



Original Article

Analysis of Fetal Chromosome Abnormalities by Karyotype

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Abstract: Prenatal screening is a crucial method employed during the fetal stage to detect and intervene early, thereby ensuring the health of newborn babies. This approach serves to enhance the overall quality of the population and mitigate the severe consequences associated with birth defects. Prenatal screening encompasses various techniques, including epidemiological investigations, fetal imaging ultrasound, maternal blood screening, and amniotic fluid screening by karyotype technique. The process involves extracting fetal chromosomes from cells present in the amniotic fluid, followed by cell culture and hypotonic treatment. Subsequently, these chromosomes are stained with G-band and examined under a microscope. The results obtained from prenatal screening in a sample of 121 patients, with an average age of 32 ± 6.69 , indicated that 21% of pregnant women had experienced a miscarriage, 23.1% had previously given birth to a malformed baby, 8.3% of families had a genetic disease, and 5% of parents exhibited chromosomal abnormalities. Among the 51 patients screened using the Double test, 26.45% of fetuses exhibited a high risk of birth defects, whereas the Tripple test identified a high risk in 9.09% of the 19 cases. Chromosome analysis of the 121 cases revealed that 15.7% of the fetuses exhibited chromosomal abnormalities, with Edwards syndrome accounting for 5.78%, Down syndrome accounting for 4.96%, chromosomal abnormalities accounting for 3.30%, Patau syndrome accounting for 0.83%, and Turner syndrome accounting for 0.83%. Age over 35 years ($r = 0.08$ and $OR = 0.63$), history of miscarriage ($r = 0.05$ and $OR = 1.38$), family history of hereditary disease ($r = 0.04$ and $OR = 1.38$), and parental chromosomal mutations ($r = 0.01$ and $OR = 1.08$) were all found to have a strong positive correlation with fetal abnormalities. Additionally, positive correlations were observed between the results of ultrasound screening ($r = 0.22$ and $OR = 5.48$) and blood screening ($r = 0.14$ and $OR = 1.22$).

Keywords: Prenatal screening, Amniocentesis, Karyotype, Chromosome, Fetal chromosome.

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1. Introduction

Prenatal diagnosis plays a vital role in the early detection of fetal birth defects. Across the globe, robust screening programs have been established to enable the early identification of genetic abnormalities, including chromosomal or genetic variations. Fetal chromosomal abnormalities occur in approximately 1 in 150 live births and represent a significant contributor to neonatal morbidity and mortality worldwide [1]. Chromosomal abnormalities can be inherited or acquired. These abnormalities can also arise during gametogenesis or early embryonic development [2]. The majority, approximately 83.0%, of these abnormalities can be attributed to trisomy 21, 18, 13, and sex aneuploidy, leading to conditions such as Down syndrome, Edwards syndrome, Patau syndrome, and Turner syndrome [3]. In Vietnam, a study conducted by the National Hospital of Obstetrics and Gynecology estimated that more than two children out of every 100 born have congenital abnormalities, resulting in a birth defect rate of 1-2% [4]. Additionally, a study conducted in the European community in 2004 revealed that approximately a quarter of premature infant deaths were caused by birth defects, with 18.0% of these cases arising from chromosomal abnormalities [5].

Chromosomal abnormalities are a significant concern in fetal malformation screening due to their relatively high frequency in the community, which imposes psychological and financial burdens on families and society as a whole. Constitutional chromosomal abnormalities are an important cause of miscarriage, infertility, congenital anomalies and mental retardation in humans [2, 6].

Chromosomal structural abnormalities can occur during the fusion of two chromosomes and break at the centromere, p, or q arm. However, in some cases, unequal segregation of chromosomes resulted in trisomy or haploid. This type of mutation can leave consequences such as Down syndrome, and Turner syndrome. The frequency of this anomaly may be 1/1000 of the population [7, 8].

The advancement of biomedical techniques has facilitated the early detection of fetal abnormalities, thereby providing a basis for early intervention solutions. Large hospitals often employ early screening methods that involve investigating historical factors, ultrasound imaging, and blood tests such as the Double and Triple tests. However, the gold standard for testing chromosomal abnormalities remains the karyotype technique [9].

At Nghe An Obstetrics and Pediatrics Hospital, karyotype analysis is a traditional genetic technique that has been studied and applied to prenatal diagnosis. However, in order to evaluate and analyze various factors associated with chromosomal abnormalities, we have undertaken research with the following objectives: to determine the rate of fetal chromosomal abnormalities using the karyotype technique and to analyze the factors related to these abnormalities in patients who undergo amniocentesis at Nghe An Obstetrics and Pediatrics's Hospital.

2. Materials and Methods

2.1. Materials

Women who visited Nghe An Obstetrics and Pediatrics Hospital between August 2020 and May 2021 opted for amniocentesis as a prenatal diagnostic procedure using the karyotype technique.

2.2. Methods

2.2.1. Sampling Method

The study was designed using a descriptive cross-sectional method.

The sample size was calculated according to the formula: $n = Z^2 \cdot \frac{p \cdot q}{(p \cdot \varepsilon)^2} (*)$

Where:

n: the sample size; α = statistical significance level (choose $\alpha = 0.05$); Z: confidence coefficient.

$Z(1-\alpha/2)$: confidence coefficient, if the confidence coefficient is 95% then $Z(1-\alpha/2) = 1.96$; e: relative value, (0.3); p: the rate of chromosomal abnormalities is 28%.

Based on the research conducted by Hoang T. Ngoc Lan in 2016 [10], which reported a chromosomal abnormality rate of 28% with $p = 0.28$, $q = 1 - p$, $q = 0.72$, so $n = 110$. Therefore, the sample size in this study is 121, satisfying the minimum sample size.

2.2.2. Surveying Method

Epidemiological investigation of interviewed patients based on the questionnaire.

2.2.3. Karyotype Method

The chromosome mapping procedure in this study followed the methodologies described in the works of Marilyn et al., in 2017 [11] and Nguyen Viet Nhan in 2010 [5].

Collect 15 ml of amniotic fluid from patients as prescribed by the doctor. Culture the amniotic fluid in BIOAMF-2 Complete Medium from Biological Industries (BI Company) for 7 days. Halt the decomposition process by adding Colcemid (180 μ l) and incubate the mixture at 37 °C with 5% CO₂ for 3 hours. Treat the cells with a hypotonic solution consisting of 4 ml KCL, 3 ml water, and 1 ml serum. Incubate the cells for 30 minutes in a 37 °C thermostatic bath.

Fix the cells using Carnoy's solution, prepared in a ratio of 3 parts methanol to 1 part acetic acid. Refrigerate the fixed cells at 2-4 °C. Prepare slides by spraying the cells onto a cooled slide within a 4 °C cabinet, followed by heating over the flame of an alcohol lamp. Stain the G-band of the chromosomes using Giemsa dye. Examine the stained chromosomes under a microscope (Niko company, Japan) at a magnification of 1000x. Analyze the results using the Lucia software.

2.2.4. Data Analysis and Data Processing Method

The collected data were processed by a medical statistical method on the computer with SPSS 20.0 software.

3. Results and Discussions

3.1. Occupational Characteristics, Age, and Number of Births of Pregnant Women

For the patients to be examined at Nghe An Obstetrics and Gynecology Hospital after screening by epidemiology and subclinical, 121 pregnant women were appointed by the doctor to have amniocentesis for chromosomal analysis. These patients have the following characteristics:

Table 1. Age and occupation characteristics of pregnant women

Characteristic	Age						Number of previous pregnancies					
	≤ 19	20-29	30-39	≥ 40	Mean	Min-Max	0	1	2	3	4	5
Quantity	5	43	53	20	32.00 ± 6.69	16-46	18	32	47	18	5	1
Percentage	4.1	35.5	43.8	16.5			14.9	26.4	38.8	14.9	4.1	0.8
Occupation												
	Government employee		Farmer		Factory worker		Self-Employed					
Quantity	23		43		24		21					
Percentage	19.0		35.5		19.8		25.7					

Pregnant women who underwent amniocentesis for chromosomal analysis had an average age of 32 ± 6.69 years. The highest age

group was between 25-29 years old, accounting for 28.1% of the participants. Pregnant women working in agriculture represented the largest

percentage at 35.5%. The majority of pregnant women were experiencing their second pregnancy, accounting for 38.8%. The findings of this study indicate that the average maternal age is slightly younger compared to the results reported by Tran Danh Cuong et al. (2012-2016) [12, 13] and Sung-Hee Han (2008) [14]. However, the average maternal age aligns with the study conducted by Chen-Ju Lin (2014), which reported a mean maternal age of 32.1 [15].

It is higher than the mean maternal age reported by Nguyen Thi Hoang Trang (2011), which was 29.02 ± 5.6 years old [12].

3.2 Screening the Fetus by Ultrasound and Chromosomal Imaging

The results of fetal screening by ultrasound results are shown in the following Table 2.

Table 2. Results of patient screening by ultrasound imaging and chromosomal mapping

Ultrasound imaging		Quantity	Percentage	Ultrasound gestational age		
Number of ultrasound images n=121; Percentage: 62.81%	Increase in nuchal translucency	16	12.60	n=121	Quantity	Percentage
	Nuchal fold	4	3.15	16-20	77	63.64
	Short nasal bone	5	3.94	>20	44	36.36
	Nasal bone hypoplasia	7	5.51	Gestational age	20.37 ± 4.42	
	Disordered umbilical cord coiling	5	3.94	Min-Max	16-37	
	Dilated ventricles	3	2.36	Chromosomal mapping analysis results		
	Cardiac anomaly	1	0.79	Mutant variant	Quantity	Percentage
	Dilated renal pelvis	3	2.36	Down syndrome	6	4.96
	Peritoneal or pericardial effusion	3	2.36	Structural abnormality	4	3.30
	Clubfoot	3	2.36	Patau	1	0.83
	Short femur bone	3	2.36	Turner	1	0.83
	Other abnormalities	29	22.83	Edwards	7	5.78
	Low risk	19	15.70	Total	19	15.70
	Healthy images		45	37.19	Healthy	102

Table 2 presents the results of fetal screening based on ultrasound images, indicating that out of the 121 fetuses examined, 76 (62.81%) displayed abnormal signs. In which 12.6% increased nuchal translucency, 5.51% had nasal bone aplasia, 3.94% shortened nasal bone and vascular plexus cyst, 2.36% had dilated renal pelvis, peritoneal effusion, pericardium, clubfoot, and short femur, and 22.83% had other

abnormalities. According to research by Devore G. R. in 2001, the ultrasound detection rate of chromosomal abnormalities is reported to be 81% [9].

Amniocentesis is a procedure performed to extract fetal cells from the amniotic fluid for chromosomal analysis, enabling the detection of genetic abnormalities at the chromosomal level. The timing of amniocentesis is crucial, as

performing it too early (before 16 weeks) carries a risk of pregnancy loss, while performing it too late (> 20 weeks) can complicate the procedure and subsequent termination of pregnancy. In this study, 77 cases (63.64%) underwent amniocentesis at the ideal gestational age. Out of the 56 patients with abnormal ultrasound images who underwent chromosomal analysis, abnormalities were detected in 12 fetuses (9.92%). This finding aligns with a study by Tran Danh Cuong in 2005, which reported a similar rate of 9.92% [13]. Another study conducted by Nguyen Thi Hoang Trang in 2011, involving 2686 cases at the National Hospital of Obstetrics and Gynecology, found that 53.7% of amniocentesis procedures were performed at the ideal gestational age [12].

In the screening of 121 patients, chromosomal mutations were found in 19 cases (15.70%): 7 cases of Edwards syndrome (5.78%), 6 cases of Down syndrome (4.96%), 4 cases of abnormal chromosome structure (3.30%), 1 case of Patau syndrome (0.83%), and 1 case of Turner syndrome (0.83%), and no cases of abnormal triploidy were detected. These findings are consistent with a study by Tran Danh Cuong in 2005, where Edwards syndrome accounted for a higher rate (30%) compared to Down syndrome (28.8%) [13]. However, there are differences compared to the study conducted by Nguyen Thi Hoang Trang, which reported Down syndrome as the most prevalent (47.5% or

116 out of 244 cases) [12]. Another study by N Yaegashi found that Down syndrome had the highest percentage (35.9% or 42 out of 117 cases), with Edwards syndrome accounting for 11.1% [16].

3.3. The Relationship Between Fetal Chromosomal Abnormalities and Some Other Factors

Table 3 displays the results of screening using the Double test and Triple test for pregnant women. Out of the 50 pregnant women screened by the Double test, 32 fetuses were identified as high risk for abnormalities (26.45%), while 19 fetuses were classified as low risk (15.70%). Among the 15 patients screened by the Triple test, 11 fetuses were determined to be at high risk for abnormalities (9.09%), and 4 fetuses were categorized as low risk (3.31%).

Among the 31 patients who underwent the Double test and were classified as high-risk, 6 fetuses were found to have chromosomal abnormalities (4.96%). Among the 11 patients who underwent the Triple test and were classified as high risk, 1 fetus was detected to have chromosomal abnormalities (0.83%). These findings indicate a strong positive correlation ($r = 0.45$) between the blood screening results and the chromosomal analysis, with the odds ratio (OR) within the 95% confidence interval (CI) is 1.22 (0.43 – 3.48).

Table 3. Relationship between blood screening results of pregnant women and chromosomal abnormalities

Blood screening test				Abnormal Chromosome		Normal Chromosome		r	OR (95% CI)
Patients		n	Percentage	n	Percentage	n	Percentage		
Double test (n=50)	High risk	31	26.45	6	4.96	25	20.66	0.45	1.22 (0.43 - 3.48)
	Low risk	19	15.70	0	0.00	19	15.70		
Tripple test (n= 15)	High risk	11	9.09	1	0.83	10	8.26		
	Low risk	4	3.31	0	0.00	4	3.31		
Not screened		56	45.45	12	9.92	44	36.36		

Table 4 presents the percentage of abnormal fetuses in different subgroups, particularly highlighting the subgroup of pregnant women over 35 years old, where the rate of abnormal

fetuses is 10.74%. The relationship between maternal age and fetal abnormalities exhibits a weak positive correlation, with $r = 0.08$, and an odds ratio (OR) of 0.63 within the 95%

confidence interval (CI) of 0.22 to 1.8. Furthermore, among the total cases, 17 fetuses (14.05%) were found to exhibit abnormalities both on ultrasound images and in the chromosomal analysis. These two factors display a relatively strong positive association, with an r-value of 0.22 and an OR of 5.48 within the 95% CI of 1.20 to 25.03. Comparing these

findings with the study conducted by Nguyen Hoang Trang in 2011, which involved 2,686 cases, it was reported that among 2,080 cases with normal ultrasound images, 31 cases (1.6%) had chromosomal abnormalities. Additionally, among 806 cases with abnormal ultrasound images, 213 cases (26.4%) had abnormal chromosomes [13].

Table 4. Relationship between fetal chromosomal abnormalities and maternal age and ultrasound images.

Patient characteristic	Age				Abnormal fetal ultrasound appearance			
	< 35		≥ 35		No		Yes	
	n	%	n	%	n	%	n	%
Normal	59	48.76	43	35.53	40	33.06	62	51.24
Abnormal	13	10.74	6	4.96	2	1.65	17	14.05
Total	72	59.51	49	40.49	42	34.71	79	65.29
r	0.08				0.22			
OR (95% CI)	0.63 (0.22 – 1.80)				5.48 (1.20 – 25.03)			

Table 5. The relationship between fetal chromosomal abnormalities and prenatal factors

Patient characteristics		History of miscarriage		History of pregnancy or childbirth with birth defects		Family history of genetic diseases		Parent(s) with abnormalities in chromosomal structure	
		No	Yes	No	Yes	No	Yes	No	Yes
Normal	Quantity	81	21	79	23	94	8	97	5
	Percentage	66.94	17.36	65.29	19.01	77.69	6.61	80.17	4.13
Abnormal	Quantity	14	5	14	5	17	2	18	1
	Percentage	11.57	4.13	11.57	4.13	14.05	1.65	14.87	0.83
Total	Quantity	95	26	93	28	111	10	115	6
	Percentage	78.51	21.49	76.90	23.10	91.74	8.26	95.00	4.96
r		0.05		0.03		0.04		0.01	
OR (95% CI)		1.38 (0.45 – 4.26)		1.23 (0.40 – 3.77)		1.38 (0.27 – 7.08)		1.08 (0.12 – 9.78)	

The analysis of pregnant women's history with indications for amniocentesis reveals the following proportions: 21.5% of pregnant women have a history of miscarriage, 23.1% have a history of birth defects, 23.1% have a family history of chromosomal abnormalities, 8.26% come from families with malformations, and 4.96% have a parent carrying a chromosomal mutation.

When examining the relationship between these historical factors and chromosomal abnormalities in the fetus, pregnant women with a history of miscarriage account for 4.13% of cases with chromosomal abnormalities. There is a weak positive relationship between these factors, with an r-value of 0.05 and an odds ratio (OR) of 1.38 within the 95% confidence interval (CI) of 0.45 to 4.26. Among women who have

previously given birth to a malformed child (4.13% of cases), there is a weak positive correlation with chromosomal abnormalities, with an r value of 0.03 and an OR of 1.23 within the 95% CI of 0.40 to 3.77. Pregnant women with a family history of genetic diseases (8.26% of cases) and those with chromosomal abnormalities in the mother or father of the fetus (1.65% of cases) show a strong positive relationship. The correlation between a family history of hereditary diseases and chromosomal abnormalities has an r -value of 0.04 and an OR of 1.38 within the 95% CI of 0.27 to 7.08. The relationship between fetal abnormalities and chromosomal abnormalities in parents has a weak positive correlation, with an r -value of 0.01 and an OR of 1.08 within the 95% CI of 0.12 to 9.78.

Research conducted by Franssen et al., suggests that abnormalities in chromosome structure in couples can be a cause of recurrent miscarriages, particularly mutations on the husband's chromosome [9]. Additionally, a study by Nguyen Bich Van in 2022 has shown a relationship between embryo chromosomal abnormalities and pregnancy loss [17]. Overall, these studies support the association between genetic abnormalities and both recurrent miscarriages and birth defects.

4. Conclusions

In a study of 121 pregnant women undergoing prenatal screening, the mean age was 32 ± 6.69 years. The high-risk group of pregnant women over 35 years old accounted for 35.5% of the participants. Additionally, 21.5% of the mothers had a history of maternal miscarriage, 23.1% had a history of birth defects, and 8.3% had a family member with a genetic disease. Furthermore, 5% of the parents had chromosomal abnormalities. Among the 51 patients who underwent the Double test, 26.45% of the fetuses were identified as high-risk, while the Tripple test revealed a high-risk status for 9.09% of the fetuses. The overall percentage of fetuses with chromosomal abnormalities in the

screened group was 15.70%. Specifically, Edwards syndrome accounted for 5.78%, Down syndrome accounted for 4.96%, structural abnormalities of chromosomes accounted for 3.30%, Patau syndrome accounted for 0.83%, and Turner syndrome accounted for 0.83%. The presence of fetal abnormalities did not show a clear correlation with certain factors such as maternal age over 35 ($r = 0.08$ and $OR = 0.63$), history of miscarriage ($r = 0.05$ and $OR = 1.38$), family history of hereditary disease ($r = 0.04$ and $OR = 1.38$), and parental chromosomal mutations ($r = 0.01$ and $OR = 1.08$). However, a positive correlation was observed between fetal abnormalities and ultrasound screening ($r = 0.22$ and $OR = 5.48$), as well as blood screening ($r = 0.14$ and $OR = 1.22$).

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