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The Role of Cytogenetic Studies in Clinical Medicine

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Abstract: Chromosomal diseases form a large group of genetic diseases. They make up the bulk of all reproductive losses, congenital malformations, and are the causes of mental and physical development in children. This article presents the results of cytogenetic studies in early pregnancy. According to statistical data, the conclusions of the research work are made.

Keywords: cytogenetics, research, chromosome, anmoaly, genetic diseases, Down Syndrome, Edwards Syndrome, Shereshevsky-Turner Syndrome and Klinefelter Syndrome.

Relevance: On December 25, 2017, the President of Uzbekistan adopted the State program "Early detection of congenital and hereditary diseases in children." This program is designed for 2018-2022 to prevent the birth of children with these diseases. As part of this program, a cytogenetic laboratory was opened on the basis of the Fergana Regional Screening Center "Mother and Child" in 2018, which serves children under 18 years old with suspected chromosomal pathology of the Fergana, Andijan and Namangan regions.

Goals and objectives of the work: Prevention of the birth of children with various developmental abnormalities and genetic diseases. The aim of the scientific work is to study the normal chromosomal set and chromosomal abnormalities underlying hereditary diseases such as Down's Syndrome, Edwards Syndrome, Shereshevsky-Turner Syndrome and Klinefelter Syndrome.

Since the opening of the cytogenetic laboratory at the Fergana Screening Center, 163 children with suspected congenital chromosomal diseases have been examined. The number of examined people from Namangan region was 30 people, from Andijan region - 22 from Fergana region - 111. Of the 163 examined, 66 children were diagnosed with Down syndrome, Shereshevsky-Turner syndrome — 10, Klinefelter syndrome — 1 child.

According to statistics, the most common chromosomal disease in the world is Down Syndrome.

According to statistics from the World Health Organization, every 700-800th baby in the world is born with Down Syndrome. This ratio is the same in different countries, climatic zones and social strata. Genetic malfunction occurs regardless of the parent's lifestyle, health, habits, and education. To date, only two causes of Down Syndrome have been established. First, the age of the mother, and the older the woman becomes, the higher the risk of having a baby with Down syndrome. After 30 years the risk is 1: 1000, and after 42 years 1:60. This is due to the aging of the eggs. As you know, their number is laid even during the period of fetal formation. The second reason is heredity.

In this, closely related marriages, as well as the presence in the family of a relative with Down Syndrome, are of some importance. In addition, scientists note a link between the grandmother's age, in which she gave birth to a daughter. The older the grandmother was when she gave birth to her daughter, the higher the likelihood that she will give birth to her grandson or granddaughter with Down Syndrome. The importance of the father's age is also not denied, the risk factor is men over 45 years old.

Research methods:

1. Ultrasound procedure

2. Clinical procedure (PAPP-A, APF / CHG, CHG + □, echographic markers (TVP) and nasal bone, etc.

For early detection of fetal chromosomal diseases, a method of screening pregnant women is carried out. In the first trimester (10-13 weeks), a PAPP-A test is performed. PAPP-A is a high molecular weight glycoprotein. A number of serious clinical studies indicate the diagnostic value of PAPP-A as a screening marker for the risk of fetal chromosomal abnormalities. PAPP-A levels are significantly reduced when the fetus has trisomy 21 (Down's syndrome) or trisomy 18 (Edwards syndrome). In addition, this test is also informative in assessing the threat of miscarriage and termination of pregnancy at a short time.

In the second trimester of pregnancy, an APF / ChG test is performed. Alpha-fetoprotein (APF) is one of the indicators of the general condition of the fetus and the likelihood of having a congenital pathology. Elevated concentrations of APF in maternal serum or amniotic fluid during pregnancy may indicate congenital spinous processes, anencephaly, esophageal closure, or multiple pregnancies.

Reduced APF values (due to maternal age) provide information about the relative risk of Down Syndrome in the unborn child. Up to 15 weeks, the concentration of APF is not reliable enough as an indicator of neural tube defects, and over 20 weeks, the APF level characterizes the functional degree of fetal maturity.

In the Fergana Regional Screening Center "Mother and Child", the blood serum of pregnant women is examined simultaneously for APF, CG and estriol.

Chorionic gonadotropin + β (ChG + β) - is produced in the placenta during pregnancy. Elevated levels indicate choriocarcinoma, gallbladder drift, or multiple pregnancies. A reduced value indicates a threat of miscarriage or miscarriage, ectopic pregnancy, gestosis, or intrauterine death. (The norm of ChG + β is given in table 1)

ChG +β, i unit /ml	Postmenopause	0-7,0
	Women	0-1,0
	Men	0-2,0
	Pregnancy: 3-4 weeks	5,8-750
	5-6 weeks	217-31795
	7-8 weeks	3697-163563
	9-10 weeks	46509-186977
	11-12 weeks	27832-210612
	13-14 weeks	13950-62530
	15-16 weeks	9040-70971
	17-18 weeks	8099-58176

Estriol is the predominant estrogen in the blood and urine of pregnant women. The combined determination of unconjugated estriol, chorionic gonadotropin (ChG) and alpha-fetoprotein (APF) in the second trimester of pregnancy is a study useful, along with other clinical data of the mother, in assessing the risk of chromosomal abnormalities of the fetus at birth. (The norm of estriol is given on tab. 2).

	Pregnant	
Free estriol, ng / ml	12 weeks	0,3-1,0 ng / ml;
	13 weeks	0,3-1,1 ng / ml;
	14 weeks	0,4-1,6 ng / ml;
	15 weeks	1,0-4,4 ng / ml;

	16 weeks	1,4-6,5 ng / ml;
	17 weeks	1,5-6,6 ng / ml;
	18 weeks	1,6-8,5 ng / ml;
	19 weeks	1,9-11,0 ng / ml;
	20 weeks	2,1-13 ng / ml;
	21 weeks	2,6-14,0 ng / ml;
	22-23 weeks	2,7-16 ng / ml;
	24-25 weeks	2,9-17 ng / ml;
	26-27 weeks	3,0-18,0 ng / ml;
	28-29 weeks	3,2-20,0 ng / ml;
	30-31 weeks	3,6-22,0 ng / ml;
	32-33 weeks	4,6-23,0 ng / ml;
	34-35 weeks	5,1-25,0 ng / ml;
	36-37 weeks	7,2-29,0 ng / ml;
	38-39 weeks	7,8-37,0 ng / ml;
	40-42 weeks	8,0-39,0 ng / ml;
	Bisexual pregnancy	
	22-23 weeks	3-18 ng / ml;
	24-25 weeks	3-20 ng / ml;
	26-27 weeks	4-21 ng / ml;
	28-29 weeks	4-22 ng / ml;
	30-31 weeks	5-25 ng / ml;
	32-33 weeks	6-39 ng / ml;
	34-35 weeks	7-39 ng / ml;
	36-37 weeks	9-38 ng / ml;
	38-39 weeks	13-40 ng / ml.

In all cases of increased or decreased APF / ChG values, repeated tests are required, clarification of the gestational age at the time of blood donation and additional diagnostic tests.

The study of the thickness of the collar space of the TCS and the assessment of the nasal bones in the fetus, as well as the facial angle, curves of blood flow rates in the venous duct and through the tricuspid valve in the fetus significantly reduces the number of women who require invasive diagnostics and increases the frequency of detection of Down syndrome and other chromosomal diseases up to 95%, and the false positive result is reduced to 2-3%. Among the numerous echographic markers of chromosomal abnormalities in early pregnancy studied in recent years, the thickness of the collar space (TCS) is rightfully considered the most valuable marker.

TCS is the area between the inner surface of the fetal skin and the outer surface of the soft tissues that cover the cervical spine. The frequency of detection of chromosomal abnormalities and fetal malformations depends on the size, and not on the appearance of the TCS.

The next most important echographic marker is the nasal bone. In 1866, Langdon Down noted that a characteristic feature of patients with trisomy 21 is a small nose. This echographic of chromosomal abnormalities marker was first reported by S. Cicero et al. in 2001

A group of specialists led by the tireless inventor of new prenatal ultrasound markers K. Nicolaides analyzed cases in which an image of the nasal bones of the fetus was absent during screening ultrasound examination at 11-14 weeks of pregnancy. According to their data, the sensitivity of this criterion in the diagnosis of Down syndrome was 73%.

The study showed cases of imaging of only one nasal bone in fetuses with Edwards and Down syndromes. For visualization of both nasal bones simultaneously, it is advisable to use a cross-section. Submitted by S. Peralta et al. (2005), 51.6% of fetuses with Down's syndrome had no images of both nasal bones at 11-14 weeks of gestation, 9.7% of cases lacked images of only one bone, and 38.7% of fetuses had both nasal bones visualized.

Despite the early diagnosis of chromosomal abnormalities, a child with Down Syndrome may be born. After the birth of a child, the preliminary diagnosis of Down's Syndrome is made, the serum is examined by the cytogenetic method.

For clinical cytogenetics, the following are important:

a) postnatal karyotyping - determination of the patient's karyotype in peripheral blood lymphocytes;

b) prenatal karyotyping - a study of the fetal chromosomes, which is carried out on the cells of the amniotic membranes (chorion / placenta), amniotic fluid and, at a later date, on the umbilical cord of the fetus.

For research, you can use any nuclear cells capable of division. In the laboratory of the Fergana Screening Center, the object of the study is lymphocytes isolated from peripheral blood.

Enough 2 ml of whole venous heparinized blood taken sterile. The best sampling method is with a coin (Sarstedtmonovette, Li-HeparinLH / 2.7ml), as it avoids infection as much as possible, which is one of the conditions for further successful cell culture. The monovet with the taken blood is thoroughly mixed and marked (full name, date of collection, date and time of collection).

The analysis is not carried out on an empty stomach. But for 1-2 days, fatty, fried foods should be excluded from the patient's diet., For 2 weeks, taking antibiotics, chemotherapeutic, hormonal drugs, etc. blood sampling in patients with acute viral-respiratory disease is carried out no earlier than 14 days after recovery ... All these precautions are necessary in order to avoid a low mitotic index of lymphocyte culture, and, therefore, repeated blood sampling. Mitotic index (MI,%) - the percentage of dividing cells from the total number analyzed. Yet in a small percentage of cases, there is a risk of low individual MI and the patient is re-drawn.

Hemolyzed or chylous blood, as well as blood with clots, is unsuitable for analysis. The blood of newborns is especially "difficult" for karyotyping. For understandable reasons, it often happens with the defects listed above, in insufficient quantities and requires repeated sampling.

Stages of processing lymphocyte cultures and preparation of chromosomal preparations:

The first day:

1. In a syringe with 0.2 ml of a working solution of heparin (1:20 water for injection), we collect peripheral blood 1-2.0 ml;
2. Prepare the culture box: wipe the surface of the laminar flow cabinet with 70 ° ethyl alcohol or another disinfecting mixture. Place the consumable in a laminar flow cabinet. Turn on UV light in laminar flow cabinet. Turn on the UV lamp indoors. Carry out UV treatment for 20-30 minutes.
3. Into sterile tubes we add 5.5 ml of culture medium RPMI-1640, fetal bovine serum or bovine serum-0.7 ml, PHA (phytohemagglutinin) -0.02 ml, 2 drops of penicillin-streptomycin or gentamycin and 10 drops of whole blood in a box room;
4. Incubate cultures for 72 h at a temperature of + 37 ° C;

Second day:

1. 50 minutes before the end of cultivation, add 40 µl of 100Y colchicine solution. Carefully transfer the contents of the vial and leave in a temrostat at 37 ° C.
2. Further, all manipulations are carried out in the laboratory room. Prepare a hypotonic solution: 0.55% KCl (dissolve 0.25 mg KCl in 50 ml of distilled water) and put in a thermostat at 60 ° C. Prepare fixative-alcohol-acetic acid in a ratio of 3: 1. Place in the freezer. Pour about 80 ml of distilled water into a clean 100 ml beaker and put 2 slides per tube into a glass of water. Cover the glass with glass with parafilm and refrigerate at + 4 ° C.

3. At the end of the colchicinization time, the cells are precipitated by centrifugation at 1500 rpm for 8 min. Remove the supernatant (supernatant) with a pipette, leaving 0.3-0.5 ml above the sediment. Break up the sediment by vigorous shaking.
4. Resuspend the sediment in 5 ml of hypotonic potassium chloride solution and incubate for 20 min (in each laboratory the time is selected experimentally for the best chromosome scatter) at 37 ° C.
5. After 20 minutes add 0.5 ml of cooled fixative;
6. Sediment the cells by centrifugation for 8 min at 1500 rpm and remove the supernatant. Resuspend pellet and add 5 ml of chilled fixative. Put in the freezer for 10 minutes;
7. Sediment the cells by centrifugation for 8 min at 1500 rpm and remove the supernatant. Resuspend pellet and add 5 ml of chilled fixative. Put in the freezer for 15 minutes;
8. Sediment the cells by centrifugation for 8 min at 1500 rpm and remove the supernatant. Resuspend pellet and add 5 ml of chilled fixative. Put in the freezer for 30 minutes;
9. Sediment the cells by centrifugation for 8 min at 1500 rpm and remove the supernatant. Put the tubes to cool at + 4 ° C.

Day three:

1. Prepare the rack-rails, remove the cell suspension and the beaker with glass slides from the refrigerator, pipetting again to break up the sediment, use a pipette to take out 30-80 µl of the suspension.
2. Remove the glass slide from the glass with water with tweezers, carefully remove the excess water by lowering the glass tip onto the filter paper, roll the remaining water drops over the glass surface, shaking it. Apply the cell suspension from a height onto the surface of wet cooled glass, and burn the fixative over the flame of an alcohol lamp.
3. Using a graphite pencil, sign the slides: the number of the test tube and the specimen. Leave the preparations to dry in an upright position.

GTG method for differential staining of chromosome preparations.

Solutions:

Sorensen phosphate buffer (pH 6.8) - consists of two solutions:

- Solution 1 - sodium phosphate: weigh 23.8 g Na2HPO4 * 12H2O or 11.8 g Na2HPO4 * 2H2O bring dis.water to 1000 ml;
- Solution 2 - potassium phosphate: weigh 9.1 g of KH2PO4, bring to 1000 ml with dis.water:

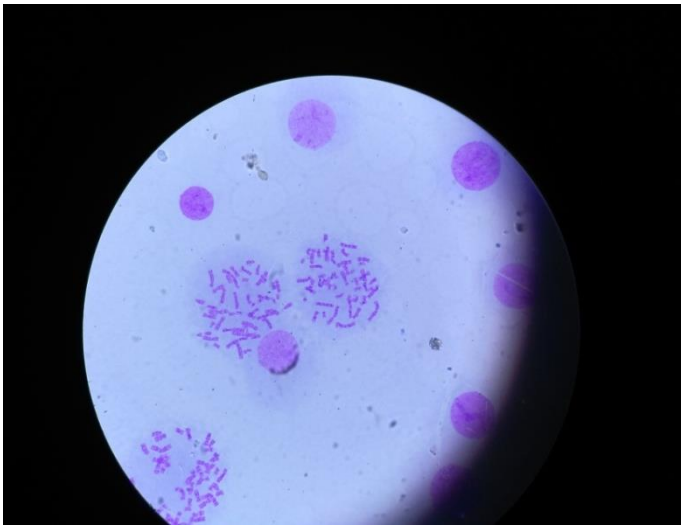
Before use, mix solution 1 and solution 2 in a 1: 1 ratio.

Trypsin working solution: Prepare 5% Giemsa solution in Sorens buffer in a Coplin or Hellendahl container.

1. Dip the preparations into the working solution of trypsin heated to 37 ° C. The processing time and the percentage of trypsin are selected empirically. Typically, they start from 10s, increasing if necessary.
2. Rinse the glasses in the disc water.
3. Stain in 5% Giemsa solution for 10-20 minutes. The staining time should be selected empirically.
4. Dry the glasses. Analyze in transmitted light.

Day four:

Fig. 1 Plates under the microscope Study preparations under a microscope and give a conclusion.



As for the recording of the conclusion, i.e. verbal description of the analyzed karyotype. The wording of the conclusions may vary from laboratory to laboratory. According to the recommendations, the conclusion on the analyzed karyotype should be written in an accessible and understandable form for a non-specialist and include data on the normal or an abnormal karyotype, balanced or unbalanced.If an imbalance is found, describe it (monosomy, trisomy, deletion, etc.) and write the name of the syndrome or disease (if any) associated with the detected mutation.

Fergana Regional Screening Center for Mother and Child

Genetic laboratory

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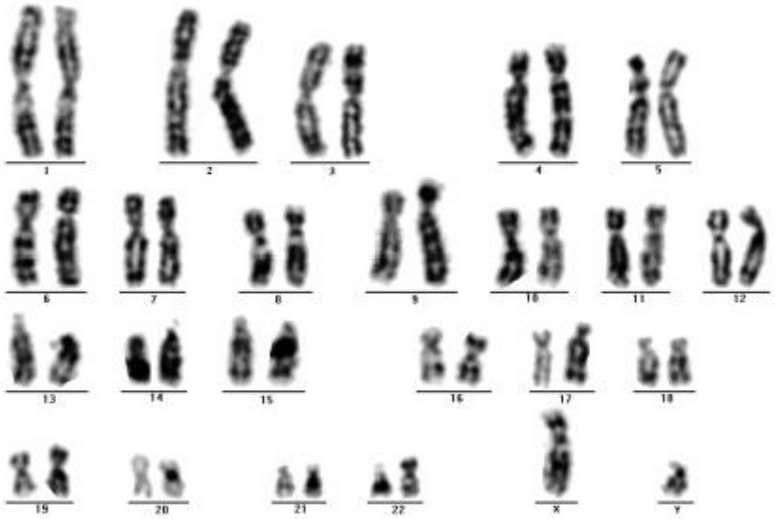
Karyotyping results	
Full Name	
Disease history №	
Research objective: Karyotyping	
Material for research: PHA stimulated peripheral blood lymphocytes	
Chromosome staining method: G stain	
Resolution level:~350-550 segments (ISCN)	Number of metaphases:11
Date of receipt of the material:	Laboratory Index:
Research result:46 XY	
Conclusion:	



Answer:

Results: The opening of a cytogenetic laboratory in the Fergana screening center made it possible for the population of Fergana, Andijan and Namangan regions to conduct free examinations and receive a clinical diagnosis near their place of residence, spending a minimum amount of time and money. In addition, these studies contribute to early detection, early diagnosis, and hence the prevention of the birth of children with chromosomal diseases.

Conclusions: Today, chromosome analysis is an extremely important diagnostic procedure in many areas of clinical medicine. Chromosomal diseases form a large group of genetic diseases. They make up the majority of all reproductive losses (miscarriages), congenital malformations, and are the causes of mental and physical development in children.

Cytogenetic methods can reveal numerical and structural changes in the chromosome set in humans (Down syndrome - trisomy on chromosome 21, Edwards syndrome - trisomy on chromosome 18, Patau syndrome - trisomy on chromosome 13, Shereshevsky-Turner syndrome - monosomy X -chromosomes, Klinefelter's syndrome - polysomy on the X chromosome, etc.). This method is based on microscopic examination of the karyotype using various methods of staining chromosomes.

With the help of light microscopy, chromosomes can be detected and examined only during mitotic cell division. This method allows you to analyze the chromosomal complex of human cells, to establish the structural features of individual chromosomes, and also to identify violations of the number and structure of chromosomes in the subject. The presence of a connection between the detected disorders and the appearance of certain pathological signs in the human phenotype makes it possible to diagnose various chromosomal diseases.

Due to the widespread introduction into practice of the method of differential staining of chromosomes, it became possible to study the karyotype in patients with suspected chromosomal pathology.

The main objective of the study is to modify the standard method for preparing chromosome preparations in order to increase the effectiveness of cytogenetic studies and to determine the significance of cytogenetic studies in identifying a hereditary disease.

A detailed study of the karyotype is carried out to solve such problems as: identifying the cause of congenital diseases of a child at the genetic level, finding the genetic causes of miscarriage and female infertility, identifying the consequences of exposure to harmful factors at work and detecting abnormal chromosomes in the fetus.

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