

CLINICAL AND KARYOTYPIC PROFILE OF CHILDREN WITH DYSMORPHOLOGY

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ABSTRACT

BACKGROUND

Chromosomal anomalies occur in 0.4% live births. The phenotypic anomalies that result from chromosomal aberrations have multiple minor face and limb anomalies which are usual associations. These assume diagnostic significance in combination. Major congenital defects can be defined rather arbitrarily as those abnormalities, if uncorrected, impair normal body function or reduce life expectancy. Most babies with two major anomalies or one major and two minor anomalies or three or more minor anomalies have a dysmorphic syndrome. The objectives of this study were- 1. to analyse the clinical and karyotypic profile of a section of dysmorphic children attending OPD and IP of the Department of Paediatrics, SATH, TVM Medical College and 2. to correlate the dysmorphology with the results of karyotyping.

Setting- Department of Paediatrics, SATH, Government Medical College, Thiruvananthapuram, Kerala.

Design- Descriptive study.

MATERIALS AND METHODS

Children who were enrolled were evaluated using a detailed proforma to analyse the clinical profile. Then 2-4 ml of venous blood was collected in sodium heparinised vacutainer with aseptic precautions and sent for karyotyping.

Subjects- 53 children referred with multiple anomalies, failure to thrive, dysmorphic facies, abnormal dermatoglyphics and other major and minor anomalies were included in the study.

Statistical Analysis- The data was collected, compiled and analysed using Microsoft Excel percentages.

RESULTS

Of the 53 dysmorphic children screened, 73.58% had abnormal karyotype. This included numerical autosomal anomalies (50.9%), numerical sex chromosomal anomalies (3.77%), structural autosomal chromosomal anomalies (7.54%) or structural sex chromosomal anomalies (3.77%). There were 3 cases of Fanconi's anaemia and a case of fragile X syndrome in the sample.

CONCLUSION

Among the 53 children, 73.58% had an abnormal karyotype. Those with two major anomalies or one major and two minor anomalies or three minor anomalies were included in the study. One major anomaly may not be indicative of a chromosomal anomaly whereas association of various major and minor anomalies may indicate a chromosomal defect. As karyotyping and further studies to detect chromosomal anomalies are expensive, selection of cases was based on inclusion criteria yields a high positivity rate.

KEY WORDS

Chromosomal Anomalies, Dysmorphology, Karyotype.

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BACKGROUND

Chromosomal anomalies occur in 0.4% live births. They are present in much higher frequencies among spontaneous abortions and stillbirths.^(1,2) Dysmorphology is the word coined by David Smith in 1966 to describe the study of human congenital defects. Congenital anomalies can arise in any part of the body and most arise in the first trimester of intrauterine life.

Some are mild, but about 3% of all children are born with serious structural defects that interfere with normal body function and can lead to lifelong handicap or even early deaths. Congenital anomalies taken together account for a large fraction of morbidity and mortality.⁽³⁾ In India, congenital malformations account for 8-10% of perinatal deaths and 13-16% of neonatal deaths.⁽⁴⁾

The phenotypic anomalies that result from chromosomal aberrations are mainly due to imbalance of genetic information. Multiple minor face and limb anomalies are usual associations. These anomalies are themselves not unusual, but, they assume diagnostic significance in combination.

Major congenital defects can be defined rather arbitrarily as those abnormalities, that if uncorrected, or uncorrectable significantly impair normal body function or reduce life expectancy.⁽⁵⁾ E.g. Down syndrome, pyloric stenosis, cleft lip, some congenital heart diseases. Overall incidence of major defects is 5-6 %. Minor anomalies are of primarily cosmetic significance. Found in less than 4% otherwise normal

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individuals, they are usually isolated and may run in families too, with an autosomal recessive inheritance. A single minor defect may be present in as many as 13% newborn babies depending on the observer. Less than 1% have two unrelated minor anomalies and perhaps 1 in 2000 have three. Though usually of no clinical significance to the patient, they may be helpful diagnostic clues, especially when several are present in the same patient. Most babies with two major anomalies or one major and two minor anomalies or three or more minor anomalies have a dysmorphic syndrome.⁽⁵⁾

Structural defects of prenatal onset may represent a single primary defect in development or a multiple malformation syndrome. The aetiology of most of the single primary defects of development are unknown but may be explained on the basis of multifactorial inheritance, where the recurrence risk is between 3-5% for the next child of the unaffected parents with one affected child. Other proposed aetiologies of these are environmental or due to inherited single altered genes which follows mendelian inheritance. Multiple malformations may be due to transcription factor mutations as in Rubinstein-Taybi syndrome, chromosomal abnormalities, teratogens and due to single gene disorders. The recurrence risk varies from 0-100% depending on whether it is a mutation/ teratogen or a case as in 21-21 translocation carrier mother in a child with Down syndrome.

SAT Hospital witnesses the birth of nearly 16000 deliveries per year. Many of these newborns are with multiple anomalies which has many genetic and prognostic implications. Many more are attending the OPD, either referred for evaluation of anomalies or for other complaints. This study was planned to analyse the clinical and karyotypic profile of a section of these children and to correlate the dysmorphology with the results of karyotyping.

Cytogenetics is the genetic analysis of cells, a discipline that has flourished since the chromosome-banding techniques introduced in 1969 by Torbjorn Caspersson and Lore Zech first provided a simple and inexpensive way to gauge the number and assess the structural integrity of chromosomes. Chromosome banding is probably the most commonly performed genetic test. Most laboratories use G-banding, named after the German Chemist Gustav Giemsa.

Aim of the Study

To assess the clinical and karyotypic profile of a group of children with congenital anomalies and dysmorphic facies attending OPD and IP of the Department of Paediatrics, SATH, TVM Medical College.

MATERIALS AND METHODS

Design

Descriptive study.

Setting

Dept. of Paediatrics, SATH, Medical College, TVM, Kerala.

Subjects

53 children referred with multiple anomalies, failure to thrive, dysmorphic facies, abnormal dermatoglyphics and other major and minor anomalies were included in the study.

Method

Children who were enrolled were evaluated using a detailed proforma. A note was made on their age, sex, parental age.

The socioeconomic status was assessed with the modified Kuppaswamy scale. Relevant antenatal and postnatal events were noted. A pedigree analysis was done in each case and the anthropometric parameters and dysmorphology were noted. Then 2-4 ml venous blood was collected in sodium heparinised vacutainer with aseptic precautions and sent for karyotyping.

The Karyotyping Method used was Human Peripheral Blood Lymphocyte Micro Culture Method. The Steps are-

- Collect 2-4 ml venous blood in sodium heparinised vacutainer with aseptic precautions.
- 6-10 drops of blood is added to 10 ml RPMI 1640 medium supplemented with 15% fetal bovine serum. Penicillin and streptomycin is added as antibiotics. 0.5 ml phytohemagglutinin is added to proliferate the lymphocytes.
- The cultures are incubated for 72 hrs. at 37°C.
- At the 70th hour, add one drop of colchicine to arrest cell division at metaphase.
- After two hours, transfer the whole content into a sterile centrifuge at 1000 rpm for 10 minutes.
- Discard the supernatant. To the cell button, add 0.075 M KCl solution and keep in incubator for 20 minutes.
- Fix the contents with methanol: acetic acid mixture in the ratio of 3:1 and keep in refrigerator for at least 30 minutes for proper fixation.
- Wash the cell pellets with fresh fixative.
- Repeat the process until we get a clear supernatant.
- These cell pellets are dropped on to a precleaned, labelled, chilled microscopic slide, air dry and stain with 10% Giemsa staining solution.
- Stained slides are observed under a research microscope and look for any numerical chromosome abnormalities.

For detecting structural abnormalities and for karyotyping, a GTG banding is done. For this 2-3 days old slides are treated with 0.05% trypsin solution and stained with 10% Giemsa containing solution. Good quality metaphases are photographed using camera attached microscope. From the prints, we cut down each chromosome and arrange them according to their size, position of the centromere and the banding pattern called the karyotype.

Statistical Analysis

The data was collected, compiled and analysed using Microsoft Excel percentages.

RESULTS

Observations

Numerical Autosomal (50.9%)

47XY, + 21 - 12
47 XX, + 21 - 07
45XX/47XX, +21 - 02
46XY/47XY, +21 - 05
46XY/47XY, +18 - 01

Numerical Sex Chromosomal (3.77%)

46XX.47XXX (12%) -01
46XX (80%), 45XO (20%) -01

Structural Autosomal (7.54%)

46XY/46XY, 13q+ -01

46XY/46XYdel (15q) -01
 46XX/46XX22qdel -01
 46XX/46XX, 22Q + (16%) -01

Structural Sex Chromosomal (3.77%)

46XX/46XXq+ -01
 46XY/46XYq+ (18%) -01

Others (7.54%)

46XY, breaks+++ -0346XY, fraXq (18%) -03(fig 1)
 46XY, fraXq (18%) -01

	Positive Karyotype No. (%)	Negative Karyotype No. (%)	Total
Mental Retardation	23 (92)	5 (18)	28
Low Set Ears	22 (92)	02 (8)	24
Upslanting Eyes	20 (71)	04 (29)	28
Downslanting Eyes	06 (86)	01 (14)	07
Epicanthic Folds	23 (96)	01 (04)	24
Micrognathia	03 (60)	02 (40)	05
Flat Nasal Bridge	18 (82)	04 (18)	22
Simian Crease	21 (95)	1 (05)	22
Clinodactyly	14 (93)	1 (07)	15
Overriding Toes/Fingers	10 (77)	3 (23)	13
Microcephaly	11 (73)	4 (27)	15
Hypotonia	25 (89)	3 (11)	28
Hypertelorism	8 (67)	4 (33)	12
Cleft Lip	1 (33)	2 (67)	3
Cleft Palate	1 (33)	2 (67)	3

Table 1. Profile of Minor Anomalies

	Positive Karyotype No. (%)	Negative Karyotype No. (%)	Total
CVS Anomaly	20 (83)	4 (17)	24
Renal Anomaly	1 (100)	0 (0)	0 (1)
GIT Anomaly	2 (67)	1 (33)	3
Skeletal Anomaly	5 (50)	5 (50)	10
Ambiguous Genitalia	1 (50)	1 (50)	2

Table 2. Profile of Major Anomalies

	Positive Karyotype No. (%)	Negative Karyotype No. (%)	Total
IUGR	18 (82)	4 (18)	22
Malnutrition	8 (62)	5 (38)	13
PIH	6 (67)	3 (33)	9
GDM	8 (67)	4 (33)	12
Bacteriuria	13 (81)	3 (19)	16
Fetal Wastage	4 (67)	2 (33)	6
Consanguinity	5 (56)	4 (44)	9

Table 3. Profile Based on Features in Antenatal History

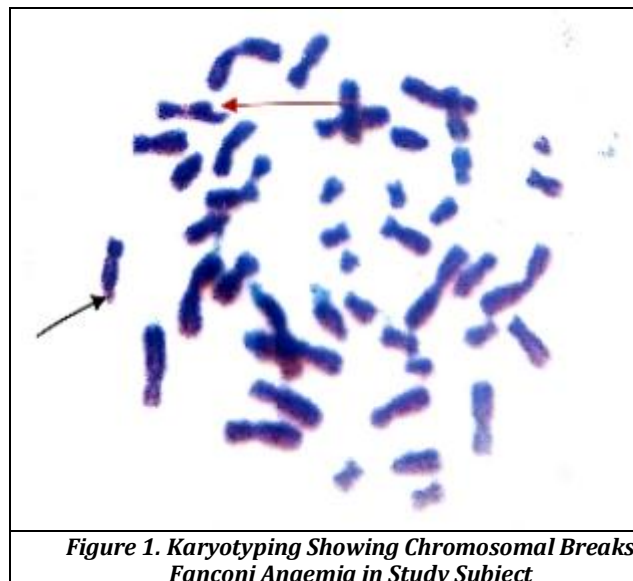


Figure 1. Karyotyping Showing Chromosomal Breaks Fanconi Anaemia in Study Subject

DISCUSSION

53 children belonging to the age group of 0-12 years with suspected chromosomal anomalies were enrolled in the study. Those with two major or one major and two minor anomalies were subjected to karyotyping using peripheral blood micro culture method and Giemsa staining. The major anomalies noted were cardiac defects like VSD, AVSD, GIT anomalies like Hirschsprung's disease etc. Minor anomalies like low set ears, hypotonia, up slant or down slant of eyes, simian crease, inner epicanthic folds were noted.

Majority of the study population (60.3%) were below one year of age, 24.5% were born between one to five years of age and the rest were more than five years of age. 75% newborn babies and 75% children one month to one-year age had an abnormal karyotype. Among the 53 children studied, 73.58% had an abnormal karyotype. Anomalies like Down syndrome, Turner syndrome, Edward syndrome, addition of deletion in chromosome 22, spontaneous breaks in Fanconi anaemia were among the cases studied.

Milia A et al in 1984 reported the results of a karyotype analysis carried out on 282 patients clinically selected for some suspicion of chromosome abnormalities. This population showed a significantly higher incidence of chromosome anomalies (21.6%) than an unselected population (0.5-0.6%)(34). Verma RS et al report in 1980 describes the cytogenetic findings in 357 cases referred for suspected chromosomal abnormalities because of abnormal clinical features. Chromosomal anomalies were found in 27.2% of the cases studied. A significantly high rate of chromosomal abnormalities was found in the population with clinical abnormalities in comparison to an unselected population ie; 0.48-0.55%.⁽⁶⁾

The male to female ratio was 28:23(chi square= 6.24). This difference was significant. Two children had indeterminate sex. One was an unvirilised male and another was a virilised female. Majority of the children with abnormal karyotype were cases of trisomy 21(49%). Of the 901 patients undergoing karyotype analysis in Ghani F et al study in 1995, Down syndrome topped the list in number. A higher incidence of Down syndrome is observed in males. As per our data also, male: female was 2:1. A group of children with a variety of clinical disorders were investigated for the possible presence of chromosomal abnormalities by Navsaria D et al in

1993. Various types of chromosomal anomalies were found which is significantly ($p < 0.01$) higher than in a control population (0.48-0.55%). The male: female ratio was 3:2 for the total population. Furthermore, in this survey population, the sex ratio of Down syndrome cases of males: females was 3:2.⁽⁷⁾

The median age of mothers with karyotypically abnormal children was 28 years and the median age of those with normal children was 25 years. Similarly, median age of fathers with karyotypically abnormal children was 32 years and the median age of those with normal children was 30 years. This difference was not statistically significant.

One study has showed that the estimated rate of all clinically significant cytogenetic abnormalities rises from about 1 per 500 at the youngest maternal ages to about 1 per 270 at age 30, 1 per 80 at age 35, 1 per 60 at age 40 and 1 per 20 at age 45.⁽⁸⁾ In the present study, the mean age of mothers of children with Down syndrome was 27.4 years and that of fathers with Down child was 33.5 years. The age of mothers ranged from 18-40 years and that of fathers ranged from 24-50 years. Previous studies showed that mean maternal age of Down syndrome infants gradually diminished, and accumulated between the ages of 31 and 34 years (36) Trimble BK et al opines that maternal age-specific risks of giving birth to a child with Down Syndrome (DS) are given by single-year age intervals. Such data are of value for more precise genetic counselling and in cost-benefit analyses of prenatal diagnostic programs.⁽⁹⁾

According to modified Kuppuswamy scale for assessing socioeconomic status, majority (86.7%) belonged to middle class and the rest to lower class. None of the subjects belonged to upper class.

One study quotes that the risk of non-chromosomal anomalies increased with increasing socioeconomic deprivation. They opine that the decreasing risk with increasing deprivation found for all chromosomal malformations and Down syndrome in unadjusted analyses, occurred mainly as a result of differences in the maternal age distribution between social classes.⁽¹⁰⁾

In this study 9 (16.9%) was born to consanguineous parents. No significant difference was observed among karyotypically abnormal children born to consanguineous and non-consanguineous parents. Consanguinity increases the risk of single gene disorders rather than chromosomal anomalies. 49% of children were first born, 41.5% were second born. More no of chromosomal abnormalities were detected in the first order of birth. Studies have shown an increase in chromosomal abnormalities among higher birth order children.⁽¹¹⁾ In this study, 60% children were with birth order 3 or 3+ were karyotypically abnormal. However, the number in this group was very low in comparison to that in first and second order of birth. Maternal antenatal events such as history of fetal wastage, symptomatic or asymptomatic bacteriuria, PIH, GDM, history of IUGR were studied, but didn't show any difference. One previous study showed that GDM was not associated with chromosomal anomalies like Down Syndrome significantly.⁽¹²⁾ The mothers of children in the study population were screened for definite history of exposure to radiation. None gave such a history. Risk of congenital anomaly were assessed in relation to parental exposure to ionising radiation acquired through work within a nuclear generating station of an electric power

company previously, but was not associated with an increased risk of congenital anomalies in the offspring of mothers or fathers.⁽¹³⁾

Pedigree tracking of study subjects showed that 5.6% had a suspected chromosomally abnormal blood relative. As karyotyping was relatively new modality at the time of the study, exact details of the anomalies in the previous generations could not be traced.

According to stature, the height of the study subjects belonged to various centiles and there was no trend or significant clustering in any of the centile ranges. Some of the chromosomal anomalies like Turner syndrome are known to have short stature while some others like Klinefelter's syndrome have tall stature.

According to weight for age (IAP classification), varying grades of malnutrition was noted in only 20% of the karyotypically abnormal children. This may probably be a reflection of the better awareness and feeding practices among the mothers of Kerala. In this study, 52% had mental retardation. Of this, 58% had an abnormal karyotype. Out of those without mental retardation, 43% had chromosomal anomaly ($p = 0.339$). Mental retardation is characteristically seen in various syndromes like Down syndrome, Edward, Patau, Rubinstein Taybi, Seckel syndrome etc. 5.6% of the study subjects had cleft lip and cleft palate was seen in same no percentages. Out of these, 2.5% had abnormal karyotype.

Of the chromosomally normal children, 15.3% had above mentioned anomalies $p = (0.145)$. Chromosomal anomalies like Patau and Edward are known to be associated with cleft lip and/ or palate. This study didn't have trisomy 13 but had a case of 13q+ syndrome which didn't have cleft lip or palate.

Low set ears were noted in 45.2%. Of this, 55% had abnormal karyotype. Of the chromosomally normal children, 15.3% had low set ears ($p = 0.023$ i.e.; < 0.05 ; odds ratio = 6.7, 95% confidence intervals 1.3-34.4). The difference was significant.

Low set ears are characteristically seen in Down syndrome, Turner syndrome, trisomies 17, 18, 13, 15, Smith-Lemli Opitz syndrome, Treacher Collins, Carpenter and Apert syndromes.

Though 7.8% of normal children had epicanthic folds, 57.55% of karyotypically abnormal children had this feature. The difference was significant ($p = 0.003$, odd ratio=16.2, 95% confidence intervals =6.04-44.7). This is seen in many conditions as Down, Zellweger, Edward syndrome.

7.6% of normal children has a simian crease, while 35% of karyotypically abnormal children had this feature. The difference was significant. ($p = 0.005$, odds ratio=13.3, 95% confidence intervals= 4.95-36.6) This is seen in many conditions, the classical being down syndrome. It may be a normal variant too.

While 62.5% of karyotypically abnormal children had hypotonia, 23% of normal children had the same. ($p = 0.024$, odd ratio=5.6, 95% confidence intervals =1.2-30). The difference was significant. Abnormalities of autosomal chromosomes are always associated with infantile hypotonia.⁽¹⁴⁾

Other anomalies like up slanting eyes, down slanting eyes, micrognathia, flat nasal bridge, microcephaly, hypertelorism were studied ($p = 0.05$). Mongoloid or up slanting eyes are seen in Down syndrome, Prader-Willi syndrome, ectodermal dysplasias. It may be a normal variant too. Antimongoloid

slant may be seen in Down, Turner, trisomy 17-18, Apert, Smith Lemli Opitz, Noonan and Treacher Collins syndromes. Micrognathia is characteristically seen in Pierre Robin syndrome, Treacher Collins syndrome, Down, Zellweger, trisomies 13 and 18, Russel Silver syndromes. Flat nasal bridge is seen in Down, Zellweger, Smith Lemli Opitz syndromes. Clinodactyly is seen in Down syndrome. Hypertelorism is increased distance between two eyes and is due to hypertrophy of lesser wing of sphenoid. It is seen in Down, Turner, Noonan, Carpenter, Di George, Rubinstein Taybi syndrome. In Edward syndrome, the index finger characteristically overlaps the third while the fifth finger overlaps the fourth. Microcephaly may be a familial feature or may be part of craniosynostosis syndromes, intrauterine infections as CMV, rubella or as part of trisomies 13 and 21, Smith Lemli Opitz syndromes.

The association of CVS, renal, GIT, skeletal anomalies and ambiguous genitalia to chromosomal anomalies were studied but were found to be significant. One major anomaly may not be indicative of a chromosomal anomaly whereas association of various major and minor anomalies may indicate a chromosomal defect.

CONCLUSION

1. Among the 53 children, 73.58% had an abnormal karyotype. Those with two major anomalies or one major and two minor anomalies or three minor anomalies were included in the study. The high rate of positive results may be attributed to the subjects were chosen strictly on the basis of the above inclusion criteria.
2. Majority of the children with dysmorphology were children less than one year (60%), of which 75% were new born. As age advanced from one to twelve years, the incidence of chromosomal anomalies were found to be less. Most of the chromosomal anomalies and malformations present at a younger age and some tend to have a reduced lifespan. Identifying the exact malformation is important in planning early intervention and future prevention.
3. Male to female ratio in the study was 28:23, there were two children with indeterminate sex due to ambiguous genitalia. One was a virilised female and the other was an un virilised male. Higher prevalence of chromosomal anomalies in males, esp. with Down syndrome has been reported. Male: Female ratio in the study was 2:1.
4. The mean maternal and paternal age of children with abnormal karyotype was 28.2 years and 33.9 years respectively. The range was 18-39 and 20-50 years respectively. There was no statistically significant association between paternal ages and incidence of chromosomal anomalies in the study. Even though a causal relationship between chromosomal anomalies and parental ages are present, no such association was observed in this study. This may be because of low sample size.
5. Majority of children with abnormal karyotype belonged to middle class, probably due to higher number of children belonging to this class, attending the hospital. There was no one belonging to upper class in this study.
6. In the study, no statistically significant association was found between consanguinity, birth order or previous

fetal wastage and chromosomal anomalies. Even though association was noted between multiparity and fetal wastage with chromosomal anomalies from references, no such association was evident in this study.

7. Antenatal events like maternal exposure to radiation and maternal medical conditions as PIH, GDM and UTI are related to fetal malformations, no such associations was noted in our study.
8. Intra uterine growth retardation is noted in case of chromosomal anomalies but mothers of children in our study group did not give such a history in majority of the cases.
9. Growth retardation, failure to thrive, and malnutrition may be associated with chromosomal anomalies, but no similar statistically significant association was noted in this study.
10. Among the minor anomalies, low set ears, epicanthic folds, simian crease and hypotonia were found to have a statistically significant association with the occurrence of chromosomal anomalies.
11. Among other anomalies, CVS, GIT, renal, skeletal anomalies and mental retardation etc, none were found to be significantly associated with chromosomal anomalies. One major anomaly may not be indicative of a chromosomal anomaly whereas association of various major and minor anomalies may indicate a chromosomal defect.

As karyotyping and further studies to detect chromosomal anomalies are expensive, selection of cases was based on inclusion criteria adopted in the study. This yielded a high positivity up to 75%. Better awareness regarding chromosomal anomalies, proper nutrition and parenting can go a long way in reducing the morbidity and mortality in affected children. Proper diagnosis of chromosomal anomalies can lead to prevention of future birth of similarly affected children. This can be achieved by instituting genetic counselling.

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REFERENCES

- [1] Hall JG. Chromosomal clinical abnormalities. In: Behrman PE, Kliegman PM, Jenson HB, eds. Nelson Textbook of Pediatrics. 17th edn. Philadelphia, Pennsylvania: Saunders 2004.
- [2] Hoyme EH. Dysmorphology. In: Behrman RE, Kliegman PM, Jenson HB, eds. Nelson Textbook of Pediatrics. 17th edn. Philadelphia, Pennsylvania: Saunders 2004.
- [3] Aase JM. Dysmorphologic diagnosis for the pediatric practitioner. *Pediatric Clin North Am* 1992;39(1):135-56.
- [4] Jones KL. Smith's Recognizable patterns of human malformations. 4th edn. Philadelphia: WB Saunders Co, 1998: p. 641-58.
- [5] Kumar MR, Bhat BV, Oumachigui A. Perinatal mortality trends in a referral hospital. *Indian J Pediatr* 1998;63(3):357-61.

- [6] Verma RS, Dosik H. Incidence of major chromosomal abnormalities in a referred population for suspected chromosomal aberrations: a report of 357 cases. *Clin Genet* 1980;17(5):305-8.
- [7] Navsaria D, Mathews T, Conte RA, et al. Chromosomal anomalies in 1,000 children referred with suspected genetic disorders. *Hum Hered* 1993;43(3):137-40.
- [8] Hook EB. Rates of chromosome abnormalities at different maternal ages. *Obstet Gynaecol* 1981;58(3):282-5.
- [9] Trimble BK, Baird PA. Maternal age and Down syndrome: age-specific incidence rates by single-year intervals. *Am J Med Genet* 1978;2(1):1-5.
- [10] Vrijheid M, Dolk H, Stone D, et al. Socioeconomic inequalities in risk of congenital anomaly. *Arch Dis Child* 2000;82(5):349-52.
- [11] Kallen K. Parity and Down syndrome. *Am J Med Genet* 1997;70(2):196-201.
- [12] Martinez-Frias ML, Rodriguez-Pinilla E, Bermejo E, et al. Epidemiological evidence that maternal diabetes does not appear to increase the risk for Down syndrome. *Am J Med Genet* 2002;112(4):335-7.
- [13] Green LM, Dodds L, Miller AB, et al. Risk of congenital anomalies in children of parents occupationally exposed to low level ionising radiation. *Occup Environ Med* 1997;54(9):629-35.
- [14] Gerald M, Fenichel M. Psychomotor retardation and regression. In: Fenichel GM, edr. *Clinical Pediatric Neurology*. 4th edn. Philadelphia, Pennsylvania: WB Saunders Company, 2001.