Iraqi Journal of Biotechnology, 2014,Vol.13, No.2 , 87-95

The Association of Maternopaternal Age and Cytomolecular Abnormalities in Iraqi Down Syndrome Children

Haider A. Salman[1] **Ban A. Majeed**[2]

[1] College of Medicine- Basrah University
[2] College of Medicine- AL-Nahrain University

Abstract: Down syndrome is the most commonly seen genetic disease in genetic outpatient units. And a common cause of mental and physical handicap. The study aimed to ascertain the relation of parental ages (maternal and paternal), and the occurrence of Down syndrome. It also aimed to detect the chromosome 21 abnormalities and their paternity origin, depending on polymorphisms using karyotyping as well as to implement the FISH technique in confirming abnormal karyotyping. Fifty six families (56 Ds child, 56 mothers, 56 fathers), were included. Patients were recruited at the genetic unit of the Teaching Laboratories in Medical City who were referred to the unit for genetic consultation. Tow laboratory techniques including Karyotyping and Fluorescent in situ hybridization were used in establishing diagnoses and polymorphisms. The results of these methods were then compared and analyzed statistically. Eighty four percent of cases were of maternal origin and 14 percent were of paternal origin. Fifty two cases of the samples were single in each family. They resulted from additional non disjunctional chromosome. Three cases resulted from translocation. One case revealed an isochromosome. There was no effect of consanguinity on the results of the study. Increasing maternal age led to the increase frequency of Down syndrome, but not the paternal age. Karyotyping had more ability to recognize the source of disease either from mothers or fathers than fluorescent in situ hybridization while the latter had higher ability to diagnose Down syndrome than karyotyping. There is recurrence risk in the families resulting from fixed translocation in mothers.

Key Wards: Down syndrome, parental ages, FISH, Karyotyping.

العلاقة بين عمر الام والاب والفحوصات الخلوية الجزيئية غير الطبيعية في الاطفال العراقيين المصابين بمتلازمة داون

حيدر أ.سلمان[1] بان عباس عبد المجيد[2]

[1]كلية الطب جامعة البصرة
[2]كلية الطب جامعة النهرين

الخلاصة: تمثل متلازمة داون الغالبية الشائعة في الامراض الوراثية في وحدات المراجعين الخارجيين والاكثر شيوعاً في المعوقين. هدفت هذه الدراسة لمعرفة علاقة عمر الامهات والاباء مع حصول متلازمة داون وكذلك معرفة دور كروموسوم 21 غير الطبيعي واصله اعتماداً على الفحوصات الخلوية الكروموسومية الاعتيادية وطريقة FISH. استخدم في الدراسة 56 عائلة راجعت الوحدة الوراثية في المختبرات المركزية لمدينة الطب في بغداد وطريقتين في الفحص الكروموسومات وهما طريقة تصبيغ الكروموسومات الاعتيادية وطريقة FISH الفلورنسية. بينت النتائج ان 84% من الحالات تبين ان اصل كروموسوم 21 امومي (من الام) و 14% من اصل ابوي (من الاب). كما بينت الدراسة ان 52 من الحالات كانت مفرده في كل عائلة وناتجة عن وجود زيادة في الكروموسوم 21 وهناك ثلاثة حالات ناتجة عن انتقالات كروموسوميه وحالة واحدة ناتجة عن كروموسوم 21 متناظر الاذرع . وكخلاصة فأن زيادة عمر الام يؤدي الى زيادة احتمالية ظهور متلازمة داون عند الاطفال وليس الاب. وان طريقة صباغة الكروموسومات العادية افضل في تشخيص اصل الكروموسومات في الطريقة الفلورنسية الطريقة الفضلى لتشخيص متلازمة داون في صباغة الكروموسومات العادية وأن هناك خطورة على العوائل من وجود انتقال كروموسومي لدى الامهات.

Introduction:

In newborn population there are three common aneuploidy in chromosomes namely, trisomy 21, trisomy 18& trisomy13. Calculated ,crude live birth rates of these conditions are 1in 826 for trisomy 21 , 1 in 6666 for trisomy 18 , and 1 in 12.500 for trisomy 13 (1). The rates of all three autosomal trisomy types increase markedly with advancing age of mothers (1,2and 3). The first description for Down syndrome was made by John Langdon Down, the British physician who described the syndrome in 1866 (cited in 4), who connect it with tuberculosis at that time. The disorder was identified as a chromosome 21 trisomy (discovery of the extra 21 chromosome) made by Jérôme Lejeune in 1959 (cited in 4). The condition is characterized by a combination of major and minor differences in structure. Down syndrome in a fetus can be identified with amniocentesis during pregnancy or in a baby at birth (4). Various workers have attempted to explore the cause of nondisjunction of chromosome 21. Important factors in the conception of a trisomy are delayed fertilization, advanced maternal age, and increased satellite DNA as well as association of other factors, such as physical, biological, and chemical mutagens.

Objectives of the study:

1. To a certain the relation of parental age (maternal and paternal), and the occurrence of Down syndrome.
2. To detect the chromosome 21 abnormalities and their paternity origin, depending on polymorphisms using karyotyping.
3. To implement the FISH technique in confirming abnormal karyotyping.

Materials and Methods:

This is a cross-sectional study extending from November 2011 to October 14, 2012, Fifty six families (56 Down syndrome children, 56 mothers & 56 fathers). All new patients with DS were recruited at the outpatients of genetic unit in medical city, teaching laboratories during the period from November 2011 till May 2012. A specialized data collection form were filled by the investigator through direct interview with the family, information including age, sex and residence of the affected child, Parental ages at the time of child's birth, relations between the parents (up to the 4th degree relation), history and number of previous affected children in the family. The family consent was taken before submitting them and their children to the study. Blood samples were submitted to two laboratory procedures: Karyotyping and FISH (Fluorescent In Situ Hybridization).

Materials for karyotyping were purchased from PAA Germany and those for FISH from Kreatech Biotechnology.

Karyotyping procedures (5):

Collecting of blood samples and culture In 1640 RPMI culture media, using a sterile Heparinized syringe, aspiration of 3-5 ml of venous blood after careful decontamination of skin by 70% alcohol.

Culture tubes were incubated at 37 c˚ for 69 hours, in a biwave incubator. Then Harvesting by Colchicine in a volume of 0.2ml was added to the samples and tubes were reincubated at 37 c˚ for 1.5-2 hours, kcl solution was added and mixed well with Pasteur pipette. Repeat washing with fixative with centrifugation until the sample become clear with pellet in the bottom of the tube. Then Dropping By a Pasteur pipette, clear liquid was taken gently without letting the liquid mixed leaving few ml at the bottom of the tube, Then by the same Pasteur pipette few drops from the precipitate were taken and dropped on the clean slide at 45 degree angle from a height of 25-30 cm, slides were left to dry and then label the slide, by steel pen on a side of the slide. Then Staining using Coplin Jars containing the following in series were prepared for staining 10 ml of Trypsine solution in a concentration of 0.025% were kept at 26-28 c˚, 10%when mixing 10 ml trypsine with 90 ml normal saline. Phosphate buffered saline solution (P.B.S) PH 7.2 then Giemsa stain.

FISH procedure (6,7):

Glass microscope slides according to standard cytogenetic procedures were used. Keep the humidity between 40% and 60% and the temperature between 18 ˚C and 25 ˚C for the slides. Drying times are very important for a good metaphase and nucleus morphology, which resulted in better FISH results. Check the density and quality of the cell suspension. Passing these standard cytogenetic slides which neither banded nor stained through several steps including pretreatment solutions, then probe preparation procedure, then Co-denaturation technique, hybridization then post hybridization procedure, all before Microscopic examination At least 5-10 cells should be examined to confirm the diagnosis, no matter the cells were in metaphase or anaphase.

For both karyotyping and FISH technique: All the patients' polymorphisms were interpreted and confirmed by photography by camera using 28x magnification power and +3 resolutions for each patient then comparing the pictures of these patients with their parents.

The data were processed with the software package SPSS (Statistical Package for Social Sciences) version 18 (SPSS, Inc., Chicago IL) and Microsoft Excel 2010. Association between different variables was measured by using Fisher's exact test, t-test or Chi square ($\chi 2$) test as indicated. P-value < 0.05 was considered as statistically significant, while values < 0.001 was considered statistically highly significant.

All the patients' polymorphisms were interpreted and confirmed by photography by camera using 28x magnification power and +3 resolutions for each patient then comparing the pictures of these patients with their parents.

Results:

The age range of mothers participating in the study was 25- 47 with a mean of 35.25 ± SD 4.840. The age range of fathers was 30-51 with a mean of 40.80 ± SD 4.534. There was no significant difference between the ages of mothers and fathers. Two of patients were siblings in one family and 3 other were siblings in another family. The rest of patients were distributed as a single child to each family. The relation degree of parents there were no relation in 37 families, 2nd degree in 5 families, 3rd in 4 families and 4th degree in 10 families. Karyotyping results: 44 patients (78.6%) show karyotype preparations of patients with DS stained by geimsa figure 1 and 2. Translocations were detected in 4 cases (7.1%) figure 3, Isochromosome 21 was seen in only one case (1.8%), Mosaic karyotyping was also detected in only one case (1.8%),There were failure of growth in 6 of patients.

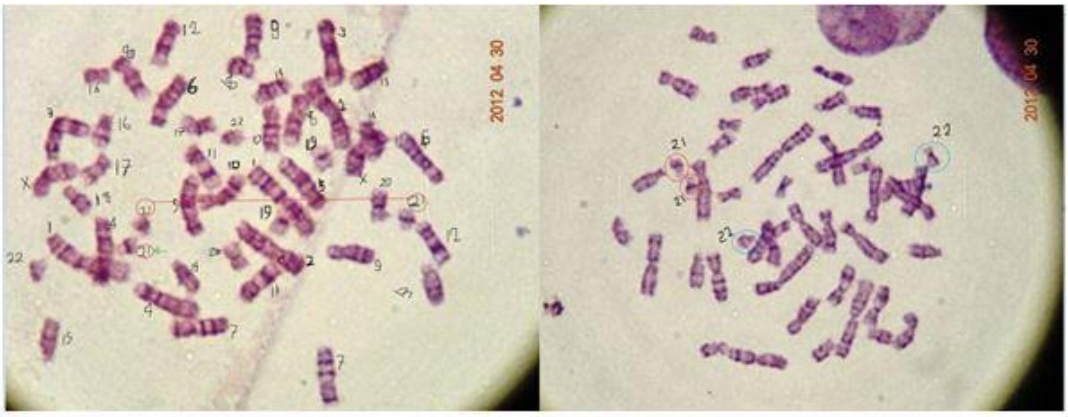


Figure 1: Karyotyping of 2 months female DS child showing trisomy 21 (marked on Lf.) compared to normal karyotyping of her father on the Rt.the recognized polymorphism seen in father and daughter

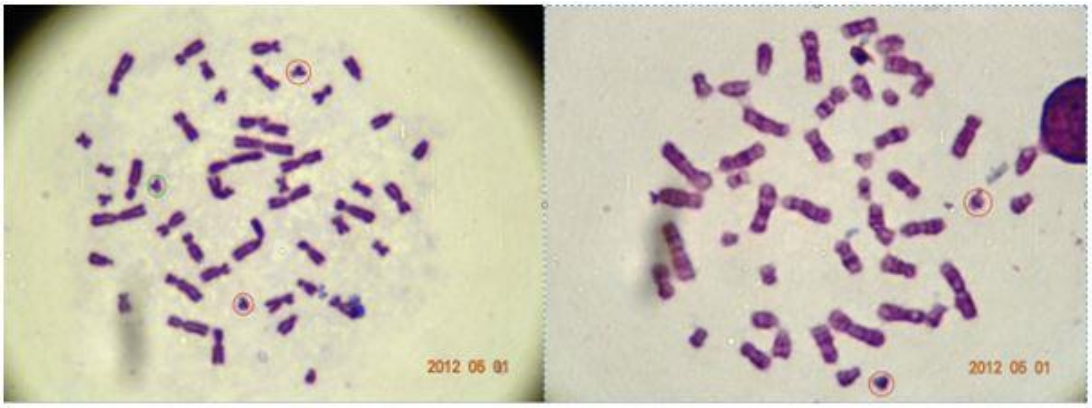
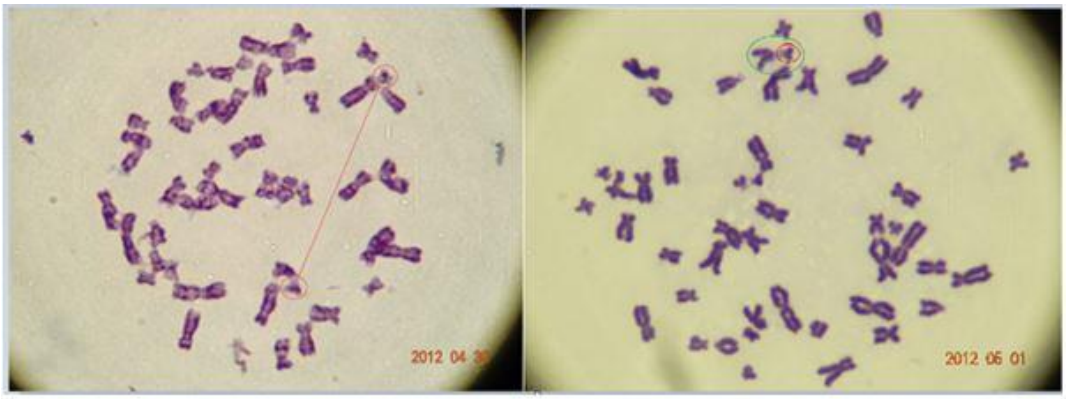


Figure 2 Karyotyping of 6 month male child showing trisomy 21 (marked on Lf.) compared to normal karyotyping of mother on the Rt. .



Figur3:Karyotype of Three months female child with DS (Lf) showing46,XX,t(21;14),on the right her mother with 45,XX,t(21;14)

FISH results:

Trisomy 21 was detected in 47 cases (83.9%) figure 4, one mosaic case (1.8%), no Isochromosome could be detected by FISH technique. In 8 (14.3%) cases there was poor labeling.

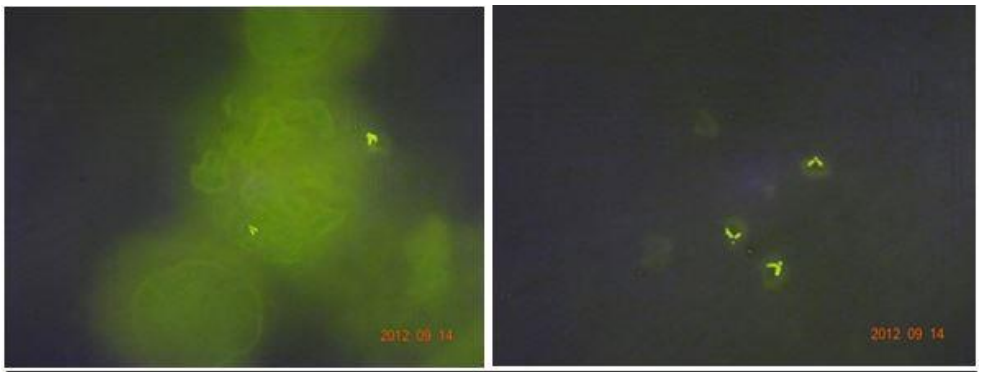


Figure 4:FISH preparation showing pictures in same family (Lf-DS child mother, RT-DS child), FISH preparation

Additional chromosome by karyotype: The source of the additional chromosome 21 was detected by karyotyping to be of maternal origin in 42 cases (75%) and paternal in origin in 7 cases (12.5%).This means 84% of the diagnosed samples are maternal in origin, While 14% are of paternal origin. Additional chromosome by FISH: Additional chromosome 21 was detected in 37cases (66.1%) to be of maternal origin and of paternal origin in 7 cases (12.5%). Sex distribution:29 females (51.8), 27 males 48.2 .

Discussions

Based on results from the NDSP (National Down Syndrome Project), and other population-based series, over 90% of nondisjunction errors leading to trisomy 21 occur in the oocyte and the majority of those occur during MI. (8). The cause of nondisjunction may not be the same between father and mother of Down syndrome, and in mothers it may relate basically to the metabolic factors that are counterparts with basic cellular biochemical functions (9).

Maternal and paternal age:

From the data that have been analyzed, it is obvious that the mean age of the mothers in this study group was 35.25 ± SD 4.840 which was significantly related to the outcome. In western mothers the mean age is 34.4 years (10). While that of tk tfhe fathers was 40.8 ± SD 4.534. Globally the evidence is contradictory.

Maternal age has for long been known to be an important etiological factor in Downs' Syndrome (11, 12, 13 and 14). Of the different chromosomal

Types causing the syndrome, especially the incidence of standard trisomy have proved to be involved with advancing maternal age (4). For other types such as translocation Down's syndrome, both spontaneous and inherited, no age Dependency has yet been proved (15).

Karyotyping and FISH:

Karyotype has diagnosed 89.3% (sensitive) of all cases and failed in 10.7 which are the so called no growth in laboratory, While FISH diagnosed 85.7 (sensitive) and failed in 14.3 which is called no labeling. It is known that 85-90% of cases can be diagnosed by Karyotyping because it only establishes diagnosis in metaphase. While that of FISH nearly 95% because it establishes diagnoses in anaphase and metaphase (16). As the P value differences between Karyotyping and FISH was found 0.115 which is non-significant, that means the diagnostic ability of both tests are the same. But still karyotyping is more sensitive. Additional 21 chromosome on karyotyping was found to be 75% of maternal origin, and 12.5 paternal, and 12.5 cannot tell, these ratios depending on polymorphisms established by karyotyping. As compared to FISH which was able to establish only 66.1 maternal and 12.5 paternal and failed in 21.4. And the P value was 0.0001 which is highly significant, meaning that Karyotyping is a gold standard in establishing the polymorphisms consequently the source of the additional chromosome (17 and 18).

Consanguinity: For the relation degree (consanguinity) P value is 0.39 for karyotype, and 0.532 for FISH by Chi square test which is non-significant in both tests, that mean in spite that consanguinity is of no importance still the clinicians ask for it. Same thing was found in many other studies concerning insignificance of consanguinity (19 and 20). **Recurrence of DS:** In the present study, families with one child of DS constituted the majority (92.9%), those with 2 DS children were 5.4%, and those with 3 constituted only 1.8%. Recurrent DS occur only in families with fixed translocations and isochromosome 21, but not in non-disjunction. Trisomy 21 is a very common cause of mental retardation and free trisomy 21accounts for 95% of the cases Recurrence of free trisomy is rare (21). However Women who have had a previous Down syndrome pregnancy have a constant absolute excess risk above their maternal age-related risk of having a subsequent affected pregnancy. This absolute excess risk is determined by the age at which the affected pregnancy occurred and is higher for younger than for older women. For example, after a Down syndrome pregnancy at age 20, this excess is 1-2% at early second trimester, and, after age 40, it is 0.04% (20). Recurrence of about 1% is well recognized (1). **Sex distribution in Down syndrome:** In our study, the percentage of female children affected

by DS was higher than males, which could be an accidental finding, because there is no control group. Data from the National Down syndrome Cytogenetic Register is used to describe the cytogenetic and epidemiology of registered cases. The overall sex ratio was (male to female: 1.23 to 1), and there was an excess of associated male sex chromosomal aneuploidy (23, 24).

References

1. Hook EB (1992) chromosome abnormalities: Prevalence, Risks and recurrence .In Brook DHJ, Rodeck CH, Fergguson – Smith MA (eds) : Prenatal Diagnosis and Screening. pp 351 – 392, Eidenburg Churchill Livingstone, ,

2. Morris JK, De Vigan C, Mutton DE, Alberman E (2005) Risk of a Down syndrome live birth in women 45 years of age and older. Prenatal Diagnosis 25:275—278.

3. JK. Mutton DE, Alberman F (2002) Revised estimates of the maternal age specific live birth prevalence of Down's syndrome. J Med Screen 9:2—6.

4. Jyothy A. Sesha K. Kumar D. (2001) Parental age and the origin of extra chromosome 21 in Down syndrome, J Hum Genet vol.46: pp. 1-2.

5. Rooney D E, Czepulkowsk BH, (1986) Human cytogenetics a practical approach.111.series;611.01816 QA431 vol.1:pp40-49.

6. Charleen MM, Robert GB (2001) Chromosome preparation, and banding technique, ENCYCLOPEDIA OF LIFE SCIENCES & 2001 Nature Publishing Group / www.els.net vol.2:pp21-26

7. Kryatic courtesy, (2012) FISH Slide preparation alternative, , whole the article.vol.1:pp1

8. Mikkelsen M, Hallberg A, Poulsen H, et al. (1995). Epidemiology study of Down's syndrome in Denmark, including family studies of chromosomes and DNA markers. Dev Brain Dysfunct 8:4-12

9. Carothers Ad, Castilla Ef, Dutra Mg, Hook Eb, (2001) Search For Ethnic, Geographic, And Other Factors In The Epidemiology Of Down Syndrome In South Ameica: Analysis Of Data From The Eclamc Project, 1967-1997, Am J Med Genet 103:149-156

10. Wisniewski KE, Wisniewski HM, Wen GY (1992). Occurrence of neuropathological changes and dementia of Alzheimer's disease in DS. Ann Neurol. 17:278-82

11. Margaret F, Judy M, Lorri P (2009), Down Syndrome Autism Dual Diagnosis Mini-ConferenceDown's Update, volume 27, issue 2: pp 1.

12. Adamrush, A, Adrian M, Hunter J., Adron A (2011) Down Syndrome Details, Contributors: Courtesy: National Human Genome Research Institute vol2:pp1-2

13. A. Hassold TJ, Burrage LC, Chan ER, et al. (2001) Maternal folate polymorphisms and the etiology of human nondisjunction. Am J Hum Genet 69:434-439

14. B. Hassold T, Sherman S, (2000) Down syndrome genetic recombination and the origin of extra chromosome 21. Clin genet 57:95-100.

15. Penrose LS. (1964). Genetical aspects of mental deficiency. Proceedings of the International Copenhagen Congress on the Scientific Study of Mental Retardation.vol.2: p 165-172.

16. Stene J, Stene E, Mikkelsen M (1984) Risk for chromosome abnormality at amniocentesis following a child with a non-inherited chromosome aberration. A European Collaborative Study on Prenatal Diagnoses. Prenatal Diagnosis 4(Spec No):pp81-95

17. Thomas E, Gesa S, (2011) Genetics of Down Syndrome, issued, Institute of Human Genetics, RWTH Aachen Institute of Human Genetics, University of Bonn Germany. 45-50. ISSN 978-953-307-631-7

18. Stylianos E, Antonarkis, (1991) Parental origin of extra chromosome in trisomy 21 as indicated by DNA polymorphisms. Vol.1:2-5

19. Jean G, David G, van d. Berg, Audrey D, Maud V, Hajo IJW 2011) Rapid testing versus karyotyping in Down's syndrome

European Journal of Hum. Genetics 19: 3–9.

20. Abdullah S, Muslim M. (2007) Consanguinity and Down syndrome. King Khalid University. Riyadh, Saudi Arabia. July, 2, 2007, Humangenetik. 27(1):45-48.

21. Hanan AH, Amira M, Azmy A, (2007) Cosanguinity and genetic disorders A jordanian profile.Saudi Medical Journal, ,Volume 28 (7):9-19

22. Tseng LH, Chuang SM, Lee TY, Komar TM (1994). Recurrent Down's syndrome due to maternal ovarian trisomy 21 mosaicism Arch Gynecol Obstet 255:213-216

23. Morris JK, Mutton DE, Alberman E, Recurrences of free trisomy 21(2005) analysis of data from the National Down Syndrome Cytogenetic Register Article first published online: New York. 18 :38-41

24. Mutton D, Alberman E, Hook EB. (1996) Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down Syndrome Cytogenetic Register and the Association of Clinical Cytogeneticists. J Med Genet;33:387-394

25. Kovaleva NV. (2002) Sex ratio in Down syndrome. A review. Tsitol Genet, Vol.36, No.6, (November-December 2002), pp. 54-69, ISSN 0563-3783.