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Original Research Article

Chromosomal aneuploidies: A tertiary care center study

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ABSTRACT

Background: Among the chromosomal aneuploidies Down syndrome is the most common type. This study was conducted to detect the frequency of chromosomal abnormalities in the paediatric patients in one year duration at a tertiary care centre.

Materials and Methods: In cytogenetic lab, clinically suspected cases were referred from different department like pediatrics, neonatology, obstetrics and gynecology for Karyotyping test for the confirmation of aneuploidies. They were sampled for Karyotyping.

Result: Incidence of aneuploidies among live-births in a year (April 2023-March2024) was 4.68/1000. Down syndrome with trisomy+21 was found in all individual (100%) of 12 cases. Among cardiac disorder, ASD, VSD and VSD+ ASD were (complex cardiac defect) account for 33.33%. Other common clinical features were also seen like slanting eyes, sandal gap, mangloid facials, single simian crease, low set ears, depressed nasal bridge, and protruding tongue

Conclusion: In this study prevalence of DS in Chhattisgarh were 4.68/1000. Most of the cases of the study were born from mother of younger age (19-27yrs). For early diagnosis clinician should counsel the younger pregnant women for such aneuploidies so that they can take appropriate measures.

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1. Introduction

Incidence of numerical and structural chromosomal disorders among live birth is 0.5% and single gene disorder, multiple gene disorders are 1% each. These abnormalities are higher in stillbirths. Most of the chromosomal disorders are Down syndrome, Patau syndrome, Edward syndrome and sex chromosome disorders. Among these most of the cases seen by physician are Down syndrome (DS). Occurrence of Down syndrome is high among the intellectually disabled children worldwide.¹ Early identification of DS is necessary to reduce the congenital disability.

Congenital aneuploidies are due to nondisjunction of chromosome. There are three rules of nondisjunction, first; most trisomy originate during oogenesis regardless of specific chromosome, second: for most chromosome, maternal meiosis I (MI) errors are more common than Maternal meiosis II (MII) errors, third; proportion of cases of maternal origin increases with maternal age.²

Trisomy 18 and trisomy 13 have higher (80%) in utero mortality while DS has 30%.^{3,4} DS fetuses are identified by markers such as double marker and triple marker and also by ultrasonography. Fifty percent of them are missed by prenatal ultrasound as they do not show any structural defects.⁴

Normally an individual has 46 chromosomes. Down syndrome is chromosomal disorder in which an extra copy

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of chromosome i.e. in chromosome number 21 (Trisomy) is present which results in many physical abnormalities such as small ears, single simian crease, upward slanting eyes, opened mouth, flat facial feature, cardiac defects, hypotonia, mental retardation. Hence all these factors show that DS have both mental & physical challenges. Major associated abnormalities are CHD (40%), atresia of duodenum (12%), Cataract nystagmus, squint, hearing defects, serous otitis media, hypothyroidism (13-15%), atlanto-occipital subluxation, delayed physical growth, malignancies like acute lymphoblastic leukemia, acute myeloid leukemia transient, lymph proliferation syndrome and myelodysplasia in Indian population.

Worldwide studies suggested that hearing loss (75%), sleep disorder and ear infection (50%-75%), eye diseases (60%), psychiatric disorders (22%), heart diseases (50%) are the major abnormalities.⁵⁻⁷

In India the birth prevalence of Down syndrome varies from 1/1230-1/1361.^{8,9}

Down syndrome has three types-

1. Trisomy-21- which is most common type of Down syndrome. Around 95% individual has trisomy-21 has 3 different copies of chromosome 21 instead of the usual 2 copies.
2. Translocation can see in only 3% of individual. It occurs when an extra part or a complete extra chromosome 21 is present, but it attached or “translocated” to a different chromosome rather than being individual chromosome.
3. Mosaic is very rare about 2% of the people with mosaic mean mixture or combination. Individual with mosaic Down syndrome some cells have 3 copies of chromosome 21, but other cells have the typical 2 copy of chromosome 21. Individual may have same feature as other individual with Down syndrome.

Screening tool commonly used in India are triple test, quadruple markers and first trimester double-marker test with or without nuchal translucency (NT).¹⁰ The risk factor for DS is mainly associated to women reproductive health like fertility regulation, abortion, maternal mortality, sexual transmitted disease and infertility. Risk of aneuploidies increases with increase numbers of abortions.¹¹ But other studies suggesting increased number of spontaneous abortions reduce the risk of chromosomal disjunction.^{12,13} In India, it is necessary to determine the prevalence of DS among the entire population. This is the prerequisite for robust management of either screening to decrease the prevalence or counseling or rehabilitation, medical care, education and welfare services. We conducted the study to robust profile and frequency of DS cases at tertiary care center in Chhattisgarh.

Aim of our study was to conduct to detect the frequency of chromosomal abnormalities in the newborn in one year

duration at a tertiary care centre.

The secondary objective was to detect the frequency of clinical signs and symptoms in these cases and correlate their occurrence with maternal age.

2. Materials and Methods

Data of live-births in AIIMS Raipur in a year (April 2023-March2024), was collected. Data and samples of clinically suspected cases of aneuploidies were collected from NICU (Neonatal intensive care Unit), Department of Pediatric and Department of Obstetrics and Gynecology and were referred to cytogenetic lab, Department of Anatomy in medical college AIIMS Raipur for karyotyping (chromosomal analysis). Consent of their parents were taken for the test. Basic clinical profile and pedigree were taken. Two milliliters blood was withdrawal in heparinized vial by lab technician with taking appropriate measures.

2.1. Inclusion criteria

1. Patients were included on the basis of their clinical features & confirmed karyotyping reports.
2. Those who had given consent.

2.2. Exclusion criteria

1. Patients were excluded who has clinical features of Aneuploidy but their karyotyping reports were normal.

Culture preparation was done under sterile condition in laminar flow cabinet. In culture media, 0.5ml blood sample was mixed in for culture and allowed it to grow for 69-70 hours in 5% CO₂ incubator. Harvesting was done by adding colchicine 80µl (1mg/ml) to culture cells and mix thoroughly and placed it in 37°C for 30 minutes in incubator. After 30 minutes culture tube was centrifuged (10mins at 1500 RPM). Supernatant was discarded and Potassium chloride (KCL) was added as a hypotonic solution and again incubated for 20-25 minutes at 37°C, after that centrifuged for 10 minutes at 1500RPM. At last Fixative (Glacial acetic acid: Methanol in 1:3ratio) was added, left it for overnight in refrigerator (4-8°C). Next day, tube was washed with fixative 3 times, till the pellet became clear.

Two slides prepared for each sample, slides were aged for 3-5 days at room temperature. After proper drying and an ageing, G-banding was done using trypsin, slides were treated for 2-3 seconds and then two times simultaneously washed with PBS buffer and stained with Giemsa. Slide was ready to observe under microscope in which metaphase chromosome was analysed.

3. Results

The total number of livebirths in last year (April 2023-March2024) at AIIMS Raipur, tertiary care center was 2563.

Fourteen suspected aneuploidy cases on the basis of phenotypic appearance and clinical examination by professionals were advised for karyotyping test. After karyotyping report it was seen that 12 cases were confirmed for DS which were further evaluated for associated cardiac anomaly by Echocardiography. Ultrasonography of brain, abdomen, pelvis and spine was performed for any other abnormalities. Other investigations were also advised such as thyroid profile to look for hypothyroidism.

Average maternal age at birth of child was 26.66 year and average paternal age at birth of the child was 30.66 year (Table 1). Trisomy 21 was the predominant type of DS seen in 100% of cases. Common symptoms were depressed nasal bridge, small nose, Low-set-ear, increased gap between 1st and 2nd toes (81.81%). Cardiac anomalies were found 27% and hypothyroidism were reported 18% (Table 2, Figure 1).



Figure 1: Showing **A)**: Slanting eyes. **B)**: Protruded tongue & Mangloid face; **C)**: Low set ear; **D)**: Depressed nasal bridge & short neck; **E)**: Single simian crease; **F)**: Sandal gap

4. Discussion

Commonest chromosomal anomalies worldwide is Down syndrome. In Chhattisgarh state we do not have data regarding DS in live birth. Our study was in tertiary care center. We have found aneuploidies 4.68/1000 live births. India study reported prevalence rate of aneuploidies 0.081-1.2/1000 live births.^{8,14–16} Our finding prevalence rate was slightly higher than the US prevalence rate for DS 1.43/1000 person.¹⁷ It could be due to our study was conducted in tertiary care center which caters to high risk pregnancies referred from all over the state including other medical colleges. During one year of study period, fifteen clinically suspected patients were investigated and twelve were confirmed for DS. These patients were evaluated carefully for associated anomalies. Out of them few cases

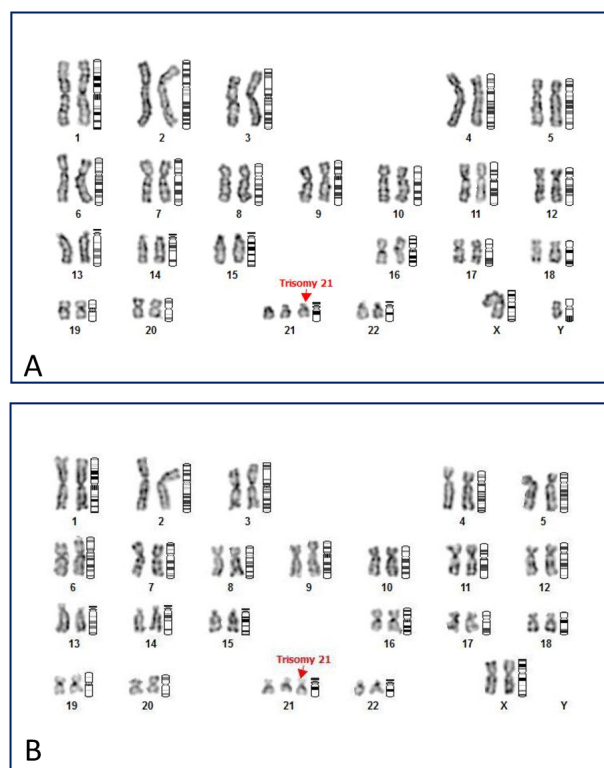


Figure 2: **A)**: Karyotype A Trisomy+21 male child. **B)**: Trisomy+21 female child

of DS are discussed here.

In our study, 66.6% of children were on the 1st birth order, 16.6% were 2nd birth order, 8.33% were 3rd birth order, and 8.33% were 4th birth order (Table 1). One study on DS showed 75% children on the 1st birth order, this finding is close to our study.¹⁷ Average maternal age in studies by Panigrahi et al,¹⁸ Lakhan and Kishor,¹⁷ was found to be 29 years, 21year respectively. For mother of children with DS, in our study average maternal age for DS was found to be 26.6 years which is closer to other studies of India. Sheth et al found that 91.6% cases of DS were from younger mother (< 35 years).¹⁹

Clinical symptoms have been discussed in Table 2. Complications like congenital heart disease were found in 33.33%. These were cases with ASD, VSD, PDA, ASD+VSD (complex). Panigrahi et al¹⁸ found 62% of cases with cardiac defects. In current study, hypothyroidism was found in 16.66% while Panigrahi et al study it was 30.28% which was higher than our findings.¹⁸ Intestinal blockage was found 16.66% in current study which were duodenal atresia and recto-vestibular fistula. Surgeries performed for that were KIMURA Dudenoduodenostomy & Posterior Sagittal Anorectoplasty (PSARP).

Table 1: Profile of children with down syndrome and their parents' age

Case No.	Age	Gender	Birth Order	Mothers Age at Birth	Fathers Age at Birth
1	4M	Female	First	29Yr	31Yr
2	3M	Female	First	25Yr	31Yr
3	4.5M	Male	First	28Yr	29Yr
4	9 Days	Male	First	19Yr	21Yr
5	1Days	Female	First	31Yr	37Yr
6	8 Days	Female	First	21Yr	23Yr
7	3days	Female	First	36Yr	40Yr
8	1M	Female	First	21Yr	25Yr
9	2Days	Male	Second	28Yr	31Yr
10	2Days	Male	Second	32Yr	33Yr
11	1M	Male	Third	23Yr	33Yr
12	6M	Female	Fourth	27Yr	34Yr
		Female-7	1 st -8	Average-26.66 Years	Average-30.66 Years
		Male-5	2 nd -2		
			3 rd -1		
			4 th -1		

Table 2: Clinical symptoms and associated complication of cases of chromosomal aneuploidy

Symptoms	Present (n=12)	Percentage
Flat occiput	9	81.81%
Small nose	10	83.33%
Low-set-ear	10	83.33%
Flattened facial features	9	81.81%
Depressed nasal bridge	10	83.33%
Open mouth	9	81.81%
Protruding tongue	5	41.66%
Broad hand, short fingers	9	81.81%
Increased gap between 1 st and 2 nd toes	10	83.33%
Hypotonia	2	16.66%
Intestinal blockage	2	16.66%
Congenital heart disease	4	33.33%
Hypothyroidism	2	16.66%

Table 3: Few cases of Down syndrome

Case	History & clinical Examination	Routine investigation & procedure done	Confirmed Karyotype
Case 1	A 20 days old female born at late preterm was admitted with complains of rhinorrhea for 6-7 days with rapid breathing, chest indrawing on examination. Birth weight was 1.9kg, length was 43 cm. head circumference was 29cm. Neonate cried immediately after birth and on clinical examination had flat facies, wide open anterior fontanelle, slanting eyes, saddle toes, protruded tongue, simian crease, hypotonia.	Blood urea/creatinine-9.1/1mg/dL. LFT- showed increased bilirubin.	Down syndrome 47, XX,+21
Case 2	A one day old male born at full term was admitted with complains of respiratory distress in the form of tachypnoea, syndromic facies and hypotonia. Subcostal retractions with downes score of 3/10 was observed. His birth weight was 3.3 kg, head circumference was 36.5cm, and length was 50cm. He Cried immediately after birth and on clinical examination had flat facies, wide open slanting eyes, saddle toes, low set ears, protruded tongue, depressed nasal bridge, single simian crease, generalised hypotonia and palmargrasp was weak.	2D echo was done which suggests of PDA 3mm. LFT- Showed increased bilirubin.	Abnormal male karyotype, consistent with down syndrome 47, XY+21 (Figure 2 A).
Case-3	An one day old female born at late preterm was admitted with complains of multiple non-bilious vomiting which was further diagnosed as duodenal atresia. Her birth weight was 1.9 kg, head circumference was 28.5cm, length was 43cm. Neonate cried immediately after birth. Baby had low set ears, Rucker bottom feet, slanting eyes, saddle toes, protruded tongue on clinical examination.	Procedure: KIMURA Dudenoduodenostomy was done under GA under AAP supine position. Routine investigation LFT test AST-50 U/L (12-38) Potassium 7.89↑ (3.5-5mmol/L)	Karyotyping confirmed-Down syndrome 47, XX,+21
Case 4:	One day old male born as late preterm was admitted with complains of respiratory distress with episodes of desaturation. His birth weight was 2.6 kg, head circumference was 33cm, length was 49cm. Baby cried immediately after birth. Baby had tachypnea and intermittent desaturation eisodes. At admission, neonate was provided oxygen by nasal prongs with 30% Fi O ₂ and 2l/min of flow. Baby had polychythemia. Facial dysmorphism showed flat facies, low set ears, epilateral folds, mongoloid telecanths, excess skin in the nape of neck, sandal gap, single simian crease.	Routine investigation- HB value 23.3, HCT-70.6, So partial exchange was done at day 6 of life with total 40 ml volume. During and after exchange transfusion vitals was stable. Post transfusion HCT was 54.8. ECHO finding- of 3.4mm ASD (aneurysmal ASD).	Karyotyping confirmed-Down syndrome 47, XY,+21 (Figure 2 A).
Case 5:	One day old female born at term was admitted in NICU with complains of Down phenotype. Weight of the baby was 1.8kg, length was 40cm and Head circumference was 30.5cm. Baby had dysmorphic features suggestive of Down's phenotype like depressed nasal bridge, mongoloid slant. Neonate was shifted in NICU for workup of Down phenotype. Baby had Mongolian slant, prominent epicanthal fold.	Prenatal testing, anomaly scan showed echogenic foci in left ventricle and later on ECHO was done which suggested 2mm membranous VSD, restrictive in nature.	Karyotyping confirmed-Down syndrome 47, XX,+21 (Figure 2 B).

Continued on next page

<i>Table 3 continued</i>			
Case 6:	Six months old female early term was admitted in pediatric ward with complains loose motion, Weight of the baby was 2kg with Down's phenotype like Small and low set ears, epicanthal fold, depressed nasal bridge, protruded tongue.	complex heart disease (ASD+VSD) in echo	Karyotyping confirmed-Down syndrome 47, XX,+21 (Figure 2 B).
Case 7:	A three months old female was admitted in ward with the complains of anorectal malformation in the form of passing of stools and urine from same opening. Weight of the baby was 3.36 kg with Down's phenotype like Small and low set ears, epicanthal fold, depressed nasal bridge, protruded tongue.	ECHO finding-Normal USG Finding-Normal	Karyotyping confirmed-Down syndrome 47, XX,+21
Case 8:	A four months old male came with complains of cough and cold for 3 days. Weight of the baby was 4kg, HC- 33cm, Ht-54. Baby had phenotype of DS like Mongoloid slats of eyes, low set ear, brachycephaly, simian crease and wide spaced gap in toes.	ECHO finding-Normal USG Finding-Normal	Karyotyping confirmed-Down syndrome 47, XY,+21
Case 9:	A one year old male was admitted with complain of loose motion. Weight of the baby was 4kg, HC- 33cm, Ht-54. Baby had delayed cry after birth. Presented to paediatric OPD with failure to gain weight and developmental delay for which he got admitted in ward. He had phenotypic features of DS.		Karyotyping confirmed-Down syndrome 47, XY,+21
Case 10:	A 9 months male at birth did not cry and had presented with complains of delayed developmental milestone, syndromic facies like up slanting of eyes, short neck, single simian creases. He had congenital hypothyroidism for which tab Thyroxine (25microgam) was advised.	USG thyroid- few enlarge lymphnodes are noted bilaterally, largest measuring 15x15mm seen in left upper jugular.	Karyotyping confirmed-Down syndrome 47, XY,+21
Case 11:	A 1 year female was reported with complain of delayed milestone, short stature, umbilical hernia and congenital primary hypothyroidism. Baby had phenotype of DS like, low set ear, brachycephaly, depressed nasal bridge.		Karyotyping confirmed-Down syndrome 47, XX,+21
Case 12:	A 1 years female child was admitted in ward with the complain of viral pneumonia with mass present at nape of neck. Syndromic baby- depressed nasal bridge, low set ears, protruding tongue and short neck.		Karyotyping confirmed-Down syndrome 47, XX,+21

Table 4: Variation in pattern of chromosomes in Down syndrome

Author's name	Year	Place of study	Variations reported
Jyothy et al.	2000	Andhra Pradesh	Study population-1001 Trisomy-87.52% Mosaicism-7.69% Translocation-4.39%
Sheth et al.	2007	Gujarat	Study population-382 Trisomy-84.8% Mosaicism-5.7% Translocation-9.6%
Podder et al.	2012	West Bengal	Case Control study Males-55(65%) & Females-30 (35%). Free Trisomy 21-91.8%, Mosaicism- 5.9%, Translocation-2.4%.
Gadhia et al.	2015	Gujarat and Western India	Study population-682 Free trisomy -92.2%, RS Translocation-7.0% Mosaicism-0.73%.
Sharath et al.	2018	Karnataka	Study population-72 Trisomy-78.7% Translocation-14.7% Mosaicism-2.6% Normal Karyotype-4%
Panigrahi et al.	2023	Chandigarh	Retrospective study 134 DS Males 74 DS Females Trisomy-97.6% Mosaicism + Translocation-2.4%
		Present study 2024	Chhattisgarh Free trisomy 100%

The study of Jyothy et al found in Andhra Pradesh, 87.92% trisomy, 7.69% mosaicism and 4.39% translocations (Table 3). This is due meiotic non-disjunction of extra chromosome 21 in maternal 79.24% and 20.76% of paternal. Study in Gujarat, Sheth et al found in 382 clinically suspected cases of DS, 84.8% trisomy, 5.7% mosaicism and 9.6% translocations. Another study was conducted in west Bengal by Poddar et al, in 55 male and 30 females. He observed free trisomy 91.8%, mosaicism 5.9% and translocations 2.4%. Gadhia et al observed among 682 confirmed cases, 92.2% free trisomy, 0.73% mosaicism and 7.0% translocations. In Karnataka, study conducted by Sharath et al in 72 clinically suspected cases of DS, 78.7% trisomy, 2.6% mosaicism and 14.7% translocations and four cases with normal karyotype. Panigrahi et al found in Chandigarh, 97.6% trisomy, mosaicism+translocations 2.4%. In our study, in 12 DS cases we found 100% free trisomy (Table 3). We did not find any mosaicism and translocation.

Our aim is to provide medical and social care to these patients as well as to prevent the occurrence of aneuploidies. Very good counseling, vigilance in the developmental process of these disorders, better nutritional support, are required to provide best care. In this study we found the cases were born to the mother in younger age, for early diagnosis. Clinician should counsel the younger pregnant women for such aneuploidies. According to the MTP rule, for special cases termination of pregnancy will be possible up to 24 weeks after taking opinion from two resisted medical practitioner. The counseling for termination of pregnancy is offered but non-directive.

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6. Conflict of Interest

None.

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