Original Article

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Incidence and Type of Abnormal Karyotype in Foetuses with Major Congenital Malformations Detected on Prenatal Ultrasound

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Abstract

Introduction: Structural fetal abnormalities occur in approximately 2-3% of all pregnancies, prenatal diagnosis, karyotyping is considered the gold standard because of its greater diagnostic accuracy to identify structural abnormalities and diagnose the most frequent aneuploidies. However loss or gain of genetic material is better studied by other techniques like array comparative genomic hybridization.

Aims: To find the incidence and type of abnormal karyotype in foetuses with major congenital malformations detected on prenatal ultrasound.

Materials and methods: All the pregnant patients with major congenital anomaly on prenatal test between January 2018 to October 2018 and total 47 patients are included in study. Routine ante natal investigations were done.

Results: Abnormal karyotype was found in 28.5% (12/42) of samples. Numerical Abnormalities were seen in 6 patients (Trisomy 21 n=5 Monosmy 1). In our series the commonest anomaly was Trisomy 21 (5/12, 41% of patients). 92.9% with structural neural abnormalities had a normal karyotype. Eleven patients had structural cardiac abnormalities of which 6 (54.5%) had an abnormal karyotype. Fifty percent of patients with renal agenesis and 40% of patients with skeletal abnormalities had abnormal karyotype.

Conclusion: Screening test should be done all pregnant patients irrespective of age and risk factors to identify the abnormal karyotype early in pregnancy which is found in significant proportion of foetuses with anomalous finding on prenatal ultrasound.

Keywords: Structural fetal abnormalities; karyotyping , aneuploidies; prenatal ultrasound.

Introduction

The genetic etiology of major congenital malformations is widely variable: monogenic syndromes are estimated to account for 2-10% of cases, whereas chromosomal abnormalities are found in 10-15% of live born with major congenital malformations and in 9-39% of fetuses with abnormal ultrasound findings, depending on the presence of a single anomaly or multiple malformations [1]. Structural fetal abnormalities occur in approximately 2-3% of all pregnancies, 10% of still births and 25% of all neonatal deaths. Examination of placental and fetal anatomy and histology fails to identify a likely pathogenic mechanism in 12% to 50% of cases [1]. Currently the main laboratory test offered to a patient with a malformed fetus after an amniocentesis or chorionic villous sampling is conventional karyotype analysis.

Similarly, a detailed postmortem examination of a stillborn with congenital malformations is not considered complete without a conventional karyotype analysis. In prenatal diagnosis, karyotyping is considered the gold standard because of its greater diagnostic accuracy to identify structural abnormalities and diagnose the most frequent aneuploidies (13, 18, 21, and X) [2].

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In already aborted fetuses, stillborn and live babies with major congenital abnormalities conventional karyotyping has got good value and has got easy accessibility. However loss or gain of genetic material is better studied by other techniques like array comparative genomic hybridization. Karyotyping can detect etiology in 5 to 40% of aborted fetuses with major congenital abnormalities [1]. Some published studies of stillbirths with abnormal anatomy cite rates of abnormal karyotype anywhere between 20 to 50%.There are only few studies addressing this issue from Indian literature. Karyotyping will give valuable information for future genetic counseling of parents.

Aim of the study

To find the incidence and type of abnormal karyotype in foetuses with major congenital malformations detected on prenatal ultrasound.

Material and Methods

This study was conducted in Department of Obstetrics and Gynaecology between January 2018 to October 2018.

Inclusion Criteria

1. All foetuses who underwent medical termination of pregnancy (<20 weeks) based on diagnosis of major congenital anomaly on prenatal tests.
2. All spontaneously stillborn foetuses who had abnormal phenotype and major congenital malformation detected prenatally.
3. All live born foetuses with major congenital malformation detected prenatally.

Exclusion criteria

1. Patients with diabetes complicating pregnancy.
2. Patients with history of exposure to radiation and teratogenic drugs.

A pre structured proforma was used to obtain data after taking written informed consent and counseling. The following data was evaluated as family history, Clinical examination.

Routine antenatal investigations are done like Complete Blood Count, BT, CT, Glucose Tolerance Test, Urine Routine & Microscopy, HBsAg, VDRL, HIV, Blood Grouping & Rh Typing, Thyroid Function Test, Nuchal Translucency scan, Triple marker, Quadruple marker, Anomaly scan, Fetal Echo and Amniocentesis and Chorionic Villous Sampling if present.

All the pregnant patients with major congenital anomaly on prenatal test were included.

Routine ante natal investigations were done. Additional investigations were done in patients with systemic diseases and obstetric complications. History regarding consanguineous marriages, drug intake, exposure to radiation was taken. Detailed examination of the abortus and the fetus was done in patients who underwent abortion or deliver a still born fetus. For Karyotyping fetal heparinised blood will be preferred. If blood is not available then tissue sample skin or placenta or umbilical cord was taken and sent to the genetic laboratory in saline.

For Chromosome Preparation most commonly circulating lymphocytes from peripheral blood were used.

For preparation of karyotype (Giemsa) banding staining methods was used to identify individual chromosomes. The chromosomes were treated with trypsin, which denatures their protein content, and then stained with a DNAbinding dye—also known as 'Giemsa'—that gives each chromosome a characteristic and reproducible pattern of light and dark bands.

The next stage in chromosome analysis involved first counting the number of chromosomes present in a specified number of cells, sometimes referred to as metaphase spreads, followed by careful analysis of the banding pattern of each individual chromosome in selected cells. The cytogeneticist analysed each pair of homologous chromosomes by automated machine.

Statistical analysis

The data was analyzed by using SPSS17. Descriptive statistics were analyzed. The Percentage and proportion were analyzed.Categorical variables were analyzed by using Chi-Square test. The continuous data was analyzed using Mann Whitney Utest.

Results

A total of 47 cases were included in the study. The maternal age ranged from 18 to 32 (24 ± 3.1) years (Fig 1). Only one patient was above 30 yrs (2.1%) and 12 patients (25.5%) were between 26 to 30 years.

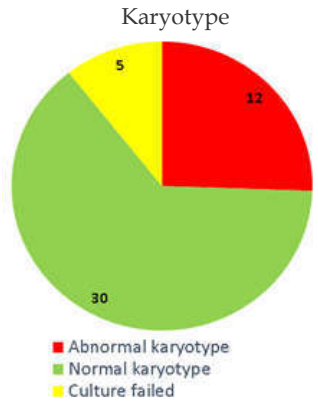

Fig. 1: Abnormal karyotypes found

Karyotype was obtained in 42 patients of which 12 (12/42, 28.5%) had abnormal karyotype and 30 (30/42, 71.5%) had normal karyotype. Abnormal karyotype was found in 23.5% (4/17) of samples in second trimester and 32% (8/25) in third trimester (Table 1).

Nuchal translucency in first trimester was done in 53%. However triple marker test was done in only 3 (6.3%) patients. All patients underwent ultrasound in 2nd trimester to detect anomalous foetuses.

Table 1: Types of abnormal karyotype found

	Disorder	**Karyotyping result**
Numerical Abnormalities		
Monosmy 1 Trisomy 21 n=5	Monosomy	45, X, 9q (h+) with single X chromosome
	Trisomy 21	47, XX, +21 Female karyotype indicating down syndrome
	Trisomy 21	47, XX, +21 Female karyotype indicating down syndrome
	Trisomy 21	47, XX, +21 Female karyotype indicating down syndrome
	Trisomy 21	47, XY, +21 male karyotype indicating downs syndrome
	Trisomy 21	47, XY, trisomy +21
Structural Abnormalities		
Translocation 2	Translocation	46, XY, +21, rob (21; 22) (q10; q10) male karyotype indicating downs syndrome. The extra chromosome is 21 translocated to chromosome 22 (Robertsonian translocation) indicating downs syndrome.
	Translocation	46, XX, +21, rob (21; 21) (q10: q10) female karyotype indicating downs syndrome. Extra 21 showing robertsonian translocation to other chromosome 21
Insertion 1	Insertion	46, XX, ins (3p; 3q) (p24.3pter; q25)
Others 3	Others (To be confirmed by Fish)	46, XX, 15p+ Female karyotype (Addl material on 15 p)
		46, XY,15p+ Male karyotype (Addl material on 15 p)
		46, XX, addl 1 (p36) female karyotype addl chromosome material seen on short arm of cromosome 1 origin of which is to be confirmed by molecular tests.FISH with specific probes for region in chromosome 13 is recommended to rule out partial trisomy 13

Table 2: Samples obtained in relation to gestational age

		Gestation age				**Total**
		<12 wks	**13 to 20 wks**	**21 to 27 wks**	**28 wks till birth**	
Types of karyotype	Normal Karyotype	0	12	2	16	30
	46, XX, 15p+/46XY15p+	0	1	0	1	2
	45, X, q9(h+)	0	1	0	0	1
	Downs syndrome	0	1	1	5	7
	46, XX, addl 1(p36)	0	0	0	1	1
	46, XX, ins (3p; 3q) (p24.3; pterq25)	0	0	0	1	1
	Cell not grown	1	4	0	0	5
	Total	1	19	3	24	47

Comparing Abnormal and normal karyotype

The mean age was not significantly different between abnormal and normal karyotype patients. The incidence of abnormal karyotype increased from 11.8% in G1 patients to 50 % in G4 (p=0.2). All the patients with history of consanguinity had normal karyotype. Twenty three percent of patients with abnormal karyotype had no history of abortions. The incidence increased to 36.4% with history of 1 abortion and 100% with history of 2 abortions (p=0.19). The abnormal karyotype had no relation to history of child deaths due to congenital abnormality or hypothyroidism.

Neural anomalies

Most of the patients (92.9%) with structural neural abnormalities had a normal karyotype. Only one patient with structural neural anomaly had abnormal karyotype (p=0.03) Table 12. The neurologic anomalies seen were hydrocephalus in 8, anencephaly in 2, vermin hypolasia in 4, choroid plexus cyst in 1, dandy walker syndrome in 3,

Table 3: Karyotype in relation to screening tests

		Karyotype			Total n=47
		Abnormal	Normal	Contaminated	
NT scan 1st trimester	Done and normal	6	16	3	25 (53.1%)
	Not done	5	15	2	22 (46.9%)
Triplemarker	Done	0	3	0	3 (6.3%)
	Not done	11	28	5	44

Table 4: Type of delivery

		Gestational age			
		<12 wks	13 to 20 wks	21 to 27 wks	28 wks till birth
Type of delivery	Induced Vaginal delivery	1	19	0	0
	Spontaneous vaginal delivery	0	0	3	17
	LSCS	0	0	0	7

Table 5: Distribution in demographic variables

		Abnormal Karyotype n(%)	Normal Karyotype n(%)	p value
Age (mean ± SD)		24.5 ± 3.3	24.2 ± 3.1	0.8a
Gravida	1	2 (11.8)	15 (88.2)	0.2b
	2	6 (40)	9 (60)	
	3	2 (33.3)	4 (66.7)	
	4	2 (50)	2 (50)	
Consanguinity	yes	0	5 (100)	0.2b
	no	12 (32.4)	25 (67.6)	
Family history	yes	0	1 (100)	1b
	no	12 (29.3)	29 (70.7)	
Socioeconomic status	Low	6 (27.3)	16 (72.7)	0.27b
	middle	5 (26.3)	14 (73.7)	
	high	1 (100)	0	
Abortion number	0	7 (23.3)	23 (76.7)	0.13b
	1	4 (36.4)	7 (63.6)	
	2	1 (100)	0	
H/O child death due to congenital abnormality	Yes	1 (25)	3 (75)	1b
	No	11 (28.9)	27 (71.1)	
Trimester	2nd	4 (23.5)	13 (76.5)	0.4b
	3rd	8 (32)	17 (68)	
Hypothyroidism	Yes	1 (25)	3 (75)	0.9b
	No	11 (28.9)	27 (71.1)	

a - Mannwhitney U test, b Chi Square test

ventriculomegaly in 5, agenesis of corpus callosum in 2, arnoldchiari malformation in 1, meningocele in 4, spina bifida in 2 and thickened nuchal fold in 3. One patient who had hydrocephalus with spina bifida had 46 XX 15 p+ karyotype. One patient with thickened nuchal fold had Trisomy 21. Remaining all had normal karyotype. Cardiac anomalies. Eleven patients had structural cardiac abnormalities of which 6 (54.5%) had an abnormal karyotype. Among patients with no structural cardiac anomalies, 77% had a normal karyotype (p=0.14) Table 12. The cardiac anomalies seen were TOF in 3, cardiomegaly in 2, arrhythmia in 1, Atrioventricular septal defects, Transposition of great arteries in 1, TAPVC in 1, ASD in 4, VSD in 8, Echogenic cardiac foci in 3, left SVC abnormal 3 vessel view in 1 aberrant SCA in 1 and complex anomaly in 1. Four of Seven patients with Trisomy 21 and 1 patient with 46, XX, addl 1 (p36) had had cardiac anomalies. Fifty percent of patients with renal agenesis and 40% of patients with skeletal abnormalities had abnormal karyotype.

Sixty percent of patients with 1 soft marker, 80 percent of patients with 2 soft markers and 100 percent of patients with 3 soft markers had a normal karyotype.

Table 6: Distribution in Soft markers

		Abnormal Karyotype (n=12) n (%)	Normal Karyotype (n=30) n (%)	p valuea
Structural neural abnormalities	Yes	1 (7.1)	13 (92.9)	0.03
	No	11 (39.3)	17 (60.7)	
Structural cardiac abnormalities	Yes	6 (54.5)	5 (45.5)	0.14
	No	7 (22.6)	24 (77.4)	
Renal agenesis	Yes	1 (50)	1 (50)	0.4
	No	11 (27.5)	29 (72.5)	
Skeletal abnormalities	Yes	2 (40)	3 (60)	0.6
	No	10 (27)	27 (73)	
Soft markers on ultrasound	0	2 (16.7)	10 (83.3)	0.4
	1	9 (39.1)	14 (60.9)	
	2	1 (20)	4 (80)	
	3	0	2 (100)	

a - Chi Square test

Table 7: Neurologic anomalies found

	Normal Karyotype n=30	46XX15p+ or 46XY15ps+ n=2	45, X, q9(h+) n=1	Down syndrome n=7	46XX addl 1 (p36) n=1	46XX ins (3p; 3q) (p24.3; pterq25) n=1	Cell not grown n=5
Hydrocephalus (n=8)	7	1	0	0	0	0	0
Ventriculomegaly (n=5)	3	1	0	0	0	0	1
Anencepahly (n=2)	1	0	0	0	0	0	1
Vermin hypolasia (n=4)	3	0	0	0	0	0	1
Choroid plexus cyst (n=1)	1	0	0	0	0	0	0
Dandy walker (n=3)	2	0	0	0	0	0	1
Agenesis corpus callosum (n=2)	1	0	0	0	0	0	1
Nuchal translucency (n=1)	1	0	0	0	0	0	0
Thickened nuchal fold (n=3)	1	0	0	1	0	0	1
Arnoldchiari malformation (n=1)	1	0	0	0	0	0	0
Meningocele (n=4)	4	0	0	0	0	0	0
Spina bifida (n=2)	1	1	0	0	0	0	0

45, X, 9q (h+) with single X chromosome

There is single X indicating Turners syndrome. 9q(h+) means material of unknown origin has been added to the long arm of chromosome 16, but the exact location the added material is unknown. This anomaly was found in 23 yr old lady with G2A1. Her first trimester NT scan was normal.Triple marker was not done. Anomaly scan done at 18 wks showed a multiloculated large cystic lesion 10x 8 cm in fetal occipital cervical region suggestive of cystic hygroma. The fetus also had features suggestive of nonimmune fetal hydrops (pleural effusion, minimal ascites, subcutaneous edema). MTP was done at 19 wks and cord blood was sent for karyotyping which was suggestive of monosmy X.

46, XX, ins (3p;3q) (p24.3pter; q25)

There is movement of intrachromosomal material from 3p to 3q. The second parenthesis tells the exact chromosome band of aberration. This anomaly was noted in a G2P1D1 (1st child died) lady. Anomaly scan at 28 wks showed bilateral pleural effusions, diffuse fetal scalp edema, bilateral club foot and polyhydramnios. An emergency LSCS was

Table 8: Cardiac anomalies seen in various karyotypes

	Normal Karyotype n=30	46XX15p+ or 46XY15p+ n=2	45, X, q9(h+) n=1	Down syndrome n=7	46XX addl 1(p36) n=1	46XX ins (3p; 3q) (p24.3; pterq25) n=1	cell not grown n=5
TOF (n=3)	1	0	0	1	0	0	1
Cardiomegaly (n=2)	2	0	0	0	0	0	0
Arrhythmia (n=1)	1	0	0	0	0	0	0
Atrioventricular septal defects (n=1)	0	0	0	1	0	0	0
Transposition great vessels (n=1)	0	0	0	0	0	0	1
Complex anomaly (RAVO, RVVO, SDS, Narrowing DTA) (n=1)	0	0	0	1	0	0	0
TAPVC (n=1)	0	0	0	0	0	0	1
ASD (n=4)	2	0	0	2	0	0	0
VSD (n=8)	5	0	0	2	1	0	0
Echogenic cardiac foci (n=3)	3	0	0	0	0	0	0
Left svc abn 3 vessel view (n=1)	1	0	0	0	0	0	0
Aberrant SCA (n=1)	1	0	0	0	0	0	0

Table 9: Other anomalies

	Normal Karyotype n=30	46XX 15p+/46XY15p+ n=2	45, X, q9(h+) n=1	Down syndrome n=7	46XX addl 1(p36) n=1	46XX ins (3p; 3q) (p24.3; pterq25) n=1	cell not grown n=5
Renal agenesis (n=3)	1	0	0	1	0	0	1
Pyelectasis (n=2)	2	0	0	0	0	0	0
Cystic hygroma (n=3)	1	0	1	0	0	0	1
Diaphragmatic hernia (n=2)	2	0	0	0	0	0	0
Fetal kyphoscoliosis (n=2)	1	0	0	0	0	0	1
Rocker bottom feet (n=1)	0	0	0	0	1	0	0
Club feet (n=5)	3	0	0	0	0	1	1
Absent fibula (n=1)	0	0	0	0	0	0	1
Short femur (n=1)	0	0	0	1	0	0	0
Single umbilical artery (n=2)	2	0	0	0	0	0	0
Polyhydraminos (n=6)	5	0	0	0	0	1	0
Clenched fist (n=2)	1	0	0	0	1	0	0
Hypoplastic nasal bone (n=5)	1	0	0	4	0	0	0
Cleft palate (n=3)	2	1	0	0	0	0	0
Micrognathia (n=1)	1	0	0	0	0	0	0
Duodenal atresia (n=1)	1	0	0	0	0	0	0
TEF (n=1)	1	0	0	0	0	0	0
Echogenic bowel (n=2)	2	0	0	0	0	0	0
Fetal hydrops (n=1)	0	0	1	0	0	0	0

done for IUD at 30 wks and cord blood was sent for karyotyping which revealed insertion in the above region.

Addl material on 15 p

46, XX, 15p + Female karyotype

This 23 yr old lady with G2P1L1 had a anomaly scan at 18 wks which showed hydrocephalus with thinned brain parenchyma and multiple dorsolumbar spina bifida. She underwent induced abortion at 19 wks and cord blood sample showed additional material on short arm of chromosome 15.

46, XY, 15p + Male karyotype

This karyotype was seen in newborn baby born to 25 yr old G3P2L2 mother. Anomaly scan at 30 wks showed cleft lip and palate.

46,XX, addl 1 (p36) female karyotype

Additional chromosome material was seen on short arm of chromosome 1 origin? partial Trisomy 13 and was advised to confirm by molecular tests FISH. This new born baby was born to 30 yr old G4P3L3 mother. Anomaly scan showed VSD and two soft markers (rocker bottom feet and clenched fist).

Downs syndrome

There were 5 cases of Trisomy 21. Two cases had Robertsonian translocation (The extra chromosome 21 is translocated to chromosome 22 in one and other chromosome 21 in one). The age ranged from 19 to 29 yrs. Only 2 cases had age more than 25 yrs. Five cases had free trisomy 21. Five of the seven cases had structural cardiac anomalies. Five had live babies at term. One had induced abortion at 19 wks and the other had IUD at 24 wks.

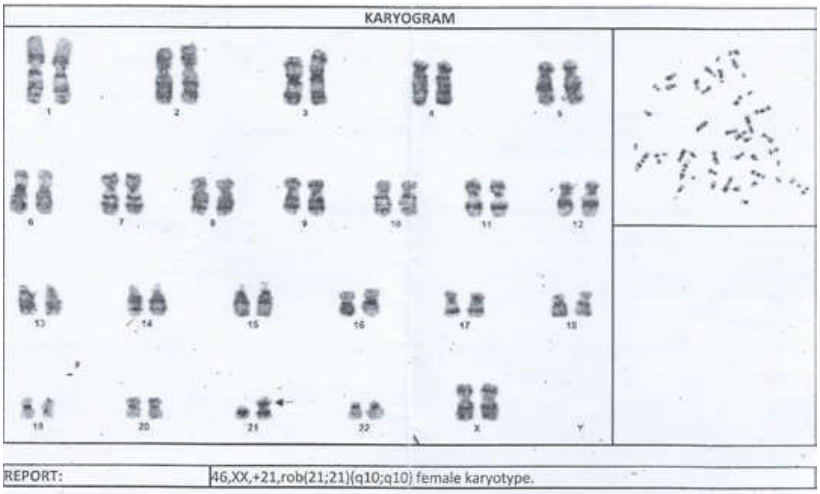

Fig. 2: Robertsonian translocation 46, XX, +21, rob (21; 21) q10; q10) indicating Downs syndrome

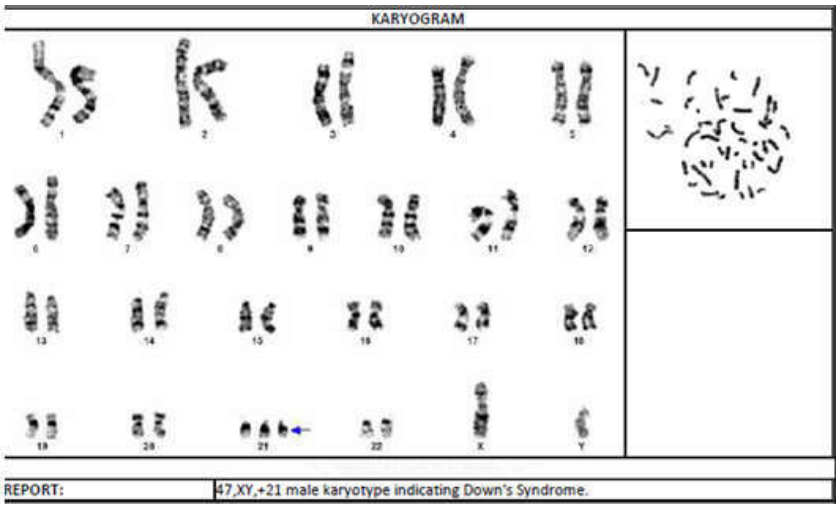

Fig. 3: Trisomy 21

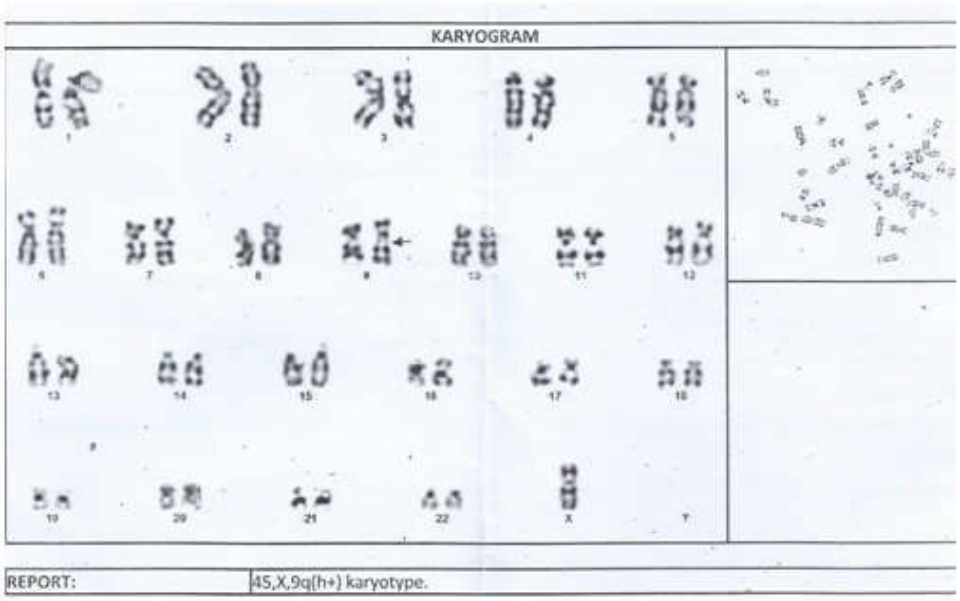

Fig. 4: Monosomy X. Turners syndrome

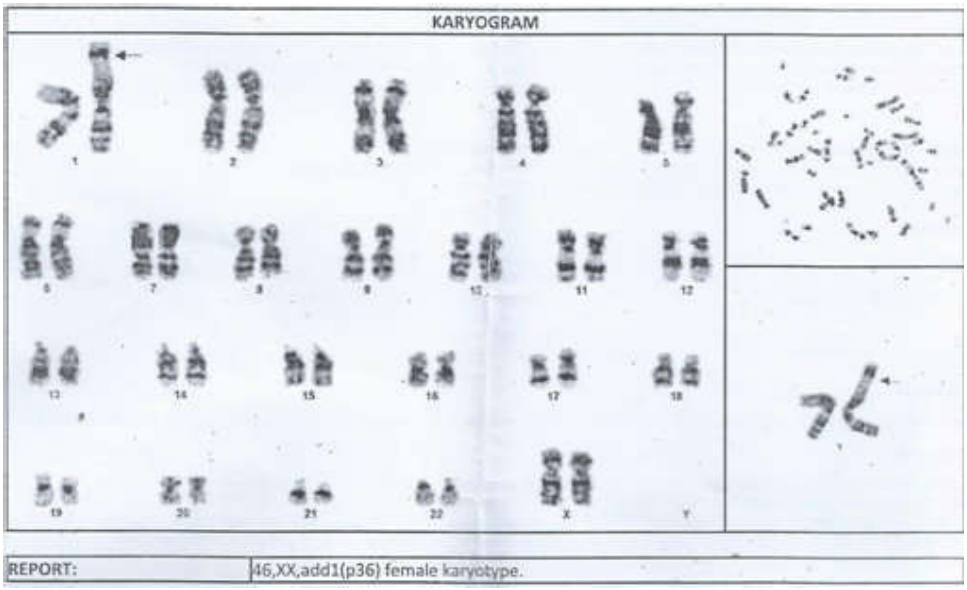

Fig. 5: Additional chromosomal material in 1p

Genetic Counselor's note: Additional chromosomal material is seen consistently on short arm of chromosome 1, the origin of which has to be confirmed by molecular tests.

Fist with specific probes for the region in chromosome 13 is recommended to rule out partial trisomy 13.

Discussion

Abnormal karyotype

In our study abnormal karyotype was found in 28.5% (12/42) cases. Our study population included prenatally detected congenital abnormalities on ultrasound.The mean age of our population is 24. The incidence of abnormal karyotype in anomalous foetuses detected by ultrasound in table 10.

In our series one patient had IUD at 24 wks and one had stillbirth at 30 wks GA. In both cord blood was sent immediately after birth. An abnormal karyotype was found in both.

Karyotype abnormalities varies from 7% to 50% depending on sample population. In a population which included 42 anomalous foetuses (15 samples between 13 to 20 wks, 3 between 21 to 27 wks and 24 samples between 28 wks till birth) we found abnormal karyotype in 28.5% samples. Most of the patients had multiple abnormalities on ultrasound.

Karyotype sample

In our study 10.7% (5/47) of samples failed to show culture due to contamination or improper sampling. 38 of 39 cord blood samples and 3 of 3 venous blood samples yielded culture. However when tissue samples were collected (3 chorionc villi and 1 umbilical cord), none of them showed the culture. Interestingly from 1 IUD and 1 stillborn we could collect cord blood immediately after birth and both of them showed the culture.

In our series 41 of 42 samples which were successfully cultured were obtained after abortion or delivery. Immediately after delivery cord blood was aspirated and sent for culture. In cases where cord blood sample was not possible, blood was aspirated from heart. If it was not possible tissue sample was sent. None of the tissue samples showed positive culture. Conventional G-band cytogenetic studies can be performed on the stillborn's blood or tissue as long as the cells are viable.

Table 10: Karyotype abnormalities in abnormal foetuses detected by ultrasound

Author	Study population	Abnormal karyotype
Karyotype abnormalities in abnormal foetuses detected by ultrasound		
Staebler 2005 [3]	428 fetal malformation on ultrasound. Mean maternal age 28.5 years (16–36)	11.2% (48/428) - abnormal karyotype 9.3% with isolated malformation and 18.8% with multiple malformations had abnormal karyotype
Nicolaides 1992 [4]	2086 Fetuses with malformations on ultrasound or IUGR or both.	14% (301/2086) had chromosomal abnormality 72.7% (222/305) had maternal age <35 yrs
ZalenSprock et al. 1991 [5]	N=210 ultrasound detected fetal malformations. GA 11 to 38 wks,	14.7% (41/288) had chromosomal abnormality 25/149 17% with single abnormality and 26% with multiple abnormalities had abnormal karyotype
Halliday et al. 1994 [6]	n=306 before 20 wks and n=241 after 20 wks ultrasound abnormalities. Maternal age <35 yrs	with isolated malformation<20 wks 18%, >20 wks 31% abnormal karyotype
Shimada S 2009 [7]	n=417 fetuses with anomaly or IUGR or amniotic fluid volume	Abnormal karyotype 17.7%
Gagnon et al. 1992 [8]	N=117 fetuses with congenital anomaly or IUGR or amniotic	19/117 (16.2%)
Present study	47 cases of prenatlly detected congenital malformations. 42 had successful karyotype	Abnormal karyotype 28.5% (12/42) 2nd trimester 23.5% (4/17) 3rd trimester 32% (8/25)
Karyotype abnormalities in various study populations		
Sheth et al. 2015 [9]	n=1728 (1,324 amniotic fluids, 366 chorionic villi and 38 cord blood samples). Mean maternal age 31.6 years (19 to 53 years)	7.2% (125/1,728)
Yashwanth et al. 2010 [1]	176 cases of congenitally malformed children (1 day to 14 yrs).	17% had abnormal karyotype 32 (37.6%) with multiple system malformations
Pinar et al. 2009 [10]	Still births of more than 20 wks GA	25.8% (60/232) had abnormal karyotype
Korteweg et al. 2008 [11]	n= 508 fetal deaths median maternal age 31 years (range 17–46 years)	13% (32/246)
Pylyp 2018 [12]	1000 products of conception in first trimester miscarriages after spontaneous conception and IVF chorionic villi was assessed in 944 cases mesodermal cells—in 66. Mean maternal age 33.7 ± 5.6 years	50.1% (501/1000) had chromosomal abnormalities
Success of karyotype samples in various series		
Gagnonetal 1992 [8]	N=117 fetuses with congenital anomaly or IUGR or amniotic fluid	karyotype failed in 12.2% cases
Pinar etal 2009 [10]	Still births of morethan 20 wks GA	Karyotyping attempted in 342 cases, failed in 66 (16)%
Kortewegetal 2008 [11]	n= 508 fetal deaths	n= 508 fetal deaths, karyotyping successful in 246 (48.4%) Success - 85% - invasive (amnio, CVS), 15%, Post partum tissue analysis, 35% success in severely macerated fetus

Maternal age

The mean maternal age in our series was 24 ± 3.1 (18 to 32 yrs). None of our patients had advanced maternal age >35 yrs. In a series by Nicolaides 1992 [4] 72.7% (222/305) of abnormal karyotype had maternal age <35 yrs. Risk of fetal trisomy increases with maternal age. This is particularly well documented for trisomy 21 but has also been demonstrated for trisomies 13 and 18. Staebler et al. [3], Halliday et al. [6] and Sheth et al. [9] have shown significant chromosomal anomalies in mothers aged less than 35 years. Our series is also consistent with the literature.

Consanguinity

Consanguinity (n=5) had no relation to abnormal karyotype in our study. Comparison between genetic diseases with different modes of inheritance showed that recessive disorders, multifactorial disorders, autosomal dominant had higher correlation with consanguinity and chromosomal disorders had the lowest one.

Miscarriage

In couples with two or more spontaneous miscarriages, the frequency of chromosome anomalies in one parent is 4% to 5% [4]. Balanced chromosome rearrangements can lead to the production of gametes with unbalanced karyotypes. In our series 42% (5/12) of cases with abnormal karyotype had history of abortion.

Types of chromosomal abnormalities

In our series the commonest anomaly was Trisomy 21 (5/12, 41% of patients). In published literature Trsimoy 21 or Trisomy 18 is the commonest anomaly. There was no case of Trisomy 18 or 13 in our series. There was one case of monosomy X in our study. This may be attributed to the small sample size of our population. There were no cases of Triploidy or tetraploidy as we didn't have any cases in first trimester. Also we didn't include molar pregnancy in our study.

In structural abnormalities we found Translocation (2), insertion (1), additional material on 15p(2) and additional material on 1p(1). The rarer anomalies have to be further confirmed by FISH or PCR.

45, X, 9q(h+) with single X chromosome

This indicates that fetus has monosmy X indicating Turners syndrome.There is increase in length of the heterochromatin on the long arm of chromosome 9, indicating that material of unknown origin has been added to the long arm of chromosome 9, but the exact location the added material is unknown. This 19 weeks fetus had large cystic hygroma in occipitocervical region and nonimmune fetal hydrops. In a large European registry of Turners syndrome the most frequent anomalies were cystic hygroma (59.5%) and hydrops fetalis (19%). The most frequent karyotype was 45, X (81.6%) followed by different types of mosaicism (16.8%). Our case also had the same anomalies. Ninety-nine percent of 45, X conceptuses result in spontaneous loss, usually by 28 weeks. Cystic hygroma with nonimmune fetal hydrops during pregnancy is the most severe form. Although 45, X is quite lethal in the fetus, those that survive to term have relatively minor problems. The reasons for this are that all

Table 11: Types of chromosomal anomalies in various series

Author	abnormal karyotype (%/ No of cases	Trisomy 21	Trisomy 18	Trisomy 13	Translocation	Mono somy X	Tripolidy	Others
Sheth et al. [9] 2015	125(7.2%)/ 1728	46/125 (36.8%)	11/125 (8.8%)	2/125 (1.6%)	20/125 [16%]	7/125 (5.6%)		Supernumary marker chromosomes 6/125 (4.8%)
Nicolaides [4] 1992	14%(301)/ 2086	69/301 (22.9%)	83/301 (27.5%)	31/301 (10.2%)			42/301 (13.9%)	
ZalenSprock et al. [5] 1991	41 (14.7%)/288.	11//41 (27%)	13/41 (32%)	3/41 (7%)	5/41 (12%)	4/41 (10%)	5/41(12%)	
Shimada S [7] 2009	74(17.7%)/417	24/74 (32.4%)	21/74 (28.3%)	3/74 (4%)		8/74 (10.8%)		Deletion/ duplication 5(6.7%) Others 6/74(8.1%)
Present study	n=42	5/12 (41.6%)			2/12 (16.7%)	1/42 (2.3%)		Addlmaterial on 15p-2 cases,insertion 3p;3q -1 Partial trisomy 13 -1

conceptions that survive have some degree of undetected mosaicism for a normal cell line [13].

Fetal hydrops Nonimmune fetal hydrops was also found to be associated with structural abnormalities in 83.3% cases, and chromosomal abnormalities in 47.3%.

Recurrence risk for Turners syndrome

In general, Turner syndrome is considered to be a sporadic condition. Recurrence in subsequent pregnancies is rare, but has occurred. It is assumed that the likelihood of recurrence is similar to that in the general population. There is no increased risk for other types of genetic abnormalities. People who have had a previous fetus or infant with Turner syndrome may consider chromosome analysis in a subsequent pregnancy, for reassurance.

Downs syndrome

There were 5 cases of Trisomy 21. Two cases had Robertsonian translocation (The extra chromosome 21 is translocated to chromosome 22 in one and other chromosome 21 in one). In our series more patients had translocation than in published literature.

Maternal age

The age ranged from 19 to 29 yrs. Only 2 cases had age more than 25 yrs. Jyothi et al. [14] in a series of 41 translocations showed that most of the translocations were born to younger mother's (< 25 years), when compared to pure trisomy. The age of our two patients with translocation were 19 and 26. This may explain the high incidence of translocation in our series as our population is young. Trisomy 21 is a genetic condition caused when chromosomes fail to separate during meiosis. The maternally derived trisomy 21 cases reported are with high mean maternal age for MI and MII errors (MMI 29.5 ± 6.8; & MMII32.0 ± 7.3, (MMI 31.06 ± 6.69 and MMII 33.84 ± 4.93**.** In an Indian study [15] a lower mean maternal age (MMI24.96 ± 4.17 and MMII 27.78 ± 3.00) was found which raised a significant concern regarding the origin of meiotic genetic error in the absence of history of older maternal age. In this study they observed that among the total number of MMI errors, 101 out of105 belonged to parents below the age of 33 years [15].

Prolonged exposure to occupational hazards of farming (pesticides), low nutritional quality along with stress are also found to be risk factors for Downs syndrome [15]. Lifestyle, environment, and occupation supersede the maternal age factor as a risk for developing chromosomal aberration in offspring. Maybe in our study these factors may have played a role in causation of Downs syndrome. It requires further studies to prove the results.

Translocation vs Trisomy

Bornstein et al. [16] compared Transloaction downs syndrome with Trisomy 21. The incidence of anomalies including structural cardiac defects were similar in both the groups. History of a prior fetus or child with downs syndrome was significantly more common in translocation group. In our series 3 of 5 fetuses with Trisomy 21 and 1 of 2 fetuses with translocation had major structural heart diseases. In total Five of the seven (71%) cases had major structural cardiac anomalies in our series.

Screening test

In our study NT scan was done in 25 patients (25/47, 53.1%) of cases. In all it was normal. However Triple marker was done in only 3 cases. Majority of patients didn't undergo screening tests. In accordance with the American College of Obstetricians and Gynaecologists (ACOG) all women should be offered aneuploidy screening before 20 weeks of gestation. ACOG says that all women should have the option of invasive testing instead of screening, regardless of maternal age [17].

An early second-trimester test based on maternal serum measurements: AFP, uE3, free beta hCG or intact/total hCG, and inhibin A together with maternal age. The quadruple test is the best serum screening test for women who present for prenatal care in the second trimester. Currently, second trimester maternal quad screening using maternal serum.

AFP, hCG, UE3, and inhibin-A yields an 80% detection rate for Down syndrome and 60% for trisomy 18 at a 5% false positive rate [15].

First Trimester

Nuchal translucency: The maximum width (in mm) of the translucent space at the back of the fetal neck as determined by ultrasound between 11 and 13 completed weeks of gestation.

Combined test

A late first-trimester test based on sonographic and maternal serum measurements: NT, free beta hCG or intact/total hCG, and PAPP-A together with maternal age.

The first trimester combined test is the best serum screening option for women whose most important goal is to obtain their estimate of risk of Down syndrome early in pregnancy. An additional measurement of alpha-fetoprotein or ultrasound screening is recommended in the second trimester for detection of open neural tube defects. Similarly, first trimester screening for hCG, plasma protein A, and fetal nuchal translucency yields an 85% detection rate for Down syndrome and 90% for trisomy 18 at a 5% false positive rate.

First and second trimester

Integrated test (full)

The integration of measurements performed during the first- and second-trimester into a single screening test result. Typically includes first-trimester NT and PAPP-A with the Quadruple test in the second trimester together with maternal age. Usually the risk is only provided once all tests have been completed in the second trimester. The full integrated test has the highest detection rate for Down syndrome, the lowest rate of procedure-related losses per woman screened, and includes screening for open neural tube defects. A recent Cochrane review [18] evaluated first and second trimester Down's syndrome serum screening tests, with or without first trimester NT. The combined test comprised of first trimester NT and PAPP-A, and second trimester total hCG, uE3, AFP and Inhibin A, and maternal age estimated a sensitivity of 95% (CI 90 to 97) at a cut-point of 5% false positive rate.

Candidates for diagnostic testing [17]

A diagnostic test is a reasonable choice for women of any age at high risk of Down syndrome or other fetal aneuploidies, such as women with.

- A positive screening test
- A previous pregnancy complicated by fetal trisomy
- At least one major or two minor fetal structural anomalies in the current pregnancy
- Chromosomal translocation, inversion, or aneuploidy in the pregnant woman or her partner
- The desire to have the most reliable information possible about their pregnancy

Neurologic anomalies

In patients with structural neural abnormalities only 1 (1/12, 7.1%) had abnormal karyotype compared to 13 (13/14, 92.9%) who had normal karyotype. (p=0.03). In our study one patient with hydrocephalus and spinabifida had 46, XX, 15p+ karyotype.

Table 12: Neurologic abnormalities in various series

Author	Neurologic anomalies
Zalen Sprock et al. [5] 1991	1/42 (2%) hadencephalocele - 47, XY, +21
Halliday et al. [6] 1994	NT defects in less than 20 wks 1/27 had abnormal karyotype NT defects in >20 wks 0/6 abnormal karyotype hydrocephalus in <20 wks 4/13 had abnormal karyotype hydrocephalus in >20 wks 3/17 had abnormal karyotype Abnormal karyotypes seen Trisomy 21 in 3, Triosmy18 in 2, t (14;21) un bal, t (14;19) un bal
Shimada S [7] 2009	11/61 (18%) Abnormal karyotype abnormalities of CNS
Gagnon et al. [8] 1992	hydrocephalus without neural tube defect (1/3: one trisomy 13)

Chromosome abnormalities are associated with spina bifida and encephalocele, but do not appear to any significantdegree to be associated with isolated anencephaly [9].

Structural Heart Abnormalities

In our patients 11 patients had structural cardiac abnormalities of which 6 (54.5%) had an abnormal karyotype. Among patients with no structural cardiac anomalies, 77% had a normal karyotype (p=0.14).

In a study by Paladni et al. Fifteen of these 31 fetuses (48%) were found to have an abnormal karyotype. Of these five of 17 (29.4%) with isolated cardiac anomalies and ten of 14 (71.4%) with cardiac and extracardiac anomalies. Detected chromosomal abnormalities included six trisomy 21, four trisomy 18, four trisomy 13, and one triploidy 69, XXX. Atrioventricular septal defects and ventricular septal defects were the cardiac malformations most often associated with abnormal karyotypes (77 and 71%, respectively). The frequency of chromosomal abnormalities in infants with congenital heart disease has been estimated from postnatal clinic data to be 5% to 10% [16]. In our series 3 of 8 (37%) with VSD, 1 of 2 (50%) with TOF, 1 of 1 (100%) with atrioventricular septal defects, 2 of 4 (50%) with ASD had abnormal karyotype. VSD was most common abnormality observed in cases with abnormal karyotype.

Advantages of conventional karyotype over CMA [18]

1. G-banding has been available for more than

38 years and has the advantage of being a widely accepted and uniform technique with an international system of cytogenetic nomenclature (ISCN). By contrast, CMA is new and more diverse in terms of techniques used, coverage, and approach to data interpretation. Unknown anomalies can increase the patient anxiety and lead to problems in prenatal counselling.

2. In the setting of multiple miscarriages, a balanced translocation in one of the parents could be the explanation for unbalanced offspring, and G-banded karyotyping should still be the standard of care for this indication [60]. It is clear that the balanced chromosomal rearrangements, such as balanced translocations and inversions, are not identified with CGH. CGH can provide important information in cases of balanced translocations. However, karyotyping is key to identifying chromosomal structure and microscopic location in such cases, and the use of CGH is justified after karyotyping. Mosaicism is diagnosed more frequently by karyotype than by CGH depending on the percentage of cells.
3. In general, traditional cytogenetic methods are still needed for single-cell analysis.
4. Other circumstances in which traditional cytogenetic methods are indicated instead of (or at least before) CMA include when the patient has a recognizable chromosomal syndrome such as trisomy 21, trisomy 13, Turner syndrome, or Klinefelter syndrome. For these circumstances, conventional cytogenetic analysis or interphase FISH analysis might provide a more rapid turn-around time, allow more sensitive detection of low-level mosaicism, and provide information regarding position to distinguish free trisomy from translocation associated trisomy.

Conclusion

Abnormal karyotype was found in significant proportion of foetuses with anomalous finding on prenatal ultrasound. Downs syndrome was the most common chromosomal abnormality found. Most of the patients with soft marker on ultrasound had a normal karyotype.

Screening test should be done all pregnant patients irrespective of age and risk factors to identify the abnormal karyotype early in pregnancy which is found insignificant proportion of foetuses with anomalous finding on prenatal ultrasound.

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