Received: 11 March 2013; Accepted: 25 April 2013
Conflict of interest: none declared.

DOI: 10.5455/msm.2013.25.131-135

ORIGINAL PAPER Mat Soc Med. 2013 Jun 25(2): 131-135

Cytogenetic Study in Children with Down Syndrome Among Kosova Albanian Population Between 2000 and 2010

Selim Kolgeci[1], Jehona Kolgeci[2], Mehmedali Azemi[3], Ruke Shala-Beqiraj[4], Zafer Gashi[5], Mentor Sopjani[2]
Obstetrics and Gynecology Clinic, University Clinical Center of Kosova, Prishtina, Kosova[1]
Faculty of Medicine, University of Prishtina, Prishtina, Kosova[2]
Pediatric Clinic, University Clinical Center of Kosova, Prishtina, Kosova[3],
Department of Histology and Embriology, University Clinical Center of Kosova, Prishtina, Kosova[4]
Policlinic "Biolab-Zafi" IVF (center), Klina, Kosova[5]

Corresponding author: Assoc prof Selim Kolgeci, Obstetrics and Gynecology Clinic, University Clinical Center of Kosovo, Muharrem Fejza street nn, Prishtinë, tel: +377(44) 208-017; E-mail: selimab@hotmail.com

ABSTRACT

Aim: The aim of this research was to ascertain the frequency of three basic cytogenetical types of Down syndrome among Kosova Albanian population and to evaluate the maternal age effect on the frequency of births of children with Down syndrome. **Methods:** Cytogenetics diagnosis has been made according to the standard method of Moorhead and Seabright. **Results:** In the time period 2000-2010 cytogenetics diagnosis of overall 305 children with Down syndrome has been realized. Of which in 285 children (93.4%) were found free trisomy 21 (regular type), and in three other children (~1.0%) were detected mosaic trisomy 21. Translocation trisomy 21 was detected in 17 children (5.6%), of which in 14 children it occurred de novo translocation, whereas in 3 other children translocation has been inherited by a parent translocation carrier. The highest number of children with Trisomy 21 due to translocation was caused by Robertsonian translocation created by a fusion of two homologous chromosomes 21 (3.3%). Analysis showed that the number of children born with Down's syndrome, from 2000 to 2010, was not decreasing among the Kosova Albanian population. **Conclusion:** Down syndrome resulted by an extra free chromosome 21 is the most common genetic cause for that condition. Robertsonian translocations present in Down syndrome children often are de novo or inherited from a carrier parent with translocation.
Key words: Down syndrome, free trisomy 21, Robertsonian translocation.

1. INTRODUCTION

Down syndrome is the most common chromosomal disorder in humans. Its prevalence in Europe is about 9.8:10 000 live born infants (1), while in the USA it is 8.5:10 000 newborns to mothers younger than 35 years of age and up to 55.3:10 000 newborns among mothers older than 35 years of age (2). Genetic cause for this syndrome is trisomy of chromosome 21 or the presence of distal part of the long arm of chromosome 21. In Down syndrome patients there are present three types of cytogenetic trisomy 21, i.e.: free trisomy 21, mosaic trisomy 21, and translocation trisomy 21 (3).

Most commonly, Down syndrome children have karyotype of free Trisomy 21, while their parents have normal karyotype. This type of trisomy 21 exclusively occurs sporadically *de novo* as a result of nondisjunction of homologous chromosomes 21 during gametogenesis to parents or during early embryonic development after fertilization (4). Analysis of chromosome heteromorphisms and many other informative markers of DNA polimorphisms of parents and their offspring with Down syndrome revealed that chromosome 21 nondisjunction occur more often during the gamete-formation process in females than in males (5, 6). Investigations found that an extra chromosome 21 mainly originates from errors in maternal side in approximately 90% of the Down syndrome cases.

In 5-10% of Down syndrome cases the extra chromosome 21 originates due to errors in father side, whereas in less than 5% of cases it results from nondisjunction of chromosomes during a post-zygotic mitosis in early embryonic development (7, 8). Important role for chromosome 21 nondisjunction has also maternal age. Older woman is more likely to have a chromosome 21 nondisjunction during cogenesis than young women (9). Therefore older women have a higher risk of having a baby with Down syndrome.

In children with Down syndrome due to translocation trisomy 21, extra chromosome 21 is joined or translocated in any other acrocentric or non-acrocentric chromosome (10). The translocation trisomy 21 present in Down syndrome patients can be created spontaneously *de novo* during gametogenesis in one of the parents or it can be inherited from parents' carrier of Robertsonian translocation or of reciprocal translocations.

Studies indicated that in the case of children having Trisomy

21 with Robertsonian translocation created sporadically *de novo*, the risk for a second future offspring trisomy 21 for their parents with normal karyotype is small. There is a significant increased risk of giving birth to a child with Trisomy 21 when one parent is a Robertsonian translocation carrier or of reciprocal translocations as they may produce balanced and unbalanced gametes during gametogenesis (11, 12). When one parent is carrier of Robertsonian translocation 21q; 21q, it has 100% chance of having a Down syndrome child as all of its produced gametes are unbalanced (13). The frequency to have one child with Down's syndrome due to translocation trisomy 21 is not influenced by the age of the mother.

Peoples with mosaic Down syndrome have two distinct cell lines with different karyotype. In some cells there are a total of 46 chromosomes having normal karyotype, while other cell lines have karyotype with Trisomy in chromosome 21 (14).

Many authors argued that restricting or reducing the births for woman's who are aged 35 years or older, mandatory prenatal cytogenetics diagnosis of fetal disorders of pregnant women aged 35 years or older, and applying the methods for prenatal screening on all ages pregnant women's reduce the number of births of children with Down syndrome (15,16).

We realized our study on the rate of birth of children with Down syndrome in Kosova Albanian population for the time period 2000-2010, when Kosova have had no conditions for full application of methods as described above in order to prevent birth of children with Down syndrome in our population .

2. AIM

The purpose of this study was to found the frequency of the 3 cytogenetic types of Down syndrome among Albanian population of Kosovo, as well as to evaluate the maternal age effect on the prevalence of Down syndrome births.

3. METHODS

Cytogenetics analysis has been realized on chromosome preparations of lymphocytes cultured from peripheral blood according to Moorhead method (17). For precise identification of chromosomes standard method for G-banding by Seabright was used (18). Cytogenetics diagnosis of all investigated cases has been carried out in the cytogenetics laboratory of the obstetric and gynecology clinic in Prishtina. Since that lab is the only laboratory for cytogenetics in Kosovo, all baby suspected of having Down syndrome were diagnosed there, thus that laboratory has most accurate information regarding to the Down syndrome cases among Kosovo's population.

4. RESULTS

During a 10 years period, 2000-2010, in the Obstetrics and Gynecology Clinic in Prishtina there have been found 305 children with Down syndrome cytogenetically diagnosed. Out of that 193 (63.3%) were males, while 112 (36.7%) females. The sex ratio has indicated the prevalence of the males for the total sample 1.72:1 (193:112) as well as for the three basic cytogenetic types of Down syndrome (Table 1). In all of the Down syndrome diagnosed cases, free trisomy 21 was present in 285 (93.4% of cases) patients, with cytogenetic formula 47,XX,+21 or 47,XY,+21 (Table 2). Mosaic Down syndrome was found in three cases (1.0%). All the parents of children having free trisomy 21 and mosaic Down syndrome have had normal

Types of DS	Male	Female	Sex ratio
Free trisomy 21	176	109	1.61:1
Mosaic trisomy 21	2	1	2.0:1
Translocation trisomy 21	15	2	7.50:1
Total	193 (63.3%)	112 (36.7%)	1.72:1

Table 1. Sex ratio among Down syndrome studied children.

karyotype. Down syndrome due to translocation trisomy 21 was detected in 17 (5.6%) children (Table 2). Cytogenetical analysis of the parents of children affected with translocation trisomy 21 revealed that in three children (1.0%) translocation was inherited by one of carrier parent of that translocation, from the mother respectively. The other 14 (4.6%) children with this Down syndrome type have had *de novo* translocation.

One child with Down syndrome had inherited his translocation 8;21 from the mother. He was a carrier of reciprocal translocation 8;21 and of trisomy 21, with cytogenetic formula: 47,XY,t(8;21)(q22;q22)mat,+21. The child's mother was a silent carrier of a balanced translocation with karyotype: 46,XX,t(8;21)(q22;q22). The child having this chromosomal aneuploidy was as a result of disjunction 3:1 of derived chromosomes 8 and 21 and their normal homologues during gametogenesis in his mother (Interchange trisomy 21).

Cytogenetic types of Down syndrome	The number of subjects	%
Free trisomy 21	285	93.4
Mosaic trisomy 21	3	1
Translocation trisomy 21	17	5.6
Total	305	100

Table 2. The frequency of free trisomy 21, mosaics trisomy 21 and translocation trisomy 21 in children with Down syndrome among Albanian population of Kosova.

Second child with Down syndrome inherited translocation from the mother who was a Robertsonian translocation silent carrier with translocation involving 14q;21q, with karyotype: 45,XX,der(14;21)(q10;q10). Karyotype of the child was 46,XX, der(14;21)(q10;q10)mat,+21 as a result of disjunction 2:1 and of adjacent-1 segregation during meiosis in her mother.

Third child with Down syndrome inherited translocation

Types of Down syndrome	Types of translocation	Male	Female	Total	
				No	%
Translocation trisomy 21	Reciprocal translocation 8q;21q	1	0	1	0.3
	Robertsonian translocation 13q;21q	1	0	1	0.3
	Robertsonian translocation 15q;21q	1	0	1	0.3
	Robertsonian translocation 14q;21q	2	2	4	1.3
	Robertsonian translocation 21q;21q	10	0	10	3.3
Mosaic trisomy 21		2	1	3	1
Free trisomy 21		176	109	285	93.4
Total		193	112	305	100.0

Table 3. Frequency of 3 basic cytogenetic types of Down syndrome and types of translocations in children with Down syndrome.

Types of translocation DS	Male	Female	Total	
			No	%
Reciprocal translocations 8q;21q	1	0	1	5.9
Robertsonian translocations 13q;21q	1	0	1	5.9
Robertsonian translocations 14q;21q	2	2	4	23.5
Robertsonian translocations 15q;21q	1	0	1	5.9
Robertsonian translocations 21q;21q	10	0	10	58.8
Total	15	2	17	100.0

Table 4.Types of translocations and their frequency in 17 children with Down syndrome studied.

also from the mother silent carrier of Robertsonian translocation 21q;21q, with karyotype 45,XX, der(21;21)(q10;q10). The child has a karyotype 46, XY,+21,der (21;21)(q10;q10) mat caused by joining of disomic gamete for chromosome 21 of the silent translocation carrier mother with normal gamete of partner.

In 16 children (5.2%) there have been detected trisomy 21 with Robertsonian translocation created by merging of chromosome 21 with another acrocentric chromosome. Most of investigated children had Robertsonian translocation formed between two homologous chromosomes 21. This translocation type is found in 10 (3.3%) investigated children (Table 3). In 4 (1.3%) other children had trisomy 21 with Robertsonian translocation between chromosomes 21 and 14. Robertsonian translocations between chromosome 21 and 13; and between 21 and 15 were present only in one child (0.3%) each. Reciprocal translocation between chromosome 21 and 8 was found only in one child (0.3%) with Down syndrome.

In this study is presented the birth frequency of children

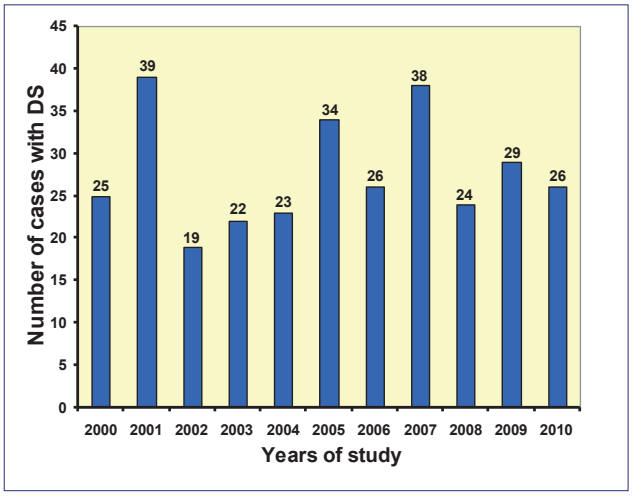

Figure 1. Graphic representation of the frequency of Down syndrome births among Albanian population of Kosova during 2000-2010 period.

with Down syndrome cytogenetically diagnosed over a period of 10 years (2000-2010) among Kosovo Albanian population (Figure 1). The greatest number of children born with Down syndrome had occurred in 2001 and in 2007, while the smallest number in 2002 and in 2003. From 2008 to 2010 there were observed minor differences regarding to the number of Down syndrome cases.

Our study focus was also to investigate the frequency of birth of children with Down syndrome to mothers of different age (Figure 2). In maternal age up to 20 years the incidence of birth

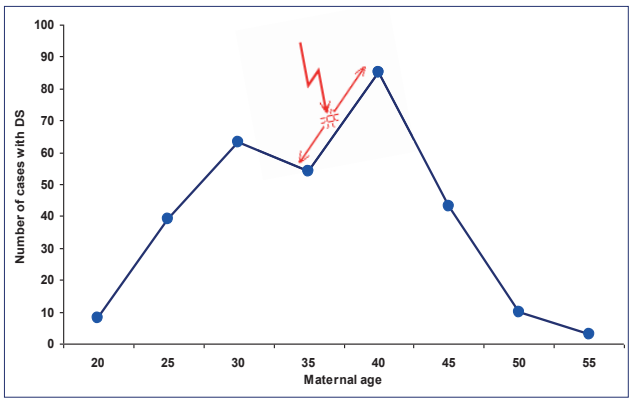

Figure 2. Graphic representation of birth of children with Down syndrome to women of various ages. After the maternal age of 35 the frequency of births of children with Down syndrome increases.

of children with Down syndrome is lower due to the smaller number of women births compared to higher risk among older women, therefore Down's syndrome children born to mothers of advanced age 21-30 years gradually increasing. This increase is in correlation with the increasement of total number of births in the general population as well, which occurs most frequently at those ages in parallel with the birth of children with Down syndrome. Although the number of children born with Down syndrome in this period at first sight seems to be higher, however if the number is analyzed for total ratio of births at this period it shows no higher incidence than in older women. There is a small decrease in the frequency of live births with Down syndrome among women aged 30-35 years. This decrease can be related to the reduction in the total number of births in general population, where the birthrate of women over the age of 35 gradually decreases until the end of their reproductive periods. In the general population, there is a parallelism on increasing the number of births of normal and of Down syndrome children by age of 35. Women aged 35 to 40 have a permanent increase of the birth of children with Down syndrome which is not in proportion with the increase of births in the general population, since at this age the number of births in the general population decreases. The increase of Down syndrome births to mothers in this age showing a direct correlation between Down syndrome and maternal age, thus the linkage of advanced maternal age to

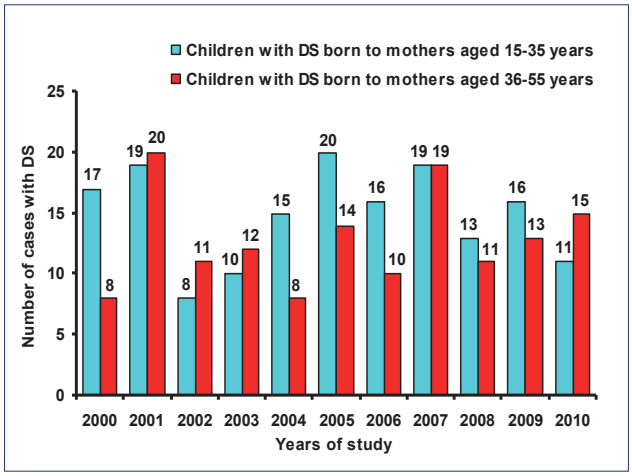

Figure 3. The number of births of children with Down syndrome among mothers aged 15-35 years compared to mothers aged 36-55 years over the 2000-2010 period.

an increased risk of Down syndrome births.

Figure 3 shows Down syndrome live births occurred among mothers aged between 15-35 years and of those 36-55 years of age in 2000-2010 time period. It should be noted that in the year 2000 (first year after the war in Kosovo) the number of Down syndrome births were two times more likely to occur to mother younger than 35 compared to those of 36 years of age or older. Most of mothers of these children have started their pregnancy in 1999, at the end of the war in Kosovo; however, there is no detail information about their war trauma experience, in order to correlate to the possible impact of trauma on non-disjunction of chromosome 21 during gametogenesis to young mothers.

5. DISCUSSION

Cytogenetics studies performed on 305 Down syndrome cases revealed that translocation trisomy 21 was found in 17 cases (5.6%) (Table 2). Our results are roughly similar to the results of other authors (3, 15, 19). Most children with translocation Down syndrome are born to mothers under the age of 35; therefore not depend on maternal age. Chromosome 21 most frequently creates translocations with acrocentric chromosomes than to other non-acrocentric autosomal or sex chromosomes. Our findings conclusively argue that as well, where Robertsonian translocation between chromosome 21 and another acrocentric chromosome have been found in 16 children, while in only one child there was present reciprocal translocation between chromosomes 8 and 21 .

The findings of some other authors (20) reported high presence of Robertsonian translocation 14q; 21q in children with translocation trisomy 21 (62.34%). In our study the most frequent type of translocation were the Robertsonian translocation 21q; 21q (58.8%) (Table 4). The second most frequent (23.5%) translocation type was the Robertsonian translocation 14q; 21q. Other Robertsonian translocation types were less present in our study cases. There has been reported that in 75% of all translocation cases it may occur *de novo*, while in 25% of cases, it can be inherited from one carrier parent, but more frequently by the mother side (21). In our present study spontaneously *de novo* translocation occurred in 82.4% of children, while in 17.6% of children inherited from a carrier parent, mothers respectively.

To prevent the birth of children with Down syndrome in translocation affected families the early detection of parent's carriers with Robertsonian translocation involving chromosome 21 is of the great importance. In the families who have children with translocation trisomy 21 arisen *de novo* i.e. when the parents have normal karyotype, the risk of giving birth to a child with translocation trisomy 21 is small (1-2%). Therefore, parents of 14 children with translocation trisomy 21 occurred as *de novo* event studied in our paper do not have high risk of giving birth to a second child with trisomy 21. Since an affected child with reciprocal translocation 8;21 and an another child with Robertsonian translocation 14q; 21q, have inherited translocation from their mother side, the recurrence risk is significantly much higher (10-15 %). By applying the prenatal cytogenetic diagnosis of embryos of each of these couple pregnancies can be prevented the spread of Down syndrome within a generation. To our knowledge, all published papers for couples which are carriers of silent Robertsonian translocation involving homologous chromosomes 21q; 21q have reported 100% risk of having a child with Down syndrome and unable to have healthy baby (13, 22). In our study a Down syndrome child inherited Robertsonian translocation 21q; 21q from a carrier mother. This family is unable to have healthy child by embryo selection through prenatal cytogenetic diagnosis due to the fact that carrier mother can only make unbalanced gametes, thus any of her child will have Down's syndrome.

The cytogenetical study conducted on 305 Down syndrome children among Albanian population of Kosovo revealed that free trisomy 21 is significantly more frequent (93.4%) than the other types of trisomy 21. Other authors also indicated high occurrence of free trisomy 21 (92-95%) in children that have Down syndrome (3, 15, 19). It shows that this chromosomal aberration is presented equally as frequent as in rest of the world. Since the free trisomy 21 is the most common genetic cause of birth of children with Down syndrome it is of great importance to undertake preventive measures in order to reduce the disorder incidence among human population. For prevention purposes, the etiological factors as a cause of birth of children with Down syndrome, should be known, and reduction measures should be taken to minimize impact.

Studies of many authors have shown that one of the factors which increase the incidence of births with free trisomy 21 is advanced maternal ages (15, 23, 24). Very often Down's syndrome babies are born to mothers who are over 35 years of age. The results of our paper have also confirmed that a woman over 35 years of age to have highest incidence of giving birth to a child with Down syndrome (Figure 2).

A relatively simple way to reduce birth incidence of Down's syndrome is the limitation or reduction of the number of pregnant women older than 35 years. The frequency of birth of children with Down syndrome is expected to be reduced up to 20-45% with the birth limiting to mothers aged over 35 years (15). Prenatal preventive diagnostic tests for Down syndrome in modern medicine can be realized through the application of either non-invasive tests (ultrasound and biochemical screening) or by invasive diagnosis methods such as chorionic villus sampling, amniocentesis, cordocentesis. Antenatal careening using different biochemical markers and ultrasound are made routine in developed countries, where their national prenatal care policies obliges amniocentesis to all pregnant women aged 35 years or over, as well as maternal serum screening for younger women. To prevent the birth of child with Down syndrome recently there are considered some other preventive strategies, such as: pre-implantation genetic diagnosis (PGD) and folic acid supplementation (25). As in Kosova currently there are no conditions for application of the above mentioned methods to prevent the birth of child with Down syndrome, during our cytogenetics study in children there was not observed a gradual decrease in the frequency of birth of Down syndrome among Albanian population of Kosova in 2000-2010 (Figure 1).

6. CONCLUSIONS

Based on the results of present study the following conclusions can be drawn:

- Free Trisomy 21 has been found in 93.4% of children with Down syndrome.
- Translocation ttrisomy 21 was present in 5.6% of children with Down syndrome.
- Most of the children with Robertsonian translocation had

translocation formed by the homologous chromosomes 21 (3.3% of cases).

- Mosaic trisomy 21 was present in 1.0% of Down syndrome children.
- Down syndrome among Kosovo Albanian population was more frequent in males (63.3%) than females (36.7%).
- Children with free trisomy of chromosome 21 are more frequently born to mothers older than 35 years of age.
- Advanced maternal age appears not to affect the frequency of giving birth to a child with translocation trisomy 21.

REFERENCES

1. Dolk H, Loane M, Garne E, De Walle H, Queisser-Luft A, De Vigan C et all. Trends and geographic inequalities in the prevalence of Down syndrome in Europe, 1980-1999. Rev Epidemiol Sante Publique. 2005; 53(2): 2587-2595.
2. Siffel C, Correa A, Cragan J, Alverson CJ. Prenatal diagnosis, pregnancy terminations and prevalence of Down syndrome in Atlanta. Birth Defects Res A Clin Mol Teratol. 2004; 70(9): 565-571.
3. Mutton D, Alberman E, Hook EB. Cytogenetic and epidemiological findings in Down Syndrome, England and Wales 1989 to 1993. J Med Genet. 1996; 33: 387-394.
4. Petersen MB, Mikkelsen M. Nondisjunction in trisomy 21: origin and mechanisms. Cytogenet Cell Genet. 2000; 91: 199-203.
5. Antonarakis SE. Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. N Engl J Med. 1991; 324: 872-876.
6. Petersen MB, Schinzel AA, Binkert F, Tranebjaerg L, Mikkelsen M, Collins FA, et all. Use of short sequence repeat DNA polymorphisms after PCR amplification to detect the parental origin of the additional chromosome 21 in Down syndrome. Am J Hum Genet. 1991; 48: 65-71.
7. Hassold T, Sherman S. Down syndrome: genetic recombination and the origin of the extra chromosome 21. Clin Genet. 2000; 57: 95-100.
8. Hassold T, Hunt P. To Err (meiotically) is human: the genesis of human aneuploydy. Nat Rev Genet. 2001; 2: 280-291.
9. Hook EB, Linsjo A. Down syndrome in live births by single year maternal age interval in a Swedish study comparison with results from a New York State study. Am J Hum Genet. 1978; 30: 19-27.
10. Kim SR, Shaffer LG. Robertsonian translocations: mechanisms of formation, aneuploidy, and uniparental disomy and diagnostic considerations. Genet Test. 2002; 6: 163-168.
11. Kolgeci S, Kolgeci J, Azemi M, Shala R, Daka A, Sopjani M. Reproductive risk of the silent carrier of Robertsonian translocation. Med Arh. 2013; 67(1): 56-59.
12. Munne S, Escudero T, Sandalinas M, Sable D, Cohen J. Gamete segregation in female carriers of Robertsonian translocations. Cytogenet Cell Genetics. 2000; 90: 303-308.
13. Kolgeci S, Azemi M, Ahmeti H, Dervishi Z, Sopjani M, Kolgeci J. Recurrent abortions and Down syndrome resulting from Robertsonian translocation 21q;21q. Med Arh. 2012; 66(5): 350-352.
14. Devlin L, Morrison Pj. Mosaic Down's syndrome prevalence in a complete population study. Arch Dis Child. 2004; 89: 1177-1178.
15. Owens JR, Harris F, Walker S, McAllister E, West L. The incidence of Down's syndrome over a 19-year period with special reference to maternal age. Journal of Medical genetics. 1983: 20: 90-93.
16. Palomaki GE, Knight GJ, McCarthy JE, Haddow JE, Donhowe JM. Maternal serum screening for Down syndrome in the United States: a 1995 survery. Am J Obstet Gynecol. 1997; 176: 1046-1051.
17. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res. 1960; 20: 613-616.
18. Seabright M. A rapid banding technique for human chromosomes. Lancet. 1971; 2(7731): 971-972.
19. Mulcahu MT. Down's syndrome in Western Australia: Cytogenetics and incidence. Hum Genet. 1979; 48: 67-72.
20. Jayalakshamma Mary Margaret, Amudha S, Tilak P, Devi R, Rajangam S. Cytogenetic analysis in Down syndrome. Int J Hum Genet. 2010; 10(1-3): 95-99.
21. Schaffer LG, Jackson-Cook CK, Stasiowski BA, Spence JE, Brown JA. Parental origin determination in thirty de novo Robertsonian translocations. Am J Med Genet. 1992; 43: 957-963.
22. Al-Alaiyan S, Al-Omran H, Kattan H, Sakati N, Nyhan WL. Down syndrome and recurrent abortions resulting from Robertsonian translocation 21q;21q. Ann Saudi Med. 1995; 15(4): 391-392.
23. Crane E, Morris JK. Changes in maternal age in England and Wales-implications for Down syndrome. Down Syndrome Research and Practice. 2006; 10(1): 41-43.
24. Young ID, Williams EM, Newcombe RG. Down syndrome and maternal age South Glamorgan. J Med Genet. 1980; 17: 433-436.
25. Cuckle HS. Primary prevention of Down's syndrome. Int J Med Sci. 2005; 2: 93-99.