°, 73°	53°, 82°	61.05°
HG-667	43°, 86°	86°	75.25°
HG-668	35°, 50°	37°	39.75°
HG-669	40°	39°, 50°	42.25°
HG-670	65°	73°	69°
HG-671	45°, 83°	35°, 68°	51.5°
HG-672	60°	35°, 68°	55.75°
HG-673	42°, 78°	38°, 65°	55.75°
HG-674	57°	50°, 69°	58.25°



Graph 1 & 2 showing the Cytogenetic Analysis of the Down's Patient

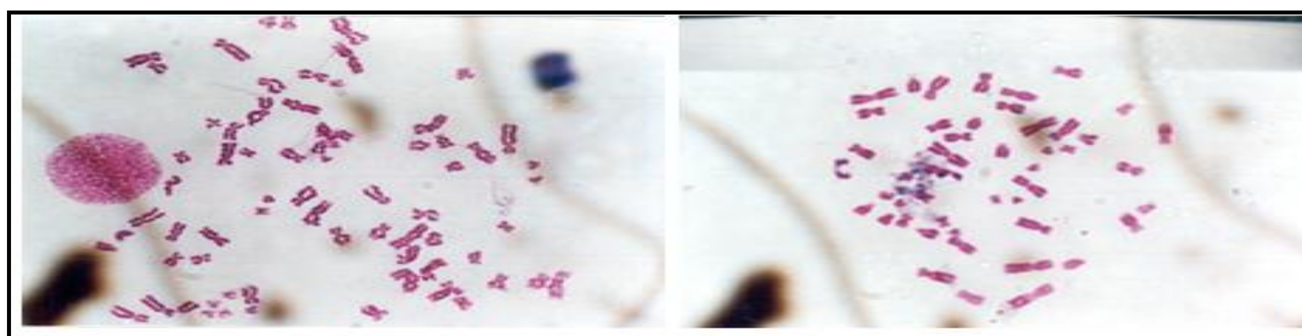


Figure 3 a, b: Showing the Chromosomal Aberrations of the Down's

© Copyright reserved by IJPRS

Table 2: Showing the Cytogenetic Analysis of the Registered Patients

Sample no.	Total metaphase	Metaphase with normal number of chromosome	Metaphase with abnormal number of chromosome				
			47 (2n+1)	Percentage of the 2n+1	45 (2n-1)	44(2n-	0thers
HG-655	60	9	34	56.66	11	5	1 PP
HG-658	60	10	46	76.66	1	3	0
HG-660	60	0	60	100	0	0	0
HG-661	60	6	48	80	0	2	2 PP 2PCD
HG-662	60	12	26	43.33	8	14	0
HG-664	60	2	44	75.33	14	0	0
HG-665	60	10	46	76.66	0	4	0
HG-666	60	0	60	100	0	0	0
HG-667	60	14	42	70	1	3	0
HG-668	60	16	36	60	4	4	0
HG-670	60	0	60	100	0	0	0
HG-671	60	2	48	80	6	4	0
HG-672	60	6	48	80	6	0	0
HG-673	60	12	36	60	8	2	2 PP

Trisomy 21st chromosomes are well presented in all the 14 successful lymphocyte cultures set for the chromosomal analysis. (Table 2) Translocation between 21 and others 21 chromosomes was also found in one of the screened cases with other associated chromosomal aberrations like Polyploidy, Precentromeric Division (PCD).[3-7] (Graph 1 & 2, Fig 3a, b)

CONCLUSION

A genetic counseling is communication process which deals with human problems associated with occurrence or a risk of occurrence of genetic disorder in a family as we handled a pediatrics malformation case where the verbal communication is absent because of IQ status and immaturity, in such a condition we have tried to give an impact by a touch therapy-a sign of love and dedication with its perfection. In view of chromosomal aberration Down's syndrome have its known abnormality with trisomy 21 and all the 20 known patients have presented in their metaphase trisomy 21. (Figure 4a-c).

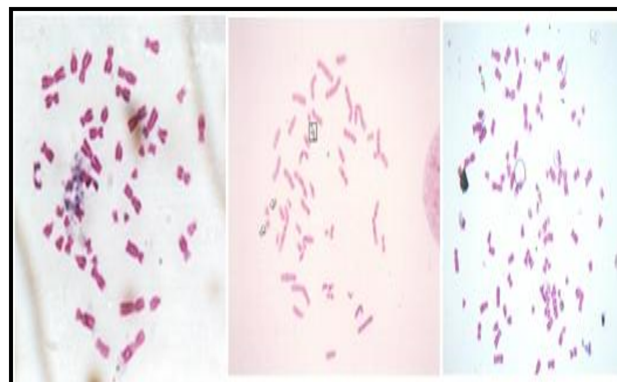


Figure 4 a, b, c: Showing the Metaphase plate with Trisomy & Hyperdiploidy

However, the few metaphases of 3 patients have shown a translocation between t (14;21) and t (21;21). Such translocation have remarkable and significance for a carrier parents and such metaphase can help to calculate the risk factor in

next generation to save the progeny with Down's syndromes.

In view of above significant genetic disorder this piece of work is an attempt to understand the phenomenon and genetic involvement of the genetic disorder with reference to Down's syndrome.

ACKNOWLEDGEMENTS

With the name of Almighty GOD we would like to acknowledge all those people, without whom, this work would not have been possible. We express our deep sense of gratitude to Hon'ble Chairman and Director Sir of Jawaharlal Nehru Cancer Hospital & Research Centre, for permitting us to work in their esteemed institute. We are also thankful to the Management of St. Thomas College, Bhilai for their supportive nature and all time response to carry on this work.

REFERENCES

1. Petersen, M. B., Tranebjaerg, L., McCormick, M. K., Michelsen, N., Mikkelsen, M., & Antonarakis, S. E. (1990). Clinical, cytogenetic, and molecular genetic characterization of two unrelated patients with different duplications of 21q. *American Journal of Medical Genetics*, 37(S7), 104-109.
2. Hook, E. B. (1981). Rates of chromosome abnormalities at different maternal ages. *Obstetrics & Gynecology*, 58(2), 282-284.
3. Mansfield, C., Hopfer, S., & Marteau, T. M. (1999). Termination rates after prenatal diagnosis of Down syndrome, spina bifida, anencephaly, and Turner and Klinefelter syndromes: a systematic literature review. *Prenatal diagnosis*, 19(9), 808-812.
4. Glover, N. M., & Glover, S. J. (1996). Ethical and legal issues regarding selective abortion of fetuses with Down syndrome. *Mental retardation*, 34(4), 207-214.
5. Pradhan, M., Dalal, A., Khan, F., & Agrawal, S. (2006). Fertility in men with Down syndrome: a case report. *Fertility and sterility*, 86(6), 1765-1773.
6. Mao, R., Zielke, C. L., Ronald Zielke, H., & Pevsner, J. (2003). Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics*, 81(5), 457-467.
7. Mao, R., Wang, X., Spitznagel, E. L., Frelin, L. P., Ting, J. C., Ding, H., & Pevsner, J. (2005). Primary and secondary transcriptional effects in the developing human Down syndrome brain and heart. *Genome biology*, 6(13), 105-107.
8. Bird, G., & Thomas, S. (2002). Providing effective speech and language therapy for children with Down syndrome in mainstream settings: A case example. *Down Syndrome News and Update*, 2(1), 30-31.
9. Patil, S. R., Merrick, S., & Lubs, H. A. (1971). Identification of each human chromosome with a modified Giemsa stain. *Science*, 173(1), 821-822.
10. Watt, J.L., & Stephen, G.S. (1986). Lymphocytes culture for chromosome analysis in human cytogenetics – A practical approach, *Irl Press Oxford P*, 1(2), 34-36.

© Copyright reserved by IJPRS