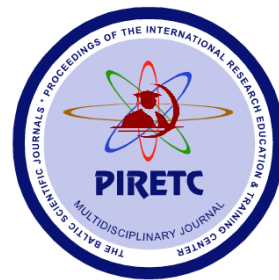


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ECONOMIC, MANAGEMENT & MARKETING SCIENCES  
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**I never think of the future - it comes soon enough. Albert Einstein**

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## BIO- PHYSICAL CONSTRAINTS ENCOUNTERED BY THE SUGARCANE GROWERS IN ADOPTION OF SSI TECHNOLOGY

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### ABSTRACT

Sugarcane is one of India's primary commercial crops. It is grown under a range of agro-climates. India ranks first among the world's sugar cane-growing countries, both in the region and producing. But at present the production of sugar cane is in crisis. Cane growers now face endless problems in low yield sugar cane production, varietal degeneration, high input costs, disease and pest incidence, soil erosion, salinity, water logging and drought, and the area under sugar cane is dwindling. At this point, the innovated "Sustainable Sugarcane Initiative" (SSI) technology was introduced as the primary option to rectify the problems in recent decades in the cultivable area to address many of the problems in open fields. It also breaks harvest times and provides a longer period of the cane crushing season for the sugar industry. Views and opinions furnished by sugarcane growers included in the study were representative the constraints encounter in the adoption of recommended SSI technology in the Cuddalore District from the aspect of bio-physical views and opinions of the whole population of the study area.

**Keywords:** Bio- Physical constraints, Sugarcane Growers, SSI Technology

### INTRODUCTION

Sugarcane dominates a prominent place on India's agricultural map covering extensive areas in the subtropics and tropics. There were 538 sugar mills in the country in 2014-15 compared to 139 mills in 1950-51 and the sugar cane acreage increased from 1, 71 million ha in 1950-51 to 4, 90 million ha in 2015-16. Under sugarcane there is hardly any possibility of additional land, primarily due to rising agricultural land availability. The industrial farming of sugarcane has contributed to a major decline in productivity. It is obvious that, in the future, the sugarcane production requirement must be achieved primarily by increasing the crop productivity. The water requirements vary considerably from 1200 to 3000 mm depending on the yield level of the crop and the climatic conditions prevailing in different regions of the country. It is grown under a range of agro-climates. India ranks first among the world's sugar cane-growing countries, both in the region and producing. But at present the production of sugar cane is in crisis. Cane growers now face endless problems in low yield sugar cane production, varietal degeneration, high input costs, disease and pest incidence, soil erosion, salinity, water logging and drought, and the area under sugar cane is dwindling. At this point, the innovated "Sustainable Sugarcane Initiative" (SSI) technology was introduced as the primary option to rectify the problems in recent decades in the cultivable area to address many of the problems in open fields. It also breaks harvest times and provides a longer period of the cane crushing season for the sugar industry. The study emphasis and illustrated the bio- physical constraints faced by the sugarcane growers during the adoption of SSI technology in Cuddalore District. The researcher felt that the study would be of much useful to the Government, foreign donors, international development agencies, private enterprises, banks, universities and to the intellectuals of the country, including the key decision makers at different tiers of the government to get a clear picture and up to date information about sugarcane growers of our country. This will also be helpful to formulate a pragmatic welfare biased dynamic sugarcane policy/program for the country.

### METHODOLOGY

Cuddalore district is one of the major sugar cane areas which occupies 21,628 hectares in the year of 2016- 2017 as noted by The Department of Economics and Statistic in Chennai. The list of SSI Sugarcane growers in the Cuddalore District in the selected taluks were obtained from the respective sugar mills in the district. In research, the researcher should keep in mind that the sample size should be taken optimum sample with respect to population size, which fulfills the representativeness, reliability, flexibility and cost effectiveness. The information collected from sample size

respondent represents the information from total population. Statistically, the maximum 30 percent is taken from a small population, but for moderate population, 5 percent and 10 percent are taken. So in this study, 10 percent of total population size was taken as per researcher convenient. The total population size (N) is 2400 therefore, the sample size (n) was taken 10 percent of the total population (N) which is 240 households from the total household population. Three Taluks, namely Chidambaram, Bhuvanagiri and Kattumannarkoil under the MRK Co-op Sugar Mill divisions, Three Taluks namely Cuddalore, Punruti and Kurinjipadi under the EID Parry Sugars (P) Ltd. Divisions. Two Taluks, namely Vridhachalam and Tittagudi under the Ambiga Sugars P) Ltd. divisions, totally all the eight taluks in the Cuddalore district were selected for this study. A well-structured interview schedule was utilised to collect the data and information through the direct contact method by interviewing the 240 respondents and the collected data are interpreted with analysing the appropriate statistical tools to generate the accurate results pertaining to this study.

## FINDINGS AND DISCUSSION

### CONSTRAINTS IN ADOPTION OF SSI TECHNOLOGY AS REPORTED BY THE RESPONDENTS

In this part the constraints encountered by the experienced sugarcane growers in their non-adoption of the SSI technology in sugarcane cultivation.

In accordance with the objectives, the constraints experienced by the respondents of various locations are presented under the head namely, Bio-physical constraints. The results are presented in Table 1.

**Table 1. Bio- Physical constraints faced by the respondents in adoption of SSI technology of sugarcane cultivation**

(n=240)

| S. No.                          | Constraints                                       | Number | Per cent | Rank |
|---------------------------------|---|--------|----------|------|
| <b>Bio-physical constraints</b> |   |        |          |      |
| 1                               | Uncertainty of canal water for irrigation         | 100    | 41.67    | V    |
| 2                               | Inundation due to floods                          | 220    | 91.66    | I    |
| 3                               | Complexity of new practices                       | 140    | 58.33    | III  |
| 4                               | Non-availability of micro nutrients at right time | 102    | 42.50    | IV   |
| 5                               | Non-availability of quality setts                 | 152    | 63.33    | II   |

### BIO-PHYSICAL CONSTRAINTS

It could be vividly observed from the Table 1 that altogether five bio-physical constraints were expressed by the respondents with regard to adoption of recommended SSI technologies in sugar cane. Among the five bio-physical constraints, inundation due to floods was the most important constraint mentioned by most (91.66 per cent) of the respondents. During rainy season, the farmers used to face uncertainties like heavy rainfall and floods which would cause inundation in the fields. This condition would have prevented the farmers to maintain the condition of alternate wetting and drying. Moreover, it may be due to the climate and location of the study area. These may be the reasons for the above mentioned constraint.

'Non-availability of quality setts' as an important constraint was reported by 63.33 per cent of the respondents. This may be due to the inadequate availability of quality setts in the local sets producers at village level. This finding is in agreement with the earlier findings reported by Reddy (2003).

'Complexity of new practices' was an important constraint reported by 58.33 per cent of the respondents. The adoption of new practices might require special knowledge and specialized skills in operating weeder, usage of Marker and maintaining the field with alternate wetting and drying. Further, it might require more skilled labourers. Due to the above reasons, most of the respondents believed that the recommended SSI technologies were complicated in nature of sugar cane cultivation. This is in line with the findings of Renjini (2000), Smitha (2002) and Punitha (2005) who also reported similar findings.

Non-availability of micronutrients at the right time was determined by 42.50 per cent of the sugarcane growers. Irregular supply of micronutrients by the Government departments and private input dealers might be the reason for reporting this constraint. This finding support with the findings of Natarajan (2008).



'Uncertainty of canal water for irrigation' was noted by 41.67 per cent of the sugarcane growers. It was found out that most of the respondents of the study area depend on water from Veeranam Ayacut for irrigating their crops. They revealed that they were uncertain about the time of release of water from Veeranam Ayacut. Because of this uncertainty, they were unable to plan the agricultural operations, which in turn would have resulted in unnecessary delay in taking up cultivations. This is in line with the findings of Jeyalakshmi (2008).

## CONCLUSION

Among the bio-physical constraints, the most important constraint faced by a majority of the respondents was an inundation due to floods (91.66 per cent). The overall bio- physical constraints encounter of the sugarcane growers on SSI was ranked as follows, Inundation due to floods (1), Non-availability of quality setts (2), Complexity of new practices (3), Non-availability of micronutrients at the right time (4) and Uncertainty of canal water for irrigation (5) respectively. Further, it was observed that the agriculture department officials may take steps to increase awareness and knowledge to rectify on those constraints through distribution of printed literature like leaflets on SSI, regular field visits, frontline demonstrations and trainings. The policy makers and the government bodies also support the farmers by initiating new programmes and giving subsidy by the way of initiating new insurance schemes for eradicating the bio- physical constraints in the adoption SSI technology of sugarcane cultivation.

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# THE CORRESPONDENCE OF POLYMORPHISM C677T OF METHYLENE TETRA HYDROFOLATE REDUCTASE GENE WITH CARDIOVASCULAR DISEASES IN THE POPULATION OF AZERBAIJAN

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## ABSTRACT

**OBJECTIVE:** The research is devoted to the studying of the C677T polymorphism of the MTHFR gene among patients with cardiovascular diseases - coronary heart disease, myocardial infarction and hypertension

**METHODS:** We used a complex of molecular genetic methods including: polymerase chain reaction in different execution modes, electrophoresis of genomic DNA and amplified gene fragments on agarose plates and nucleotides of individual fragments to determine the type of mutation.

**RESULTS:** As a result of sequencing of a fragment of the MTHFR gene in 72 patients, in 27 cases (37.5%), the mutation C677T MTHFR (Ala677Val) in the heterozygous state (C / T) was identified. When comparing the obtained mutation values among people in the control and experimental groups, it was found that the frequency of occurrence of the C677T MTHFR mutation (Ala677Val) among the group of patients was 15.3% higher than that in people in the control group ( $p < 0,05$ ).

**CONCLUSION:** As a result, a high frequency of these mutations was established within the group of patients with severe forms of cardiovascular disorders. For the first time there also were determined the frequencies of these mutations in the population of Azerbaijan and was postulated that these frequencies are mainly correlated with the frequencies described in other populations of the world.

Proper prophylaxis by detecting the C677T mutation of the MTHFR gene will allow doctors to conduct qualified treatment of cardiovascular diseases

**Keywords:** genetic polymorphism, reductase, polymerase chain reaction, cardiovascular diseases.

## INTRODUCTION

Methylenetetrahydrofolate reductase (MTGFR) is a key enzyme in the folate cycle and catalyzes the reaction of converting folic acid into the active form, which is involved in the synthesis of the amino acid methionine, which is responsible for DNA methylation during cell division and eliminating the excess of the amino acid homocysteine, which has a pronounced toxic effect [6, 25]. An increased concentration of homocysteine in the blood leads to the development of early myocardial infarction and thrombovascular disease. Now it has been proven that there are several allelic variants of the MTHFR enzyme. However, only two variants of the allele have practical significance; one of them is the C677T allele. Replacement of nucleotides cytosine (C) to thymine nucleotide (T) at position 677 of the gene have resulted in a decrease of enzyme activity to about 35% of the average value. Persons who inherit this variant of the genotype from both parents are significantly more (14-21%) susceptible to diseases of the cardiovascular system and also congenital pathologies in the offspring [1, 124]; [2, 27]; [5, 44]; [3, 145]; [4, 17].

The purpose of our study is to establish the relationship of polymorphism C677T of methylenetetrahydrofolate reductase (MTHFR) gene with cardiovascular diseases in the population of Azerbaijan



## MATERIAL AND METHODS

To study the genetic polymorphism of the MTHFR gene we used a complex of molecular genetic methods including: polymerase chain reaction in different execution modes, electrophoresis of genomic DNA and amplified gene fragments on agarose plates and nucleotides of individual fragments to determine the type of mutation.

To identify mutations and establish the frequency of occurrence among individuals, we have examined 180 people. The research material was venous blood on the anticoagulant EDTA (or heparin) in an amount of 2 ml from 72 individuals (39 men and 33 women) with diseases of the cardiovascular system (coronary heart disease, myocardial infarction, hypertension) from 18 to 67 years. The history of arterial hypertension, established by history, ranged from 2 to 26 years. As a control group, venous blood of 108-x practically healthy individuals from 20 to 52 years old (60 men and 48 women) was used.

## CONCLUSION

In 24 cases out of 108 practically healthy individuals included in the control group, the mutation C677T MTHFR was identified. All of 24 healthy individuals had a heterozygous mutation (C/T). The phenotypic mutation rate was 22.2%. The phenotypic frequency of the normal genotype (C/C) without this mutation was 77.8%. The genotypic frequency had a fraction of unity equal to 0.2222. The frequency of the T and C alleles in unit fractions was 0.1111 and 0.8889, respectively.

72 patients with CVD also have been examined, and in 27 cases (37.5%) the C677T MTHFR mutation in the heterozygous state (C/T) was identified. When comparing the obtained mutation values among individuals in the control and experimental groups, it was found that the frequency of occurrence of the C677T MTHFR mutation among the patient group was 15.3% higher than this indicator in people in the control group ( $p < 0.05$ ). Also, the frequency of the mutant gene (T - 0.1806) among patients was 0.0695 units higher than those in the control group (0.1111;  $p > 0.05$ ). Therefore, there is a relationship between the presence of the C677T MTHFR mutation and CVD.

Persons with CVD are divided into the following groups: 1. patients with a mild clinic; 2. patients with a moderate clinic; 3. patients with a severe form of the disease.

The frequency of the C677T mutation in patients with mild hypertension is 22.22% (4 cases out of 18). For individuals with a moderate form of hypertension, the mutation rate was 30.0% (9 cases out of 30). Among patients with severe hypertension, the highest incidence was 54.17% (13 cases out of 24).

The frequency of mutations of C677T in patients with mild form of hypertension is 22.22% (4 cases out of 18). For individuals with a moderate form of hypertension, the mutation rate was 30.0% (9 cases out of 30). Among patients with severe hypertension, the highest incidence was 54.17 % (13 cases out of 24). The frequency of the severe C677T allele (0.0903) was on average 3.3 times higher than in the group of patients with mild (0.0278) and 1.3 times higher than with a moderate form of hypertension separately (0.0694).

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## HISTORY OF FORMATION OF THE FAUNA OF THE FISH MYXOSPOREA OF THE WATER BODIES OF AZERBAIJAN

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Despite the fact that all water bodies of Azerbaijan belong to the basin of Caspian Sea, among the myxosporeas of our fish, there are only 3 species from Ponto-Caspian marine faunistic complex formed by brackish-water of Pontic Lake-Sea. In the Neogene, in the drainless water bodies of Near East, Western Asian faunistic complex arose. Its representatives began to penetrate into the water bodies of Caucasus, after it joined its southernmost tip to the mainland and turned from an island into a peninsula. From the myxosporeas of our fauna, this complex includes species that parasitize mainly on *Labeobarbus* and barbel. In the Neogene, in the water bodies of Northern Palaearctic, the most widespread freshwater faunistic complex, i.e. boreal plain complex (in the sense of Yakovlev, 1964) was formed. In our fauna, it is represented by three environmental groups. From these, palearctic group includes specific myxosporea of northern pike and a number of species with a wide range of hosts. Representatives of the next, Ponto-Caspian group are inferior to the Palaearctic species in the degree of eurythermy and are therefore more numerous in the southern and low-land areas than in the northern and mountainous. Among the myxosporeas of the fauna of the republic there are 16 of them. They are found in fish belonging to various faunal complexes. Our amphiboreal group consists of 6 species with interrupted habitat, including Europe and the Far East. The vast majority of myxosporeas of boreal low-land origin is euryhaline and can parasitize in the body of many fishes. Therefore, these forms could easily penetrate from the north, for example, from Volga basin, into the brackish waters of the Caspian Sea. Passing the sea, through rivers, on migratory fish, they also got into the freshwater bodies of our region. Due to periodically occurring phases of desalination, this process has been significantly simplified. In Neogene, when the mountain range of the Greater Caucasus did not reach its present height, boreal forms could pass into the freshwater bodies of the Caucasus also through the upper reaches of the rivers. In the era of productive strata, as a result of strong regression, the northern boundary of the sea water body corresponding to modern Caspian Sea receded far to the south and the rivers flowing now into the Middle Caspian Sea temporarily turned into tributaries of the so-called Paleo-Volga. In such conditions, typically freshwater boreal forms of the fish and ichthyoparasites penetrated the southern freshwater tributaries.

Approximately in the Neogene, in the lowlands of Palearctic, a brackish-water faunistic complex was formed, in particular, sticklebacks and their highly specific parasites, including 3 species of myxosporidia. Their penetration into our region could follow the same paths as boreal lowland forms. Moreover, given the limnophily nature of the former, apparently, the possibility of their crossing the upper reaches of the rivers should be excluded.

In the post-tertiary period, when the Khvalyn (Caspian) basin renewed its connection with the Ancient Euxin (Black Sea) basin, the entrance of the Mediterranean into atherins, Common goby and pipefish, as well the latter parasite, myxosporea *Sinuolinea sakinchanumae* was happened in the Caspian Sea.

Salmon fish and 2 species of their myxosporeas in the water bodies of Azerbaijan are representatives of boreal piedmont-faunistic complex, which formed in Siberia and Northern Europe from some of boreal low-land part. Their penetration, apparently, should be attributed to already post-glacial period, when the nelma moved into the Caspian Sea from the Arctic basin, which then turned into a passage inconnu.

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## FEATURES OF THE MECHANISM OF ANTI-MUTAGENIC EFFECT OF SUMAC FRUIT EXTRACT AT THE STAGE OF INITIAL DAMAGE OF THE DNA MOLECULE IN THE CASE OF THE LAST MUTATION

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During the last mutation at the stage of primary DNA damage in wild-type E.Coli K-12 cells and AB 1885 (uvrB), JC 5519 (recBC), JC 9238 (recF) V and JC 7689 (sbc B) strains that are its derivatives, sumakh extract was used in a priori detected dose of 0.01 mg/ml. In the experiments, mutagens UV, 4-nitrocholine -1 – oxide (4NXO) V and mitomycin C were used, which differ in the spectrum of primary damage to the DNA molecule formed by them. All this allows us to believe that the main factor of mutagenesis modification and cell death by sumakh fruit extract is the position of reparative enzymes, which, being in a state of defect, encode the genes of uvr B, rec BC V, and rec F. In the schemes of experiments using the studied extract, cells with a reparatively damaged genome are more sensitive to the mutagen compared to wild-type cells. Increased sensitivity of the E. Coli uvr B strain to influencing factors, in comparison with wild-type cells, is associated with the presence of a nuclease independent of uvr AB in cells of this series, which is a product of uvr A and uvr B genes (Howard-Flanders, Boyce, 1996). Increased sensitivity and relatively equal sensitivity in E. Coli strains rec BC and rec F in the first case is associated with the activity of rec BC – dependent nuclease (exonuclease V) (Witkin, 1974), and in the second case – with a partial weakening of the process of post-replication repair of the rec F gene defect (Ganesan, Seawell, 1975). In turn, increased sensitivity of sbc B cells in the series is associated with inactivation of exonuclease I activity (Tapacov, 1982). The results given below were obtained by adding an extract from sumakh fruit to the schemes of experiments with the influence of mutagens. In experiments using damaging factors UV and 4NXO in wild cell mutants, as well as rec BC and sbc B strains, a decrease in mutagenesis is observed. Thus, in uvr B and rec F cells of the series, the genome-protecting properties of sumakh fruit extract are observed in practice. In experiments where an artificial mutation was achieved using Mitomycin C, the positive effect of the extract was observed in wild-type cells and sbc B mutants, but no positive effect was observed in uvr B-, V - rec BC – rec F-genotype cells.

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## MOLECULAR-GENETIC RESEARCH OF PHENYLKETONURIA IN AZERBAIJAN FAMILY

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### ABSTRACT

Phenylketonuria gene has an identified R261G (G-A) mutation. The study of erythrocyte enzyme preparation for family members have shown low electrophoretic mobility for G6PD which was unknown in the world studies. A new biochemical variant was identified on the basis of physic-chemical indications of G6PD enzyme, and PKU inherited metabolic disease with R261G (G-A) mutation were found in the family G.M., who live in Tekle village of Masally area. Phenylketonuria and G6PD enzyme deficiency were identified in one family in Tekle village of Masally area of Azerbaijan Republic. Heterozygous and homozygous genetic types of phenylalanine-4-hydroxylase gene mutation R261G (G-A) were identified. A new unknown to scientific literature biochemical polymorphism of G6PD enzyme was identified.

**Keywords:** disease, polymerase chain reaction, enzyme, biochemical polymorphism, family, identification

### INTRODUCTION

The family with identified inherited metabolic disease of phenylketonuria lives in Masally administrative area. Masally area itself is located in South-East of Azerbaijan Republic on the slopes of Talysh mountains in subtropical zone. Members of proband's family possess deficiency of glucose-6-phosphatedehydrogenase. Phenylketonuria gene has an identified R261G (G-A) mutation. The study of erythrocyte enzyme preparation for family members have shown low electrophoretic mobility for G6PD which was unknown in the world studies. Based on the Michaelis-Menten constant ( $K_m$ ) applied to G6P, substrate values have manifested high biochemical polymorphism.

The goal of nutrition management of phenylketonuria (PKU) is to maintain blood phenylalanine concentrations between 120 and 360  $\mu\text{mol/L}$ . The diet for PKU includes medical foods low in or devoid of phenylalanine and limited quantities of phenylalanine from intact protein sources. Frequent monitoring of blood phenylalanine concentrations is key to successful diet management. Frequent adjustments in the diet are needed to achieve desired blood phenylalanine concentrations as well as to promote normal growth and feeding development. A variety of PKU medical foods and modified low-protein foods are available to accommodate different nutrient needs and taste preferences throughout the life span. Maintaining the diet is challenging for many patients with PKU; alternative therapies are available, but most still require some degree of diet modification [6,9].

The phenylalanine-4-hydroxylase (PAH) gene is located on the long shoulder of chromosome 12 in q22-24.1 site. The length is 90 thousand nb and consists of 13 exons. Synthesized protein consists of 451 amino acid residues. Phenylalanine amino acid coming with food in oxidation process turns into different amino acid -tyrosine as a result of phenylalanine hydroxidation process. In the result of mutation in PAH gene this phenylalanine into tyrosine transformation fails [7,9]. Up to 1 % cases of phenylketonuria are presented with atypical forms. The disease is inherited as to autosome-recessive type. The prevalence rate differs in different population groups. For example, in Europeoid inhabitants in the USA it is 1 to 10000. The highest rate is in Turkey, which is 1 to 2600. In Finland and Japan the rate of phenylketonuria is extremely low: even less 1 newborn to 100000 births. In Slovakia in some gypsy populations there were found ultrahigh rates of phenylketonuria because of inbreeding: 1 case for 40 newborns.

According to the World Health Organisation data, there are around 100 million people suffering from glucose-6-phosphatedehydrogenase (G6PD) enzyme activity deficiency. More than 400 abnormal variants were identified, and around  $\frac{1}{4}$  of them are endemically different.

One part of those abnormal G6PD variants could be characteristic for only one certain ethnic group, and another part - for several ethnic groups. A group of people with enzyme deficiency resulted with hemolytic crisis after some specific medicines, and other people - just after eating food cooked with beans [7].

Thus, the goal of our studies was to identify gene mutations in people with PKU diagnosis and to study the physico-chemical specificities of abnormal G6PD enzyme in the proband's family members[8].

## MATERIAL AND METHODS

Venous blood samples with heparine anticoagulant were used as the study subjects. Blood was sampled from G.M. (proband) family members, who are inhabitants of Tekle village of Masally area, Azerbaijan Republic.

PKU diagnostics was carried out by means of IFA method. In identification of PKU gene mutations, complex of molecular-genetic methods were used [1,5].

Genomic DNA was isolated from venous blood, using readymade kits by QIAGEN (Germany) company. Intactness and quantity of isolated genomic DNA were identified by means of electrophoresis in 1.7% agarose gel, as well as gene fragments after polymerase chain reaction (PCR). Electrophoretic apparatus and power source were BioRad (USA) manufactured. Marker for identification of synthesized DNA fragments was DNA Ladder 100 bp.

The content of PCR: 0,1-1,0 µg of genomic DNA, 0,25 µM of each dNTP, 25 µl buffer (67 mM Tris-HCL, pH 8,8: 16,6 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0,01% Twin-20, 1,5 unit DNA-polymerase. 2 µg of primers for each of exons 3,5,7,11 and 12.

Regime of PCR for PKU gene was as follows: 95°C-2 minutes, (94°C-45', 58°C-45', 72°C-45' 30 cycles), 72°C-7 minutes and pause at 4°C for 10 minutes. PCR was conducted in amplifier – Professional Thermocycler, Biometra, (Germany).

Purification of DNA fragments after the first PCR stage a set of magnets was used: «AgencourtAMPure XP PCR purification» and SPRIPlate 96 Super Magnet Plate. After that purified DNA fragments were used for the further researches. The second PCR was conducted in the regime: 95°C-2 minutes, (95°C-30', 52°C-58°C - 30', 78°C-2 minutes 30 cycles), 72°C-10 minutes and pause on the amplifier at 4°C for 10 minutes. Then the standard procedure on the apparatus GENOMELabGeXP™ Sequencing for the identification of nucleotide sequence of each DNA fragment was carried out.

G6PD enzyme activity was measured by means of modified fluorescent method [2,3].

Purification of enzyme preparations and study of the characteristics were carried out according to the WHO standardized methods [4].

## RESULTS AND DISCUSSION

The world scientific literature researches show that European populations have mainly R408W, P281L, R261Q, R158Q, R252W, I65T, IVS10nt546, IVS12nt1. PAH gene mutations prevail over the others. These mutations are located in 3,5,7,11 and 12 exons of the gene [4,10]. With this purpose we have done amplification of PAH gene exons 3,5,7,11 and 12 genomic DNA fragments, got from lymphocytes of the G.M. family members: two parents and six their children, by means of polymerase chain reaction with 5 primer groups.

Fragments of exon 3 of 112 nb, exon 5 of 162 nb, exon 7 of 218 nb, exon 11 of 222 nb and exon 12 of 177 nb were amplified.

Total nucleotide sequencing was done only for exon 7 as an example, where R261G mutation was identified. Being a point mutation we have found a substitution of guanine with adenine. The result of mutation was on protein level, and arginine amino acid was substituted with glutamine amino acid.

Homozygous form was identified in 4-year-old girl. Heterozygous form carriers were both parents and one sibling. So, family members manifested one homozygous and three heterozygous forms of R261G mutation.

It's worthwhile noting, that proband's parents are children of two sisters. Marriage is identified as a 3rd consanguineous parallel marriage type.

G6PD enzyme deficiency was as low as 5.2-12.5% of the normal activity in three brothers in the family. According to the obtained activity it was relates to the II activity class. All three enzyme preparations showed low enzyme (0-10%) activity. Each of the three kids had erythrocyte hemolysis and anaemia after eating food with beans. All three enzyme preparations have the indication of pH-optimum in the normal range (pH 7,5-8,5). All enzyme preparations under research have shown low electrophoretic movibility. Based on the G6P substrate, constant of Michaelis-Menten (K<sub>m</sub>) indication of all the enzyme preparations was high (146,7µm). For analogue of 2dG6P substrate was high disposal degree identified. Enzyme preparations, obtained from the inhabitants of Masally area Tekle village, have manifested such physico-chemical characteristics of G6PD deficiency which was new and had no analogue in the world scientific literature.



Thus, a new biochemical variant was identified on the basis of physic-chemical indications of G6PD enzyme, and PKU inherited metabolic disease with R261G (G-A) mutation were found in the family G.M., who live in Tekle village of Masally area.

## CONCLUSION

Phenylketonuria and G6PD enzyme deficiency were identified in one family in Tekle village of Masally area of Azerbaijan Republic.

Heterozygous and homozygous genetic types of phenylalanine-4-hydroxylase gene mutation R261G (G-A) were identified.

A new unknown to scientific literature biochemical polymorphism of G6PD enzyme was identified.

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## EARLY PRENATAL DIAGNOSIS OF FETAL ANEUPLOIDY AND NEURAL TUBE DEFECTS

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10-15% of fertilized eggs cannot be implanted (1). Pregnancy losses are observed in 22% of cases before the diagnosis of clinical pregnancy (2). Cytogenetic study of spontaneous miscarriages reveals chromosomal abnormalities in 50-65% of cases (3, 4, 5). 17.4% of Polyploidy, 23.7% of X monosomy, 49.8% of trisomy (mainly, Chromosome 16 trisomy) were found in miscarriage specimens in which chromosomal abnormalities were detected (6). 50% of fertilized human eggs undergo reproductive loss, and chromosomal and gene mutations play a significant role in the occurrence of these losses (7). The use of genetic testing methods allows to explain the causes of reproductive losses: spontaneous abortions, perinatal death and disease. If a woman has a history of 2 or more miscarriages, genetic counseling is crucial. In this case, genealogical examination should be carried out taking into account not only, the family history of the couple, but, also miscarriages, stillbirth, intrauterine growth retardation, congenital malformation, mental weakness, infertility.

During genetic counseling and cytogenetic examination:

1. Reasons identified as a result of genealogical and cytogenetic analysis should be explained to the couple.
2. The risk of miscarriages and congenital malformations of the fetus should be assessed;
3. It is necessary to explain the importance of prenatal diagnosis in subsequent pregnancies and the possibility of in vitro fertilization with donor eggs and sperm in the presence of a gross pathology in the family.
4. The importance of cytogenetic testing in cases of miscarriage, stillbirth and neonatal death should be emphasized (8).

Screening tests with a comprehensive assessment based on several indicators allow early detection of some fetal chromosomal abnormalities, defects of the neural tube during the first and second trimester of pregnancy. The improvement of non-invasive diagnostic techniques plays an important role in early detection of chromosomal abnormalities in the fetus. Of these, the screening test used in the first trimester of pregnancy is of particular importance.

Indications for biochemical markers in the first trimester of pregnancy:

- When the mother's age is over 35 and the father's age is over 40.
- History of  $\geq 2$  spontaneous miscarriage
- Taking medication before fertilization or in the first weeks of pregnancy
- Presence of bacterial and viral infections (TORCH syndrome)
- The birth of children with chromosomal abnormalities in the family or close relatives
- Probability of radiation from one or both parents
- The desire to have a healthy child, etc. (8, 9).

The risk of fetal trisomy increases with advancing maternal age. In prenatal diagnosis of aneuploidy (trisomy 13, 18, 21) and neural tube defects, ultrasound data are combined with the age of the mother, two or more biochemical indicators. It should also be noted that prenatal diagnosis of aneuploidy should not be carried out taking into account only the mother's age. Amniocentesis should not be performed on women over 40 years without prenatal screening. Because the risk of chromosomal abnormalities with a negative test may be  $< 1/200$  (10, 11). According to the UK National Screening Committee (UK NSC) diagnostic tests with a sensitivity of  $> 75\%$  and a false positivity of  $< 3\%$  can be used. These tests should be used between 10 and 20 weeks of gestation. First trimester screening should be done before 3 weeks and 6 days of pregnancy. There is still no reliable source for prenatal diagnosis of Down's syndrome before the week 10 of pregnancy. A blood test combined with an ultrasound (which checks the thickness at the back of the fetus's

neck, known as its nuchal translucency), human chorionic gonadotropin (hCG) and pregnancy-associated protein A (PAPP-A) are evaluated simultaneously in the 1st trimester screening. Early diagnosis makes it possible to identify the risk before week 14 of gestation and make a timely decision. At a meeting, in 2007, it was stated that the sensitivity values of a diagnostic test conducted only with biochemical or ultrasound examination were lower (8, 11, 13). Triple or quad screening are performed in the second trimester. As 15% of pregnant women seek antenatal care in the final weeks of pregnancy, triple or quadruple screening is recommended in the second trimester. Human Chorionic Gonadotropin (hCG) and unconjugated estriol (uE3) and  $\alpha$ -fetoprotein (AFP) are detected by triple marker screening tests in the maternal plasma. The quadruple tests includes hCG, AFP, uE3 and additionally Inhibin A.

Screening may be done in the following weeks of pregnancy:

- ✓ PAPP-A test is measured between 10 weeks 0 days and 13 weeks 6 days in the first trimester
- ✓ Second trimester screening is done between 15 weeks 0 days and 20 weeks 0 days;
- ✓ Weeks 9 to 10 of gestation are more suitable for PAPP-A determination. From 10 to 13 weeks of gestation, its level gradually decreases.
- ✓ The first trimester combined test is performed between weeks 11 and 14 of pregnancy, taking into account NT and PAPP-A.
- ✓ Pregnant women seeking antenatal care in the last weeks of pregnancy, a quadruple (possibly triple screening) test is performed between weeks 15 - 20 of pregnancy.
- ✓ The cut-off is 1/150 for the first trimester screening and 1/200 for the second trimester screening (13).

Invasive tests such as amniocentesis and Chorionic villus sampling (CVS) are recommended if a high risk is identified. A karyotype can be defined in a sample obtained in this way and a final diagnosis can be made. CVS is performed at week 11-13 of pregnancy and amniocentesis is done from 15 weeks.

NIPS (Prenatal Cell-Free DNA Screening -cfDNA) is a non-invasive screening test. It is designed to detect chromosomal abnormalities without risk during pregnancy. Fetal DNA is found in the mother's blood and if any changes are detected, invasive confirmation and genetic counselling is recommended (12). False-negative response is 1% for trisomy 21, 3.6% for trisomy 18 and 9.4% for trisomy 13. When the Y chromosome is found, the sex is determined. The main objective of genetic examinations –development of healthy generation through family planning. The main purpose of genetic research is to develop a healthy generation through family planning.

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## ROBERTSONIAN TRANSLOCATION PATIENT WITH RECURRENT MISCARRIAGE

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### ABSTRACT

Translocation is a type of chromosomal abnormality, which results in a chromosome breaks where the portion of it reattaches to a different chromosome. This includes balanced and unbalanced translocation, with two main types: reciprocal -and Robertsonian translocation. Robertsonian translocation is a type of translocation caused by breaks at or near the centromeres of two acrocentric chromosomes. The reciprocal exchange of parts gives rise to one large metacentric chromosome and one extremely small chromosome that may be lost from the organism with little effect because it contains few genes. The resulting karyotype in humans leaves only 45 chromosomes, since two chromosomes have fused together. Robertsonian translocation is one of the major chromosomal rearrangements with a prevalence rate of 0.1% of the general population and 1% of the infertile population. These chromosomal translocations are mainly observed in group D including 13, 14, 15 and group G including 21 and 22 chromosomes

**CASE:** A non-consanguineous couple (32-year old male and 26-year old female) with the complaint of repeated miscarriages attended to the "Afgin Genetic Diagnosis Center" for cytogenetic evaluation. They had a history of three repeated miscarriages in the past four years of their marriage. The first abortion was two months from pregnancy the second was a tubal pregnancy. The third was a missed abortion from a of 7-weeks pregnancy. None of the abortions got cytogenetically evaluated and there were no such histories of repeated abortions in any other family member.

**MATERIALS AND METHODS:** Two milliliters of peripheral blood was obtained from both partners in heparinized tubes to harvest white blood cells for karyotyping 30 to 40 metaphases were analyzed and the karyotype was interpreted using the Smarttype Karyotyper Analyzer. The chromosomes were identified and classified according to the guidelines by the International System for human Cytogenetic Nomenclature (ISCN, 2011)

**RESULTS:** In this study, we report a Robertsonian translocation rob (14; 15) in a female patient with a history of repeated miscarriages. The resulting balanced karyotype has only 45 chromosomes including the translocated one, which is the result of a fusion of the long arms of two acrocentric chromosomes. Chromosomal analysis of the male partner showed normal 46, XY karyotype. Chromosomal analysis revealed an abnormality in the female partner with 45, XX, rob (14; 15) (q10; q10) chromosomal constitution

**DISCUSSION:** The presence of a balanced chromosomal rearrangement in a parent results in an increased risk for structural chromosomal defects in future pregnancies. It is estimated that in about 70% of couples with at least two spontaneous abortions, one parent carries a balanced chromosomal rearrangement such as inversions, translocation, etc. The carrier of a Robertsonian translocation has a normal phenotype but is at risk of producing unbalanced gametes and, therefore, unbalanced offspring. In general, the prevalence of chromosomal abnormalities is higher in females than in males. Cytogenetic analysis of couples with recurrent abortions is mandatory to evaluate the probable presence of any chromosomal aberrations. This will offer valuable data for the appropriate genetic counseling strategies. Physicians should be aware of the condition as at least 5% of these couples with repeated abortions exhibit chromosomal abnormalities as the cause. Such cases have to be analyzed as early as possible to arrange for adequate genetic counseling and to allow couples to make an informed reproductive decision regarding subsequent pregnancies. Prenatal diagnosis should be offered to these couples in the case of future pregnancies.

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## GENOTYPE-PHENOTYPE OF NLRP3 GENETIC MUTATIONS OR POLYMORPHISMS

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### INTRODUCTION

Inflammasomes - are the essential part of the natural immune response and are multiple intracellular protein complexes that severely control different aspect of adaptive immunity. Generally, sensory molecules recognize different pathogenic(PAMP)or endogenous sterile distress signals(DAMP)they activate caspase-1 using adapter proteins such as ASC, leading to the processing and release of pro-inflammatory cytokines such as interleukin(IL)-1 $\beta$  and IL-18. NLRP3 inflammasome pathway is known to be the most researched among 7 different inflammasomes. Mutations detected in the NLRP3 gene which encodes the sensor protein chryphoria, are known to cause 3 different diseases.

### MATERIALS AND METHODS

Venial blood was obtained from 50 different patients and DNA were isolated. Mutagenesis was performed by sanger DNA sequencing methods and results were read by Finch TV, CLC Sequence Viewer – QIAGEN Bioinformatics and MEGA7 biomorpho -logical programs. Mortality evaluations were done according to NCBI, PolyPhen-2, INFEVERS and many different databases.

### RESULTS

2 synonymous mutations (Ala240Ala and Arg260Arg) were found in both of the study groups which classified as polymorphism 6 different mutations which were detected from the study group, were found to be related with conditions. 10 patients carried Gln703Lys mutation, 4 patients carried Ser726Gly mutation, 3 patients carried Val198Met mutation, 2 patients carried Ile313Val mutation, 2 patients carried Thr913Met mutation, and 2 patients carried Ser331Arg mutation.No mutations were found on NLRP3 gene in the healthy control group.

### DISCUSSION

One of the associated mutation found by us (Thr913Met) was new, and had been reported to INFEVERS database. Most of the mutations found to be located on the 3<sup>rd</sup> exon of NLRP3 gene as well as 4<sup>th</sup> and 8<sup>th</sup> exons.Mutations cause increase in function of NACHT, NAD, and LRR parts of the protein structure. The effect of each mutation on protein structure was further investigated.

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## THE ROLE OF HYPOXIA IN THE DEVELOPMENT OF SECONDARY BRAIN DAMAGE IN PATIENTS WITH BRAIN STROKES

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### ABSTRACT

Adverse outcomes of treatment of acute brain strokes are largely due, first of all, to the action of a hypoxia. The brain's own oxygen reserves are negligible, and even the short-term hypoxia can lead to hard consequences. There is no clear definition of parameters of assessing the oxygen starvation of the cell in the literature, which could unambiguously and reliably indicate the presence or absence of it. These parameters would exactly could determine the outcome of brain damage, remain insufficiently studied.

The aim of the study was to assess the diagnostic and prognostic significance of hypoxia criteria in patients with acute cerebral strokes.

### METHODS

A prospective method was used in the work. The study involves 75 patients. There are 62.6% with hemorrhagic strokes, and 37.3% with ischemic strokes. According to the outcome of the disease, regardless of the diagnosis, the groups of patients were identified: with a favorable outcome (54.6%) and with an unfavorable outcome (45.3%). The results were compared.

### RESULTS

The diagnostic criteria of hypoxia - indicators of LDH and CPK enzymes, lactate and glucose, parameters of ABS and blood gas composition by clinical groups at admission and at treatment stages.

It was noticed that the level of LDH on the 2nd day compared to the initial values was statistically significantly higher in both groups of patients: in the 1<sup>st</sup> day -by 6.5% ( $p = 0.005$ ), in the 2<sup>nd</sup> day -by 5.25% ( $p = 0.005$ ). On the 3<sup>rd</sup> day of the disease, the increase in LDH in the 1st group remained at 15.0% ( $p = 0.021$ ), while on the 2<sup>nd</sup> -at 31.5% ( $p = 0.05$ ). Although there was a slight decrease in LDH on the 4<sup>th</sup> day of the disease in the 1<sup>st</sup> group, but it exceeded the initial indicators by 11% ( $p = 0.022$ ). The same pattern had a place to be in the 2<sup>nd</sup> group of patients, where the LDH level decreased slightly, but remained elevated by 24.9% ( $p = 0.012$ ) on the 4th day, compared to the baseline values. The interesting fact is that during the study of CPK indicators, was noted that at admission of patients from both groups, there were significantly high levels of this enzyme ( $468.97 \pm 192.0$  and  $256.17 \pm 110.5$  g/l), compared to acceptable levels (less than 190 g/l). On the 2<sup>nd</sup> day, the 1<sup>st</sup> group had a statistically significant increase of 22.5% ( $p=0.040$ ), on the 3<sup>rd</sup> day of the disease, its level was 13.75%, higher than the initial one. Dynamically, on the 4th day, though there was a decrease of 67.36% from the initial level, this indicator remained above acceptable standards. In the 2<sup>nd</sup> group of patients with ischemic stroke, the CPK index compared to the baseline levels was as follows: on the 2nd day-there was a statistically significant increase of 26.4% ( $p=0.012$ ), on the 3rd day-it exceeded the baseline by 15.2% ( $p=0.003$ ), on the 4th day – a statistically significant decrease of 30.53% ( $p=0.009$ ). Thus, the study of LDH indicators showed statistically significant increases in both study groups of patients, both at their admission to the hospital and in the dynamics of their treatment. In the study of CPK, there was noted more significant increase in the 1<sup>st</sup> group of patients. However, more statistically dependent relationships were found in the group 2 of patients.

Studying the parameters of the CBS and gas composition, the following results were revealed: patients with compensated forms of acidosis were admitted in both groups, but in the 1st group, against the background of a slight increase in the level of carbonate ions to  $31.37 \pm 16.2$  mmol/l, the pH level was  $7.36 \pm 0.055$ , in the 2nd group: with acceptable HCO<sub>3</sub> indicators,  $26.31 \pm 6.17$ , the pH level was  $7.38 \pm 0.05$ . In dynamics, in the 1st group for 3<sup>rd</sup> -4<sup>th</sup> days there was a decrease of the partial pressure of oxygen in both to  $45.11 \pm 9.05$  mmHg. CT- $48.17 \pm 11.03$  mmHg. St, and a

statistically significant decrease in  $\text{HCO}_3$  to  $26.70 \pm 3.04$  -  $27.13 \pm 3.60$  mmol / l. However, the pH remained at the level of reliable compensation. In the 2nd group of the studied patients, the indicators of the gas composition of the blood underwent more significant changes. Against the background of reducing the oxygen partial pressure to  $44.24 \pm 13.43$  -  $47.93 \pm 10.19$  mmHg, according to the acceptable indicators of  $\text{pCO}_2$  and  $\text{HCO}_3$ , the pH level on day 3 reflected the presence of a decompensated form of respiratory acidosis in patients with pH of  $7.23 \pm 0.7$ , but this indicator was statistically insignificant.

Analysis of the diagnostic data of lactate and blood glucose shows that at the admission to the hospital they were at the initial state, all patients also had elevated levels of both lactate –  $2.41 \pm 1.5$  and  $2.06 \pm 1.58$  mmol/l, and blood glucose –  $8.78 \pm 2.94$  and  $7.97 \pm 2.32$  mmol/l. There was a gradual statistically significant decrease in the level of both lactate to  $1.32 \pm 0.7$  and  $1.03 \pm 0.3$  mmol/l, ( $p=0.001$ ) and glucose: up to  $7.31 \pm 1.29$  and  $6.56 \pm 1.02$  mmol/l ( $p=0.001$ ), dynamically on the 2-3-4 day of the disease in the 1<sup>st</sup> group of patients. Results of the study of LDH and CKD, lactate and glucose, CBS parameters and blood gas composition in patients with acute brain strokes with favorable and unfavorable outcomes. All admitted patients with brain strokes were diagnosed with initial hypoxia with hyperfermentemia, lactatemia, and hyperglycemia against the background of acidosis. In dynamics, during the treatment in a group of patients with a favorable outcome of the disease, the decrease in creatinephosphokinase was observed by 52.5%. On the contrary, there is an increase of LHD on 38.1 per cent, CPK by 34%, saving excess lactate in 2.2, glucose in 1.3 times against the background of decompensated acidosis from  $7.33 \pm 0.69$  in the group of patients with unfavorable outcome, which was due to more severe disease in this group of patients and the subsequent fatal outcome in the progression of the disease.

## CONCLUSION

Analyzing the dynamics of treatment of patients, with favorable and unfavorable outcomes, it was found out that the results of increased activity of diagnostic criteria for hypoxia (creatinephosphokinase, lactate dehydrogenase, lactate, glucose and decompensated acidosis) indicated a severe degree of hypoxic damage to brain tissue. Subsequently, they can serve as prognostically considerably diagnostic criteria for the adverse outcome of the disease in patients with acute brain strokes.

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## STUDY OF RHEOLOGICAL ASPECTS AND ATTEMPT TO THE THEORETIC RHEOLOGICAL RESULTS WITH GENETIC BUILDING IN THE HYPERTONIC PATIENTS AND IN THEIR FAMILY MEMBERS (HYPERTONIC)

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### ABSTRACT

We made a small clinical laboratory research with compliance with the Helsinki Declaration; we have used of a special method of informed consent between patients, doctor, which was approved by administration of research institutions. We selected patients who had hypertension within the age of 60 to 80 years old. We selected 12 families which consist of (mother, father, one child). Therefore, we have two group. I group - 19 patients with hypertension (some mother and father were not alive at moment of our research) and II group - the children of the patients with hypertension, III group - control (this group contents was 20 healthy adults same age).

We used an innovation Georgian method for counting RBC aggregation index. This method due on count the ratio of the area of aggregated of RBCs to the area of non-aggregated of RBCs in unit volume.

Process of aging is one of the most interesting issues for scientists, many different researches had been done to identify how different agents affects human body to decrease or loose the abilities which had before.

In this article we tried to explain this process from rheological view we, rheologists will try to find the answers through the way of fluid flow and their aspects in body, and the role of high blood pressure on patients with hypertension and their family members in this process. First we should consider that the process of aging in human body is not fully recognized yet, but there are some important factors such as DNA methylation which has big affect on this.

**Keywords:** aging, rheology, RBC aggregation, hypertonia

### INTRODUCTION

To start the research, we have made small clinical laboratory work with the compliance of the Helsinki Declaration [1]; We have used a special form of informed consent between patients, doctor, which was approved by administration of research institutions. We selected hypertension 12 families which consist of (mother, father, one child) and patients within the age of 60 to 80 years old. Therefore, we had two group. I group – consist of 19 patients with hypertension (some mother and father were die to moment of our research) and II group – which consist of their children - patients with hypertension, III group - control group (this group contents was 20 healthy adults in the same age). Detailing by age in table 1. We used an innovation Georgian method for counting RBC aggregation index. This method work is due to the counting the ratio of the aggregated area of RBCs to the non-aggregated area of RBCs per unit volume [2,3].

### RESULTS

In the table 1 Table 1: Averaged age in groups.  $M \pm m$

| Group   | Years  |
|---------|--------|
| Control | 71±3.2 |

|   |        |
|---|--------|
| Parents (men)                             | 81±2.7 |
| Parents (women)                           | 82±5.3 |
| Children (without differentiation of sex) | 63±2.8 |

Table 2: RBC aggregation index in three difference group (soma time with differentiation of sex and some time without differentiation of sex). M±m

| Parameter             | Control | Parents (Men) | Parents (Women) | Children |
|-----------------------|---------|---------------|-----------------|----------|
| RBC aggregation index | 31±4.0  | 45±6.2        | 44±7.2          | 55±4.8   |

Abbreviations: RBC - erythrocyte

## CONCLUSION

Statistically processed values are describing, that the RBC aggregation in in I group was less than in II group. But for both groups, the RBC aggregation rate was increased in a considerable amount to compare with the normal rate (control group). The presence of genotypic factors that influences the life time is proved by the fact that the process of RBC aggregation in blood of long-livers is slowed down. But in the children of these parents, who had hypertension, the aggregation capacity of the erythrocytes was much increased in comparison with their parents, despite the fact that aggregation usually depends on age, it is in direct proportion of the this function  $y=f(x)$  basically, the possible increase of hypertonia disease in relatives (especially first degree relative) of people who suffering from this disease. Continuation of the work in this direction is very important. It is necessary to look for new connections between hypertension and genetics in order to deepen biomedical fundamental knowledge, which may favorably affect practical cardiology and gerontology.

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## THE CONTRIBUTION OF GENETIC TESTING TO MEDICINE - THE EXPERIENCE OF GEORGIA

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### INTRODUCTION

Since the completion of the Human Genome Project, remarkable advances have been made in understanding the human genome's contribution to health and disease; the wealth of scientific discovery generated over the past 10 years is unparalleled in the history of biomedicine, and the rate of discovery is accelerating [1]. The traditional study of single gene disorders (genetic medicine) has developed into an understanding of how multiple genes interact with environmental factors: genomics [2].

Personalized medicine is health care that tailors interventions to individual variation in risk and treatment response. Although medicine has long strived to achieve this goal, advances in genomics promise to facilitate this process. Relevant to present-day practice is the use of genomic information to classify individuals according to disease susceptibility or expected responsiveness to a pharmacologic treatment and to provide targeted interventions. [3]

Inherited thrombophilia is a genetic disorder of blood coagulation resulting in a hypercoagulable state, which has been suggested as a possible cause of recurrent thromboembolism. Family and twin studies have established a heritable component to venous and arterial thrombosis. For the vast majority of patients, thrombosis is a complex, multifactorial disease caused by a combination of numerous, often unknown, environmental and genetic factors [4]. Since the placenta is rich in blood vessels, it is reasonable to infer that clot formation within the placental vasculature may result in placental insufficiency, intrauterine growth restriction and other complications. Intrauterine growth restriction (IUGR) is defined as estimated fetal weight below gestational age, according to ultrasound data, and birth weight below the 10th percentile of the birth weight for gestational age reference curve. Fetal growth restriction (FGR) affects 5–10% of pregnancies, leading to clinically significant fetal morbidity and mortality.

**Aim:** The aim of this study was to determine the intensity of connection between inherited thrombophilia (Factor V Leiden, Prothrombin G20210A and MTHFR C677T gene mutations) and IUGR.

### MATERIALS AND METHODS

48 Georgian women with pregnancy complication (IUGR of unknown origin) and 100 controls (women with three or more uncomplicated pregnancies) were investigated for detection of inherited thrombophilia (Factor V Leiden (FVL), Prothrombin (PTH G20210A) and Methylenetetrahydrofolatereductase (MTHFR C677T) gene mutations) by PCR analyses.

Studied gene mutation were detected by the molecular-genetics methods, which implied the following stages:

I. Extraction of genomic DNA: The genomic (nuclear) DNA was isolated from the peripheral blood leukocytes by a commercially available DNA extraction kit (Pronto Diagnostics).

For the detection of mutation in the extracted DNA, was used Pronto kits (Pronto Diagnostics, Israel) [5], which detects Single Nucleotide Substitution by a single nucleotide primer extension reaction, followed by Enzyme Linked Immuno-Sorbent Assay (ELISA).

II. Identification of mutation stages in genomic DNA

1. DNA amplification by Polymerase Chain Reaction (PCR), Gene Amp PCR System 9700 (Applied Biosystems) and Pronto BRCA Amplification Mix;
2. Detection of amplified DNA by gel-electrophoreses
3. Wild type and mutation-positive allele detection by a single nucleotide primer extension reaction using Gene Amp PCR System 9700 (Applied Biosystems) thermocycler;
4. Wild type and mutation-positive allele detection by Enzyme Linked Immuno-Sorbent Assay (ELISA);
5. Date detection by photometer-reader.

The PRONTO Product line is for *in vitro* diagnostic use and is accredited to the highest international quality standards of production including GLP/GMP, EN46001, ISO 9001 and ISO 13485 and is CE certified.

## STATISTICAL ANALYSIS

Statistical analysis was performed on SPSS v. 21 statistical software.. The difference was considered to be significant when  $p < 0.05$ .

## RESULTS

Relationships between IUGR and FVL (12.5% in patients and 0% in control;  $\chi^2(1, N=148)=13.028$ ,  $p=0.001$ ) and Prothrombin (8.33% in patients and 1% in control;  $\chi^2(1, N=148)=5.343$ ,  $p=0.038$ ) mutations were significant. Relationship between IUGR and MTHFR mutation (6.25% in patients and 1% in control;  $\chi^2(1, N=148)=3.399$ ,  $p=0.1$ ) was weak. The combined double and triple mutations were seen in 4 cases, which was not seen in control group. It is important to note that 46(95.83%) patients had a history of other pregnancy complications and thrombosis, 19(39.53%) patients had a history of stillbirth.

## CONCLUSION

There are few studies concerning the association between IUGR and thrombophilia. Our study reconfirms role of studied mutations (totally 23%) in IUGR and also in other pregnancy complications. Anticoagulation therapy in pregnant women produces specific risk at the time of delivery, where bleeding and clotting risks interface. Altered metabolism rates of anticoagulants in pregnant women should also be considered. Low-molecular-weight heparins (LMWHs) and unfractionated heparin are the mainstay of treatment, as they don't cross placenta. Further research should help to clarify who should receive thromboprophylaxis, how to prevent adverse pregnancy outcomes in women with inherited thrombophilia. Clinical trials on treatment are essential since they will provide physicians with the information to determine whether or how they should modify their clinical practice.

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## THE ROLE OF CYTOGENETICS IN ROUTINE CLINICAL APPLICATIONS

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### ABSTRACT

**Introduction:** Chromosomal irregularities include numerical and structural chromosomal anomalies and are examined by cytogenetic methods. These methods include conventional cytogenetic and molecular cytogenetic methods. In this study, it was aimed to detect numerical and structural anomalies that are encountered more frequently with cytogenetic methods and to discuss possible consequences of genetic diseases after clinical evaluation.

**Method:** In this study, 286 cases with postnatal chromosome analysis indication were included. Phytohemagglutinin (PHA) -induced peripheral blood lymphocytes cultures were used for the study. The chromosomes of 20 G-banded metaphases (500-550 band level) were examined for numerical and structural chromosome abnormalities. Twenty G banded metaphases were evaluated according to the 2016 International System for Human Cytogenetic Nomenclature (ISCN).

**Results:** Chromosome anomalies were detected in 30 cases (9%). 20 cases (6,9%) of them were trisomy 21 (down syndrome), 8 cases (2,7%) were gender anomaly, and 2 cases (0.6%) were balanced translocation carriers.

**Conclusions:** Cytogenetic analysis results are consistent with the rates reported in the literature. Balanced translocation carriers are healthy but unbalanced gametes may occur during gametogenesis. Therefore, preimplantation (PGD) genetic diagnosis is recommended in all pregnancies and pregnancy follow-up is recommended. The family is given genetic counseling.

**Keywords:** cytogenetic, traslocations, choromosome analysis,

### INTRODUCTION

Clinical cytogenetics is the study of the number, structure and heredity of chromosomes. Chromosome disorders form a major category of genetic disease. They account for a large proportion of all reproductive wastage, congenital malformations, and mental retardation and play an important role in the pathogenesis of malignant disease(1). Clinical Indications for Chromosome Analysis;

- Problems of early growth and development
- Stillbirth and neonatal death
- Fertility problems
- Family history
- Neoplasia
- Pregnancy in a woman of advanced age

Mutations in the genetic material can sometimes cover a very large area of the chromosome. If this irregularity is large enough to be observed in the light microscope, it is defined as "chromosomal anomaly or irregularity". There are 2 types of chromosomal anomalies; numerical and structural anomalies. They occur at different stages of the cell cycle. Numerical chromosomal anomalies are the number of chromosomes in the cells more or less and these are polyploidy and aneuploidy. Polyploidy is the increase in the number of chromosomes in cells by the exact number of the haploid number. Haploid chromosome number is expressed as "n" and is equal to the number in gamet cells. Aneuploidy, on the other hand, is called the increase or decrease of the basic chromosome number that is not the multiple of it (2). Aneuploidy is the most common and clinically significant type of human chromosome disorder, occurring in at least 5% of all clinically recognized pregnancies. Most aneuploid patients have either trisomy (three instead of the normal pair of a particular chromosome) or, less often, monosomy (only one representative of a particular chromosome). Either trisomy or monosomy can have severe phenotypic consequences. trisomy for a whole chromosome is rarely compatible with life. By far the most common type of trisomy in liveborn infants is trisomy 21 (karyotype 47,XX or XY,+21), the

chromosome constitution seen in 95% of patients with Down syndrome (1,3). Monosomy for an entire chromosome is almost always lethal; an important exception is monosomy for the X chromosome, as seen in Turner syndrome. It is known that the most common chromosomal mechanism is meiotic nondisjunction. This refers to the failure of a pair of chromosomes to disjoin properly during one of the two meiotic divisions, usually during meiosis I. The consequences of nondisjunction during meiosis I and meiosis II are different (4). Structural rearrangements result from chromosome breakage. Structural abnormalities are present in about 1 in 375 newborns. Structural anomalies constitute 21% of all chromosomal anomalies. Structural rearrangements are defined as balanced, if the chromosome set has the normal complement of chromosomal material, or unbalanced, if there is additional or missing material (5). Unbalanced rearrangements; deletions, duplications, marker, ring chromosomes, isochromosomes and dicentric chromosomes. Duplication of part of a chromosome leads to partial trisomy; deletion leads to partial monosomy. Any change that disturbs the normal balance of functional genes can result in abnormal development (6). Large deletions or duplications involving imbalance of at least a few million base pairs can be detected at the level of routine chromosome banding, including high-resolution karyotyping. Detection of smaller deletions or duplications generally requires more sophisticated analysis, involving FISH or microarray analysis (7). Balanced rearrangements; inversions (paracentric or pericentric), translocations (reciprocal translocations or robertsonian translocations) and insertions. Reciprocal translocations are mutual displacement of fragments broken in homologous and non-homologous chromosomes. Robertsonian translocation is the changes that occur between acrocentric chromosomes (13,14,15,21,22). Molecular cytogenetics provides a much higher-resolution study of chromosome structure and allows chromosomal rearrangements to be identified in nondividing cells. It is likely that new syndromes will be delineated due to very subtle changes of chromosome structure in the near future, further expanding the clinical role of cytogenetic analysis(8). The most common indications for postnatal analysis are the occurrence of a recognized chromosomal syndrome, the occurrence of multiple congenital anomalies or developmental problems suggestive of a syndrome, a history of recurrent miscarriage, or a family history suggestive of a possible chromosomal abnormality(4).

## METHOD

Chromosomal analysis is integrated into the routine practice of medicine. The identification of a chromosomal abnormality may provide a diagnosis, prognostic information, and knowledge of genetic recurrence risks for a family. Cytogenetic analysis is most effectively performed if the cytogeneticist is aware of the indications for testing and can customize the study to the clinical question. Although a standard level of scrutiny is expected for all routine studies, in many instances special studies or focus on specific chromosomal regions may be clinically appropriate. "Routine" chromosomal analysis consists of counting chromosomes in 20 cells and karyotyping two cells (1). These karyotypes are banded at the 400 band level or better. Most laboratories use G-banding for routine analysis, although Q- or R-banding may be used in some laboratories (9). Chromosome analysis was performed from the peripheral blood sample according to the appropriate protocol. Phytohemagglutinin (PHA) -induced peripheral blood lymphocytes cultures were used for the study. The chromosomes of 20 G-banded metaphases (500-550 band level) were examined for numerical and structural chromosome abnormalities. The twenty metaphase areas stained with giemsa trypsin banding technique were evaluated according to the 2016 International System for Human Cytogenetic Nomenclature (ISCN). The karyotype results are normal but those with continuing clinical suspicion were examined by advanced methods (fish, array).

## RESULTS

Chromosome anomalies were detected in 30 cases (9%). 20 cases (6,9%) of them were trisomy 21 (down syndrome), 8 cases (2,7%) were gender anomaly, and 2 cases (0.6%) were balanced translocation carriers. Only one of 20 cases with down syndrome showed mosaic structure (46, XX / 47, XX + 21). Two of the Turner syndrome-related cases show mosaicism (45, XO / 46, XX). In the cases where mosaic was detected, the number and the ratios of 100 cells were determined by means of the FISH method. The karyotype of 45, XO / 46, XX del (X) in one case and 45, X / 46, X, i(X) karyotype in one case were detected. 46 XX, chromosome structure were detected in one male patient. In this case, 21 hydroxylase enzyme deficiency was detected. Klinefelter's syndrome was found in two identical twins brothers (47, XXY). The karyotype of 46, XX, t (12; X)(q24.3;q22) and the other 45, XY, t (13; 14) (q10; q10) were detected in one of the two cases with balanced translocation carrier. The chromosome analysis of the parents who were translocation carriers was also performed, it was seen to appear as de novo, there was no transition from the family. In cases with

different clinical additional findings, microarrays were planned for cases that could not be explained by chromosome analysis.

## CONCLUSION

Chromosome analysis is the first step for genetic diseases. Cytogenetic analysis results are consistent with the rates reported in the literature. In this study, clinical features of Down syndrome patients were also evaluated. Almost all of the cases were given genetic counseling in our center. With the definitive clinical diagnosis, consultancy should be given to the family appropriately. Clinical violence may be variable in cases with mosaic. In this case, a milder phenotypic effect is expected in mosaic patients and disease course includes variability. Detection of numerical and structural chromosomal anomalies is informative in terms of risk assessment while having pregnancies and children. Cytogenetic study was performed in cases with Down Syndrome pre-diagnosed and down syndrome was detected in 78.1%. Similarly, in our study, we evaluated down syndrome by cytogenetic analysis and found it compatible(10). In a study, 21% chromosomal anomaly was detected in a cytogenetic study in primary amenorrhoea patients(11). We detected chromosome anomaly in patients with suspected gender anomaly similar to this study. Karyotype analysis is required for patients with suspected chromosomal anomaly or whose cause cannot be elucidated. Thanks to cytogenetic studies, early diagnosis and treatment can increase the quality of life of the patients, but also reduce the problems they will experience to the lowest possible level. Translocations occur best with cytogenetic methods, especially they can be detected by chromosome analysis, the conventional cytogenetic method. It is very important to detect balanced translocation carriers. Because, balanced translocation carriers are healthy but unbalanced gametes may occur during gametogenesis. Therefore, preimplantation (PGD) genetic diagnosis is recommended in all pregnancies and pregnancy follow-up is recommended. The family is given genetic counseling.

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## THE ROLE OF PREIMPLANTATION GENETIC DIAGNOSIS IN ASSISTED REPRODUCTIVE TECHNOLOGIES PROGRAM

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### ABSTRACT

The correct selection of embryos is one of the main predictors of the success of extracorporeal fertilization. To date, this is performed visually by an embryologist using a high-resolution microscope, that is, a so-called "morphological" analysis of embryos is carried out.

But the introduction in recent years of the procedure of preimplantation genetic diagnosis shows that not always morphologically qualitative embryos can be genetically complete or vice versa. Preimplantation genetic diagnosis is the diagnosis of genetic diseases in a human embryo before implantation into the uterine wall, that is, before the stage of the transfer in the program of extracorporeal fertilization.

Typically, for analysis, a single blastomer biopsy is performed in an embryo in the division stage (6-10 blastomers) or a trophoctoderma (outer layer of cells) biopsy in the blastocyst stage (fifth day of embryo development). The main advantage of preimplantation genetic diagnostics is that when using it there is no selective termination of pregnancy, and the probability of having a child without a diagnosed genetic disease is quite high [3 .8].

The literature presents contradictory data on the plating effect of preimplantation genetic diagnosis on outcomes of extracorporeal fertilization [1, 5, 7]. Some authors say that preimplantation genetic diagnosis does not increase the chances of pregnancies in extracorporeal fertilization, while others note a high frequency of detection of genetic abnormalities in this procedure. But world statistics show that despite the increasing use of preplantation genetic diagnostics, the rate of pregnancy in assisted reproductive technology does not exceed 35-40% [2, 4, 6]. Therefore, it is of interest to study the structure of chromosomal disorders of embryos on the basis of preimplantation genetic diagnosis in the program of assisted reproductive technologies, as well as the impact of this procedure on the results of pregnancies.

### MATERIAL AND METHODS

The chromosomal pathology of embryos was studied in 86 women with different outcomes of extracorporeal fertilization. Preimplantation study of embryos was performed by FISH method in 42 women with positive results of *extracorporeal fertilization* and in 44 women with negative results of *extracorporeal fertilization*. The quality of the embryos was evaluated on the third day of cultivation. All patients were subjected to a special examination before extracorporeal fertilization: hormonal profile, infectious status, peripheral karyotype, hysterosalpingography, hysteroscopy with pathohistological examination of endometrial biopsy were studied. In men, it was mandatory to study sperm with morphological sperm index, genetic sperm analysis (FISH) and DNA fragmentation. The criteria for exclusion were women with monogenic diseases and men with pronounced forms of pathozoospermia. Controlled ovarian hyperstimulation was carried out according to standard antagonist protocol from the 2nd-3rd day of menstrual cycle with recombinant follicle-stimulating hormone preparations in combination with human menopausal hormone preparations. The eggs were taken 35-36 hours after the ovulation trigger was introduced. All patients received intracytoplasmic sperm injection (icsi method). The embryo biopsy was performed on day 3 after fertilization at the blastomer or blastocyst 6-10 stage. In-situ fluorescence hybridization was used to detect numerical and structural chromosomal disorders. Statistical data processing was performed using the SPSS statistics 17.0 application package. To assess the significance of intergroup differences of several independent samples, the Kruskal-Wallis test was investigated. In the case of two samples, the Mann-Whitney U-criterion for unrelated sets was applied. Evaluation of compliance of inserted parts of genotypes with Hardy-Vastiberg law was carried out by  $\chi^2$  criterion in comparison with expected frequencies of genotypes of equilibrium distribution. The reliability of differences in the frequency of occurrence of qualitative signs was determined by the  $\chi^2$  criterion.

## RESULTS

The results of the study on the characteristics of embryos subjected to preimplantation diagnosis are shown in Table 1. A total of 220:111 embryos in group A and 109 embryos in group B. The patients of each of the study groups were divided into subgroups by age under 35 years and over 35 years. In Group A, the number of embryos subjected to preimplantation diagnosis was 52 among women under 35 and 59 for women over 35. In Group B, women under 35 had 48 embryos subjected to preimplantation diagnosis, and women over 35 had 61 embryos.

Table 1. Characterization of embryos subjected to preimplantation diagnosis

| Indicator                                     | Group A n=42 |      | Group B n=44 |        | Total n=86 |
|---|--------------|------|--------------|--------|------------|
|   | < 35 years   |      | > 35 years   |        |            |
|   | abc          | %    | abc          | %      | abc %      |
| In total embryos, the undergone PD            | 52           |      | 59           |        | 220        |
| No embryo pathologies by chromosomes          | 36           | 69,2 | 35           | 59,3   | 106        |
| Embryo pathology by chromosomes available     | 16           | 30,8 | 24           | 40,7   | 114        |
| Embryo pathology by chromosomes within groups | 40           | 36,0 | 74           | 67,9** | 114        |

Note: \*- \*\*  $p < 0,05-0,01$  compared to group A of identical age

A study of the structure of the chromosomal pathology of viable embryos in comparison groups showed the following (Table 2). Thus, in group A, 41.7% embryos were diagnosed with 21 trisomy (Down syndrome), in group B, this syndrome was observed in 40.0% embryos ( $p > 0.05$ ). Patau syndrome (trisomy 13) and Edwards syndrome (trisomy 18) were diagnosed in 25.0% and 16.7% viable Group A embryos, comparable to similar data in Group B, where the frequency of the said syndrome diagnosed in embryos was 20.0% and 13.3% ( $p > 0.05$ ), respectively. There was no significant difference in the frequency of Klinefelter syndrome (XXY) and polysomy Y (XYY) in viable embryos ( $p > 0.05$ ).

Table 2. The nature of chromosomal pathology in pathological viable embryos

| Viable embryos                  | Group A n=42 |      | Group B n=44 |      | Total    |
|---------------------------------|--------------|------|--------------|------|----------|
|                                 | 12 abc       | %    | 15 abc       | %    | 27 abc % |
| Klinefelter syndrome (XXY)      | 0            | 0    | 1            | 6,7  | 1 3,7    |
| Turner's syndrome (X0)          | 1            | 8,3  | 1            | 6,7  | 2 7,4    |
| Daun syndrome (trisomy 21)      | 5            | 41,7 | 6            | 40,0 | 11 40,7  |
| Patau syndrome (trisomy 13)     | 3            | 25,0 | 3            | 20,0 | 6 22,2   |
| Edwards's syndrome (trisomy 18) | 2            | 16,7 | 2            | 13,3 | 4 14,8   |
| Polysomy Y (XYY)                | 1            | 8,3  | 2            | 13,3 | 3 11,1   |

Thus, the study of the preimplantation characteristic of embryos in the in vitro fertilization program revealed a higher value for embryos without chromosomal pathology in the group with effective in vitro fertilization and lower values for the

relative frequency of embryos with chromosomal pathology compared to the group with negative results of in vitro fertilization.

In a group with a favorable outcome of extracorporeal fertilization, women over 35 were more likely to encounter viable embryos and less likely to encounter non-viable embryos. The nature of chromosomal pathology in the women studied showed no reliable difference between comparison groups.

A fairly large number of morphologically normal but genetically abnormal embryos are also determined. If preimplantation genetic diagnosis were not carried out, the choice of embryologist would undoubtedly fall on those embryos that have reached the blastocyst stage. As a result, this would lead to a negative *extracorporeal fertilization* result.

At the same time, there are also those embryos that have been genetically healthy but morphologically disabled. All these data indicate that, in order to obtain a high-quality embryo and positive *extracorporeal fertilization* results, it is necessary to improve both the protocols of controlled ovarian hyperstimulation, the drugs used, the embryological stage and the preimplantation genetic diagnostics procedure itself.

Despite the conflicting data, the analysis of the world literature data and the results obtained by us during the study showed the great advantages of preimplantation genetic diagnostics.

Having the wide diagnostic capabilities of preimplantation genetic diagnostics within the framework of assisted reproductive technology, it is possible to select and transfer embryos with the absence of chromosomal pathologies into the uterine cavity, reduce the risk of miscarriage and multiple pregnancy, and also increase the chances of successful implantation and the birth of a healthy baby.

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## THE SMPD1 GENE MUTATIONS IN TWO SIBLINGS WITH NIEMANN-PICK TYPE A/B DISEASE

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Purpose of work. Niemann–Pick type A/B disease in children was investigated. Niemann–Pick type A disease is the most severe form that begins in infants, characterized by an increase in the hepatosplenomegaly and progressive damage to the nervous system. Type B is characterized by a chronic course, hepatosplenomegaly, the nervous system is usually not involved. Both types are associated with mutations in the SMPD1 gene and sphingomyelinase deficiency. In addition to these, there is an increased level of cholesterol and lipids in the blood and thrombocytopenia. The sphingomyelinase breaks down sphingomyelin into ceramide and phosphocholine in lysosome membranes, and its deficiency leads to excessive accumulation of sphingomyelin. The SMPD1 gene occupies a locus on chromosome 11p15.4-15.1. The disease is inherited as an autosomal recessive trait (1, 2, 3, 4).

Material and methods. Study materials were collected on DBS cards as dried venous blood spots from Republic Children's Clinical Hospital departments and Baku Health Center. The method of tandem mass spectrophotometry was used to determine the activity of acid sphingomyelinase in dried blood stains. Using direct automatic sequencing, all 6 coding exons of SMPD1 gene were studied in the ARCHIMED Life laboratory (Austria). General blood analysis and ultrasonic scan were made with common methods.

Patients A.L. (born 2000) and A.Z. (born 2006) live in the city of Ganja. Those born from a blood-related marriage are a cousin of a parallel type. Low activity values of the acid sphingomyelinase enzyme in dry blood spots were obtained for A.L.- 0.29  $\mu\text{mol/liter hour}$  for A.Z.- 0.3  $\mu\text{mol/liter/hour}$  (norm> 0.9  $\mu\text{mol/liter/hour}$ ).

Results. Genetic analysis of a DNA sample extracted from the blood of patients A.L. and A.Z., revealed two mutations of the SMPD1 gene: G→A transversion at position 1345 (1345G→A) and cytosine nucleotide duplication at 188 position (188dup. C). Both siblings have a double heterozygous state for two different mutations - 1345 G→A/188 dup. C, which are characteristic of Niemann–Pick disease type A/B.

The replacement of the guanine nucleotide with the adenine nucleotide at position 1345 in exon 5 of the SMPD1 gene in the heterozygous state leads to the replacement of the glutamine amino acid with lysine at position 449 (Glu449 Lys) in the patient protein.

A mutation of 188 dup. C in the first exon of the SMPD1 gene in the heterozygous state was also identified. Mutation in the protein leads to the replacement of the amino acid leucine with serine at position 64 (Leu 64 Ser).

Conclusion. Thus, both siblings have a double heterozygous state for two different mutations of the SMPD1 gene - 1345 G→A/188 dup. C. Therefore, the identified mutations of the SMPD1 gene indicate Niemann–Pick type A/B disease.

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## BIOCHEMICAL POLYMORPHISM, GENETIC HETEROGENICITY, GENE MAPING AND PREVENTION OF MUCOPOLYSACCHARIDOSIS HEREDITARY DISEASE IN THE POPULATION OF THE REPUBLIC OF AZERBAIJAN

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**The purpose of the work:** is to study the biochemical polymorphism, genetic diversity of mucopolysaccharidosis lysosomal hereditary disease in the population of the Republic of Azerbaijan, to compile a gene map of the disease for the population of the Republic and to develop the ways to prevent this disease.

**Materials and methods:** It was discovered within expeditions in the regions of the Republic of Azerbaijan, during genetic screening among sick children treated at the CRH. Fluorimetric method and liquid chromatography were used for genetic screening.

**Results:** Lysosomal enzymes for genetic screening of myggp polysaccharidosis hereditary diseases:  $\alpha$  - L - iduronidase, Iduronatsulfatase, Heparan - N - sulfatase,  $\alpha$  - N - acetylglucosaminidase,  $\alpha$  - N - acetyl - CoA: Glycosamine - acetyltransferase, N - acetylglucosamine - 6 sulfatase, Galactosamine - 6 sulfatase,  $\beta$  - galactosidazole, Arylsulfatase B,  $\beta$  glucuronidase, N - acetylglucosamine -1- phosphotransferase activity were studied.

Direct sequencing of IDS, HGSNA, GALNS and ARBS genes was performed by the Senger method. By using the method, disease-causing mutations within the IDS, HGSNA, GALNS, and ARBS genes were identified. The method was developed in the laboratory of GENTOGENE, Rostock, Germany.

**Conclusion:** Based on the results of our biochemical and molecular genetic research, treatment and prevention methods will be developed separately for each type of mucopolysaccharidosis.

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## PROGNOSTIC SIGNIFICANCE OF PD-L1 STUDIES IN CERVICAL DYSPLASIA

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### ABSTRACT

The article is included the clinical and morphological features of 35 patients who had been diagnosed and treated with cervical dysplasia and reflected the immunohistochemical results of PD-L1 expression in atypical cells. By standardized immunochemical methods, receptor levels vary depending on the degree of intraepithelial dysplasia. In patients with CIN I, cells were not stained at all (negative PD-L1 reaction), PD-L1 expression in CIN II was 1+ and 2+ in 76.9±5.6% , and 3+ in 23.1±5.3% of patients ( $p<0.05$ ). In patients with CIN III, 3+ cases (severe staining) were detected in 66.7±4.2% and cases of 2+ (moderate staining) in 33.3±4.9% of women. Thus, expression of the PD-L1 receptor, known as anti tumor immunity regulator is closely related to dysplastic processes that begin in the tissue. By other words, the detection this receptor expression in patients with CIN diagnosis(especially CIN III) can play an important role in the selection of treatment tactics and assessment of prognosis.

Cervical cancer(CC) accounts for 6.6% of malignant neoplasms among women, with more than 57,000,000 new cases being diagnosed worldwide each year, and the number is steadily increasing (1,2,3). Detection of precancerous diseases in CC screening is one of the key mechanisms to combat this problem (4). For this reason, diagnosis, adequate treatment, and prognostic assessment of cervical intraepithelial neoplasia (CIN) are of great importance in the management of CC.

**The purpose of the study.** To determine the relationship between PD-L1 receptor expression detection and histological grade in cervical dysplasia.

**Materials and methods.** Results of diagnosis of 35 patients with CIN that conized were included in to the study. There was performed standart immunocytochemical method (VENTANA Bench Mark Ultra) using PD-L1-monoclonal rabbit antibodies (VD21R, Medaysis firm) was applied newly obtained incisions after deparaffinization. The reaction results are evaluated by the number of staining cells as follows: 1+ - the number of poorly stained cells is less than 10%; 2+ - moderate staining in 10% of cells; 3+ - Strong staining in ≥10% of cells.

**Results.** Patients ages 28 to 61 years, with an average age of  $37.3 \pm 2.6$ . The majority of patients were women between the ages of 28 and 49 - 32 (91.4 ± 1.9%), 20 of them ( $57.1 \pm 2.4\%$ ) were women between the ages of 28-39. Only one woman had a diagnosis of CIN between the ages of 60-69 ( $2.9 \pm 3.2\%$ ,  $p<0.05$ ). Thirty ( $85.7 \pm 3.7\%$ ) of the patients were reproductively activite, and 5 ( $14.3 \pm 4.0\%$ ,  $p<0.05$ ) were in the pre- or menopausal period. In 31 of the women ( $88.6 \pm 2.6\%$ ), no complaints were reported, and the rest reported mucosal-bloody vaginal discharge ( $11.4 \pm 2.9\%$ ). All patients underwent cytological examination, colposcopy and biopsy on their cervix. The results of the cytological examination in 30 cases ( $85.7 \pm 4.1\%$ ) coincided with subsequent histological findings. Of these, 26 ( $74.3 \pm 2.8\%$ ) were diagnosed with HSIL (high grade intraepithelial squamous lesion), and 4 ( $11.4 \pm 2.7\%$ ) were diagnosed with LSIL (low grade intraepithelial squamous lesion). After histological examination, patients were divided into 3 groups: CIN I - 7 ( $20 \pm 3.5\%$ ), CIN II - 13 ( $37.1 \pm 4.5\%$ ), and CIN III - 15 ( $42.9 \pm 2.8\%$ ). ill. In 12 ( $34.2 \pm 3.4\%$ ) of patients, the depth of epithelial damage was up to 1.4 mm, 9 of them ( $75.0 \pm 5.1\%$ ) were women in the CIN III group. This is  $60.0 \pm 3.9\%$  of the incidents in the group. Immunohistochemical staining was not recorded during CIN I; About in CIN II - PD-L1 expression was 1+ in 6 cases ( $46.1 \pm 5.2\%$ ), 2+ in 4 ( $30.8 \pm 3.7\%$ ) and 3 ( $23.1 \pm 5.3\%$ ) ,  $p<0.05$  was rated as 3+. CIN III was defined as 3+ in 10 patients ( $66.7 \pm 4.2\%$ ,  $p<0.05$ ) and 2+ in 5 ( $33.3 \pm 4.9\%$ ), with no staining reported. In PD-L1-positive cases, acute expressed koilocytosis was observed. Lymphocytar infiltration was also reported in light microscopy analysis.

**Final.** In our study CIN was most commonly found in young, reproductively-active women ( $85.7 \pm 3.7\%$ ,  $p<0.05$ ). The depth of intraepithelial invasion depends on the degree of dysplasia, and in  $60.0 \pm 3.9\%$  of patients with CIN III it was 1.4 mm. PD-L1 expression is observed in atypical cells, which varies depending on the degree of dysplasia. In case of negative reaction in CIN I, strong staining (3+) in CIN II was observed in 3 cases ( $23.1 \pm 5.3\%$ ) and in CIN III at  $66.7 \pm$

4.2% (10 cases) shows strong staining ( $p < 0.05$ ) and severe koilocytosis. In addition, during CIN III, poor staining cases were not reported at all. Although we encounter lymphocytic infiltration in microscopic imaging, the relationship between this symptom and degree of dysplasia should be investigated in detail.

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## MOLECULAR BASIS OF HEMOGLOBINOPATHIES IN AZERBAIJAN

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**Introduction:** Thalassemias are defined by the absence or decrease of globin chain(s), which may lead imbalances of globin chains, ineffective erythropoiesis, hemolysis, and eventually to a variable degree of anemia. With the estimated carrier frequency of  $\beta$ -thalassemia is 4%–8.6% in country, the importance of premarital genetic testing, informed family counseling and preventive medical treatment cannot be overstated <sup>1-4</sup>. Screening for traits by hemoglobin electrophoresis and biochemical analysis has become a routine application test prior to the issuance of a marriage license throughout Azerbaijan.

**Methods:** We aimed to evaluate prevalent mutations spectrum, the effect and their co-inheritance and/or role of polymorphisms (BCL11A, HBS1L-MYB and *Xmn1* SNP) on disease phenotype by using reverse dot-blot hybridization using customized strips, sequencing analysis of the complete genes and MLPA.

**Results:** We report here a summary finding of *HBB* gene analysis for 265 patients and *HBA1-HBA2* genes analysis for 28 patients, along with their family members totaling 570 individuals.

**$\beta$  thalassemia** - 66 were found to have homozygous, 76 possessed compound heterozygous and 80 possessed heterozygous mutations. Overall, a total of 416 alleles were found to have 34 mutations. The first 20 frequented mutations covered 87% of the entirety of all mutations. The summary range is as following: c.25\_26delAA (p.Lys9Valfs) 27.9%; c.93-21G>A (IVS1+110G>A) 11.3%; c.315+1G>A (IVS2+1G>A) 8.9%.

**$\alpha$  thalassemia** - Genotyping of 45 alleles revealed 9 mutations, the 3.7 kb deletion is the most frequent mutation (35.6%), followed by 20.5 kb deletion (24.4%) and  $\alpha 2$  polyA2 (*HBA2*: c.\*92A >G, 13.3%).

**Conclusions:** The results may help inform decisions in the design and implementation of prevention strategies and diagnostic approaches. Through examination of the cumulative results we established the distribution pattern of hemoglobinopathies in Azerbaijan which facilitated a more focused molecular genetic approach to prevention. Wide range sequence analysis of *HBB* gene from 5' promoter (-150) to 3' promoter region (\*150) provide additional contribution to diagnose and whole gene sequencing is important to clarify of patient with thalassemia intermedia clinical findings. We will demonstrate in presentation that despite the high degree of molecular heterogeneity, the above approach, combined with the advent of PCR-based techniques and improved methodologies of early fetal sampling have made heterozygote screening and prenatal diagnosis of feasible in Azerbaijan.

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## KOSOVO DURING 1989-1999

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### ABSTRACT

The people of Kosovo are rightly described as people who have suffered through many years of injustice. Kosovo's path to liberation and detachment from Serbian injustice was difficult and with profound consequences. Kosovo's efforts for freedom and independence were long-lasting, but they took an active start during the 1980s and 1990s. The abolition of Kosovo's autonomy given to it by the constitution of 1974 enabled Kosovo to actively begin the path to liberation and independence.

This written material interlinks the events that took place during 1989-1999 that followed Kosovo until its liberation. Protests ranging from miners to students of the University of Prishtina, the Constitution of Kacanik, then political movements, military movements alluding to the KLA's emergence, talks and various international efforts to find a solution more peaceful on the Kosovo's issue, the Rambuje talks, the Reçak massacre and many events that followed this journey of Kosovo alluded to NATO's humanitarian intervention and the Kosovo's issue to appear before the world as a perennial injustice to an innocent country and population.

Milosevic's capitulation through the intervention of NATO and the international community led Kosovo to embark on a new journey through the international protectorate and transition that would allude to Kosovo's independence.

The purpose of this analysis is to highlight the challenges that Kosovo has faced over the years, with an emphasis on the political and diplomatic level. Through descriptive, analytical and comparative methods we will attempt to elaborate on Kosovo's journey through 1989-99, as well as to highlight the significance of events during this decade, events which influenced the Kosovo's process to result in liberation and later with the proclamation of the independence of Kosovo.

Keywords: Kosovo, protests, massacres, talks, KLA, NATO intervention

### INTRODUCTION

Kosovo's road from 1989 to 1999 constitutes a period, a field of many difficulties and problems that weighed on the back of the people of Kosovo. During this time Kosovo and its problem became a matter of daily life, which was covered with problems, challenges and injustice to it.

The narration of Kosovo's journey from 1989-1999 aims to unveil and analyze the political path that Kosovo pursues during this period, as well as the events and decisions that were made during this period that influenced in Kosovo's favor or disadvantage.

Since the abolition of autonomy until 1999, the Kosovo's issue has taken on a different shape and importance. During this period, the ruling, academic, and political elite up to the people of Kosovo set in motion to liberate the country from occupation and build an independent and free country.

Thus the journey of Kosovo during this period can be divided into several stages which were characterized by the development of events within them. We have divided this period into three phases in which the events have been elaborated, coordinating them with their significance and time period.

The years 1989-1995 are characterized by the abolition of Kosovo's autonomy, the Constitution of Kacanik which defined Kosovo as an independent state, a referendum held by the people of Kosovo, the education of Kosovo Albanians, and the movement of intellectual circles.

The years 1995-1998 are characterized by the events of the Dayton Accord which ignored the Kosovo issue and devoted itself only to Bosnia and Herzegovina. From Dayton, Kosovars received the message that the course of policy and action from peaceful ones should be shifted to more concrete and tougher actions. During this period the KLA came out openly. These years followed the events of student demonstrations, which continued later with the attacks on Prekaz against the Jashari family. This period was characterized by massacres and attacks on innocent populations. The Milosevic-Rugova meeting also happened during this period. At the same time, the involvement of the international community became more active which continued to show its importance and role in the Kosovo issue.



The years 1998-1999 were marked and crowned with the massacre of Rečak, which became a major driver of the international community's military actions against the Milosevic regime. Further the Rambuje Conference was held, which yielded no results. Then the international community, NATO launched attacks on the Milosevic regime, which then capitulated. At the same time, the UN Security Council adopted Resolution 1244, and Kosovo began another period after its liberation, a period which would be that of state-building .

## THE YEARS 1989-1995

The Serbian government has historically aimed to annihilate "everything Albanian" in Kosovo. Indeed, "planned crime and genocide, prepared 'scientifically' and professionally and institutionally implemented ", has begun to be systematically implemented in Kosovo after World War I to culminate in Slobodan Milosevic's Serbian regime. Thus, "promoting hate speech towards the Albanian people ", especially during the thirties and nineties of the last century, could only lead to the denial of the right of Albanians to exist. (Stavileci, 2005)

The efforts of the people of Kosovo to create an independent state date back a long time, but they took on the form of a political organization and other forms mainly from the late 1980s and early 1990s, when the breakup of the former Yugoslavia began. The years 1988-1989 date back to mass protests by Albanians against Serbia's policies that were repressive of Kosovo, resulting in Slobodan Milosevic's removal of Kosovo's autonomy, which returned Kosovo as part of Serbia.

Replacement of leaders (Azem Vlasi and Kaqusha Jashari), Albanians in Milosevic's party mechanisms in the province, and their replacement (with Rahman Morina) sparked protests. Trepca miners played a key role in the protests, who went on strike calling for a list of demands: the list demanded the resignation of Rahman Morina and the cancellation of Milosevic's "discriminatory policy ", though the first demand was "that not to give up the fundamental principles of the 1974 Constitution. " (Malcolm, 2011). It is believed that the miners' strike was the one that shook the foundations of the former Yugoslavia.

In 1989, the Assembly of Kosovo met in extraordinary circumstances under pressure from forces and tanks, which alluded to the abolition of the Autonomy of Kosovo. After the violent collapse of the Autonomy of Kosovo on March 23, 1989, which was rightfully experienced by the Albanians as a conquest of Kosovo by Serbia, Belgrade took over all the institutional and social mechanisms in Kosovo. Through the Yugoslav military-police apparatus, it began to take over all the structures of power that had been blamed on the "Serbian tragedy in Kosovo" until then. On this occasion it turned to severe repression against all those who had prevented the adoption of constitutional amendments or who in any form resisted the foreseen changes (Buxhovi, 2015). Thus, Serbia invaded Kosovo, filling thousands of Serb policemen and soldiers who carried out repressive measures, aperture, violence and caused the arrests and isolation of many intellectuals: politicians, professors, various creators, journalists, etc. The Kosovo Academy of Sciences and Arts was also closed down, and the educational process was blocked by introducing Serbian curricula into schools. A vicious anti-Albanian campaign had begun to deprive the people of Kosovo of all their rights by causing some form of planned violence against them by Serbia.

Although repression continued to disregard this, the Assembly of Kosovo, on the 2-nd of July, 1990, promulgated a constitutional declaration defining Kosovo as a Republic independent of Serbia. Thus, on September 7, 1990, in Kacanik, a meeting of the delegates of the Assembly of Kosovo was held. There, the Republic of Kosovo will be proclaimed and its first Constitution, to be called, the Constitution of Kacanik, will be approved (Buxhovi, 2015). The Assembly of Kacanik determined the holding of the Referendum on Independence, as well as holding of free parliamentary elections within a few months from which the formation of the Government of the Republic of Kosovo would take place.

In September 1991, Kosovo's people organized a referendum on the country's independence and sovereignty. In the independence referendum held from 26 to 30 September 1991, 87.01% of the Kosovo population voted, while 99.87% of participants declared Kosovo as a sovereign and independent state (Buxhovi, 2015). Whereas elections throughout the Kosovo's region were organized on May 22, 1992, where private houses were used as voting centers. From this election, Ibrahim Rugova is elected president of the country and forms the government with Prime Minister Bujar Bukoshi. After the proclamation of the Republic of Kosovo, the tension had reached its peak (Meier, 2006).

The education of Kosovo Albanians and the intellectual circles created during the 1980s made Kosovo's political course shift. On this occasion, two organizations have played a key role: the Association of Philosophers and Sociologists of Kosovo, on the one hand, and the Association of Writers of Kosovo, on the other. The first one had become a center of intellectual protest against Serbian politics and had organized many public gatherings and petitions (Malcolm, 2011).

The second was forced to assume the political role due to the circumstances, becoming a political movement that was formally established in 1989. This political movement was the "Democratic League of Kosovo" which became known by its LDK initials and the president of the Writers' Association, Dr. Ibrahim Rugova became the leader of the LDK. In the elections of 1992, Rugova was appointed to the post of President of the Republic of Kosovo. Rugova had conceived of his policy as peaceful, calling for a peaceful resolution of the Kosovo problem, preventing violent revolt and with the help of the international community, demanding international political intervention in various forms on the Kosovo's issue. Leader Rugova led in a solid way the peaceful resistance of the people of Kosovo to the Serbian regime. Institutional resistance and the state of Albania had prevented Kosovo from getting involved in the war ahead of time, though this cost the people with great suffering from Serbian repression. Rugova's pacifist policy made it possible for Kosovo to remain calm during the wars waged in Slovenia, Croatia and Bosnia and Herzegovina.

### THE YEARS 1995-1998

As the situation continued to worsen, Rugova's and LDK's peaceful policies began to be criticized. The Dayton Agreement, 1995, which reached an agreement on the cessation of war and the resolution of the Bosnia and Herzegovina problem, left Kosovo out of Dayton. The Dayton Peace Agreement brought Kosovars even more bitter disappointment. It was clear from the beginning that the international community's hopes for peace in Yugoslavia did not leave much to Kosovars' demand for independence. No representatives of the Kosovo's Government were invited to the talks and the fact that the Kosovo issue needed a pressing solution was completely ignored (Petritsch & Pichler, 2002). Dr. Ibrahim Rugova urged US President Clinton to include Kosovo in the Dayton conference, while the US President announced that Kosovo and its problems remain for special treatment (Buxhov, 2015). The Albanians increased the demand not to continue with civil resistance but to return to active resistance alluding to war.

Serbian policy towards Kosovo during these years had general strategies aimed at forcing Albanians to leave the country. Repression, violence, mistreatment and denial of human rights and freedom were made in the most cruel way by Serbs.

Military guerrilla groups began to form over the years that carried out actions against Serbian and military police forces in different parts of Kosovo. This indicated that peaceful politics was gaining momentum by returning to active resistance, which resulted in the KLA appearing on the scene. The "Kosovo Liberation Army" appeared publicly on November 28, 1997 at the funeral of teacher Halil Geci in Lausha, Drenica. From this moment the "Kosovo Liberation Army" will become an inevitable military but also a political reality in Kosovo, which from day to day will come to the forefront of the international deployment factor, no matter what its attitude towards it, initially termed a terrorist organization and later a "part of the rebellion" against Belgrade's military violence, until it even became an "international partner" (Buxhov, 2015). The emergence of the KLA and fears of a violent escalation brought the international community to the scene, which was found to be compelled to act decisively (Petritsch & Pichler, 2002).

The purpose of the KLA was to liberate the country from the Serbian occupation and to make military, political and diplomatic triumphs in order to free the people of Kosovo of the long-lasting suffering. Although Rugova's peaceful policy differed from that of the KLA, their common goal was to liberate and build Kosovo. So everyone in their own way and opportunity contributed to the Kosovo's issue.

On October 1, 1997, demonstrations by University of Prishtina students would change the course of politics on the Kosovo's issue, giving it a new dimension. These active developments by the youth (students) were actively escalated by engaging in open confrontation with the Serbian police and military forces. This development was to be expected, and neither politics nor diplomacy nor other maneuvers would be able to stop things from getting where they would go, so to NATO's armed intervention of March-June 1999 (Buxhov, 2015).

In March 1998, Serbian police and military forces attacked Prekaz, addressing the family of Commander Adem Jashari, whom they had targeted and knew well for their contributions to Kosovo. Serbian forces carried out massacres against the Jashari family by barbarically executing the entire family and not saving even the children from the massacre. The Drenica massacres in Qirez, Likoshan and eventually Prekaz against the Jashari family shook world opinion and highlighted Serbian intentions (Buxhovi, 2015). Attacks by Serbian forces also took place in the village of Glogjan and in the Decan region with the aim of destroying the KLA, which faced resistance from the KLA forces who successfully resisted.

Serbian forces continued unprecedented attacks and massacres on innocent and defenseless populations on the territory of Kosovo. Albanian villages, suspected of collaborating with the KLA, would be besieged by regular military forces, including artillery and tank units, and shelled. Civilians would flee or fall victim. Smaller units would then be sent

to villages, often killing entire families or tribes of Albanians who had failed to find shelter in the surrounding areas (Weller, 2011).

### 1998-1999

The meeting of Rugova and Milosevic on May 1998, to talk about finding a peaceful solution was the clearest evidence of the peaceful behavior of Albanians even under circumstances when Serbian forces were in open action massacring and killing Albanians.

Ibrahim Rugova was sent to Belgrade to talk with Milosevic in coordination with the Americans. Following talks between Rugova and Milosevic in Belgrade, US Ambassador Christopher Hill began moves to draft an agreement. The deal failed, which put a lot of tolerance on Milosevic to the detriment of the Albanians. Then, Milosevic confronted US diplomat Richard Holbrooke, who, after noticing the old Milosevic vaults, immediately confronted the ultimatum and coercive demands.

In coordination with international powers and Ambassador Holbrooke, Milosevic was presented with a catalog of demands, and if these demands would be disregarded then they would be followed by military action. Milosevic was informed by envoy Holbrooke of the package and the ultimatum demand that led Milosevic to agree. Milosevic was told that if he did not accept the catalog of demands put forward by the Contact Group, NATO would launch attacks without the mandate of the United Nations Security Council. Milosevic was forced to comply with the Holbrooke package.

On October 16, 1998, Belgrade and the OSCE signed an agreement on the deployment of two thousand troops of this organization's verification mission that will be deployed in Kosovo immediately. Most of them will be Americans, among whom will be the head of the OSCE verification mission in Kosovo, diplomat William Walker (Buxhovie, 2015).

The massacre of Reçak occurred on January 15, 1999, where civilians were massacred by Serb forces. There were young people, old people, women and children in this massacre. The massacre was unveiled by OSCE monitors and mission chief William Walker, with the international press present. Walker allowed world television cameras, the BBC, CNN, AP, to shoot this horror. Walker publicly stated that this was a massacre against the civilian population. In January 1999, the massacre of forty-five Albanians in Reçak raised tensions (Ahrens, 2010). Belgrade reacted harshly to Walker and denied the massacre. The Serbian regime, as might be expected, refuted Walker's allegations, pointing out that all those killed in Reçak were "Albanian terrorists" who had fought against the Serbian police (Shala, 2000).

The macabre massacre of innocent civilians sought to cover up Serbs by muddying the epithets of terrorism, denouncing world media and missionary Walker. Images of Reçak, piled up on a hill, along with Walker's attitudes, would shock the world. Walker will not hesitate to conclude that in his life, for many different diplomatic missions and views of the saddest, he had not seen such a thing (Shala, 2000).

Washington's reactions were immediate, requiring NATO and Europeans to prepare for air strikes on Yugoslav forces in Kosovo, but after the proposal was rejected, a political proposal was made (by the major powers, the 'Contact Group'), which alluded to the preparation of a Conference on Kosovo. Serbs and Albanians will be threatened that if the settlement is not reached and the conference fails, then they will be bombed (Ahrens, 2010). Thus the actions of the United States of America with their strong diplomacy created the American 'formula' that was called by Mrs Albright, "Withdrawal of Serbia from Kosovo". The conference took place in Rambuje, near Paris in France which is known as the Rambuje Conference.

The Rambuje talks began on February 6, 1999. A delegation of the Serbian Government and a delegation from Kosovo consisting of the LDK and Ibrahim Rugova as well as the KLA and its leaders, who were united in a delegation, were invited. The Rambuje was attended by the Serbian and Kosovo delegations, the two sides already in open conflict with each other, Serbia to rule and the Albanians in Kosovo to break away from Serbian rule (Bytyçi, 2012).

The conference begins in February and ends this month with no final results. It was called for its continuation in Paris some time later. The three major powers (the United States, Great Britain and France) proposed this solution to the crisis: the withdrawal of Serbian security forces; the deployment of a NATO-led peacekeeping force; building democratic institutions; making a definitive decision on Kosovo's constitutional status after three years. The Albanian delegation (Hashim Thaçi, Ibrahim Rugova, Rexhep Qosja), who ruled out Kosovo's eventual remnant under Yugoslavia, accepted the plan under intense American pressure,... the Serbian delegation objected (Schmitt, 2012). So the talks failed without results.

During the talks, Serb forces increased pressure, violence and expulsion on the population of Kosovo. Milosevic neglected international diplomacy, noting that he would solve the Albanian problem in his own way.

During 1999 Serbian forces, police, paramilitary, Serbian armies, acted on the population of Kosovo in a cruel way by killing, massacring, deporting and burying in mass graves, innocent civilians and committing genocide against the people of Kosovo. There were kidnapped persons who were considered missing after the war, many women raped, mistreated and killed. These inhumane acts perpetrated by Serbian forces would cause Kosovo's path to liberation to be paved and raised in macabre cases to innocent populations, and diplomacy to develop over the bodies and blood of an innocent nation that was subject to politics and the actions of a cruel dictator.

After Rambuje's failure, events quickly took a more active course. While Serbia was short of achieving its goal - Kosovo without Kosovo's Albanians - Russia and China thwarted the United Nations' attempt by the three major Western powers to obtain legitimacy for military intervention. In the face of mass deportations, NATO decided to intervene even without a decisive UN mandate.

On March 24, 1999, NATO launched air strikes against Serbia, which continued until June 10, 1999 (Schmitt, 2012). During this time, the Albanian population was subjected to horrific violence by Serb forces. Milosevic had planned through a plan called "Operation Horseshoe" to expel Kosovo Albanians and to Serbise them, a plan prepared by the Serbian intelligence service and state leader Milosevic. With the implementation of this plan many Kosovo 's Albanians were expelled to Albania and Macedonia.

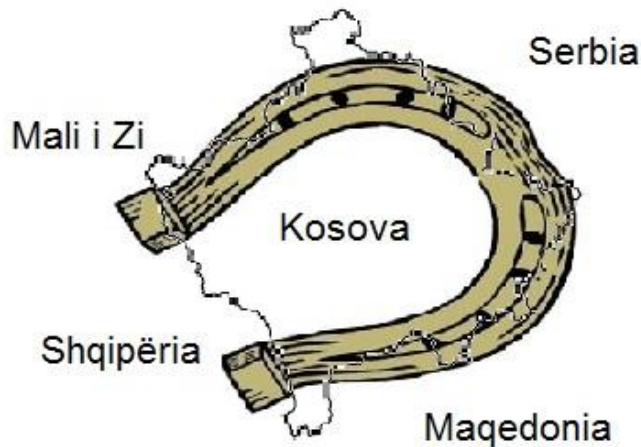


Figure 1. Operation " Horseshoe "

On June 10, 1999, Milosevic signed the capitulation and NATO suspended air strikes. The UN Security Council, based on a compromise made between Russia and the West, has prepared a resolution under which Kosovo is placed under UN international administration. On June 10, 1999, the UN Security Council adopted Resolution 1244.

The Kosovo crisis and the military intervention of the Atlantic Alliance enabled the return of refugees, the placement of Kosovo under international protectorate, the removal of all Serbian military, paramilitary and police forces from Kosovo, and the prospect that it would be resolved through a status negotiation process. Whereas in the concrete plan, the conflict in Kosovo caused great material, human, social, economic and psychic damage to its citizens (Bytyçi, 2012). From this period Kosovo begins a journey of building everything from the beginning after many devastation and suffering experience.

## CONCLUSION

Kosovo's path since the abolition of autonomy has become more difficult and with more consequences. As Kosovo was unfairly removed from its autonomy then the signs were clear to the people of Kosovo that any boundaries had been exceeded and any rights to them had been violated. The years that passed since the abolition of autonomy were years of suffering and decision-making that would result in the eventual liberation of Kosovo from the long-lasting injustice.

Protests by miners and the Kacanik constitution, which prepared the way for the referendum held, made the Kosovo's road start an unstoppable and triumphant process. The peaceful efforts of Ibrahim Rugova and the Albanian people resulted in Kosovo being out of a state of war during the secession of Slovenia, Croatia and Bosnia and Herzegovina. With the KLA staging, it became clear that the time had come to act by force in order to dispel injustice. The ensuing protests and the efforts of the people of Kosovo in co-operation with international factors made Kosovo appear and be seen clearly from the world suffering from injustices, injustices that violated human rights and international law. The Reçak massacre was the clearest shaking testimony that gave signals to the world about crimes against humanity. Attempts to resolve the Kosovo's issue peacefully failed despite many efforts by the international community. So it became clear that Milosevic was non-negotiable and that the only solution to the Kosovo's crisis was to intervene by force. NATO's humanitarian intervention and Milosevic's capitulation gave the clear message that delaying interventions had numerous consequences that impact many aspects of the domestic, regional and international context, and especially directly on the innocent population. Milosevic's capitulation on June 10, 1999 prompted the adoption of Resolution 1244 that same day, by which Kosovo was put into an international protectorate that would in the future bring stabilization to the country and its preparation for eventual status with a view to Kosovo becoming a state, independent and sovereign in full compliance with international law and human rights.

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## PRE-IMPLANTATION GENETIC DIAGNOSIS IN THE PROGRAM OF ASSISTED REPRODUCTIVE TECHNOLOGY

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Pre-implantation Genetic Diagnosis (PGD) is the diagnosis of genetic disorders in human embryos prior to implantation into the endometrium, i.e. before the phase of transfer on the program of in vitro fertilization (IVF). A biopsy of one blastomer in an embryo that is at the cleavage stage (6-10 blastomeres) or a biopsy of the trophectoderm (the outer layer of cells) at the blastocyst stage (day 5 of embryo development) is typically performed for analysis. The main advantage of PGD is that there is no selective termination of pregnancy when it is used and the chance of giving birth to a child without any diagnosed genetic diseases is quite high [1,3,15].

There are discrepant data in literature on the effectiveness of PGD as part of the program of assisted reproductive technologies (ART) [2,6,8].

According to some studies including ASRM (American Society for Reproductive Medicine) data, application of PGD doesn't increase the frequency of pregnancies with in vitro fertilization (IVF). This may be due to imperfection of the technique of the blastomer sampling procedure or the choice of a laboratory screening method to diagnose aneuploidy and microstructural chromosomal abnormalities simultaneously in all chromosomes. The method of array comparative genomichybridization (CGH) showed high performance for clinical studies on embryo transfer within ART (69-70%). While there is the high genetic abnormalities detection rate in PGD based on many studies, the frequency of pregnancies with this method doesn't exceed 30-40% [4,7,11].

Study of the structure of embryo chromosomal disorders based on pre-implantation genetic diagnosis in the program of assisted reproductive technology as well as the impact of this procedure on the results of pregnancies is, therefore, of particular interest.

### Study Materials and Methods

We studied chromosomal abnormalities of embryos in 86 females with different IVF outcomes. Pre-implantation study of the embryos was conducted by the FISH method in 42 females with positive IVF outcomes and in 44 females with negative IVF outcomes. The quality of the embryos was assessed on the third day of culture.

All female patients underwent a special examination before IVF: the hormonal panel was studied (FSH, LH, estradiol, TSH, free T3, free T4, TSH, thyroperoxidase antibodies, prolactin, progesterone, Anti-Mullerian Hormone, testosterone) and infectious status (TORCH-complex infection, STDs), papanicolau test, peripheral karyotype, determination of the vitamin D level in the blood, hysterosalpingography, hysteroscopy with pathohistological examination of endometrial biopsy material. Males underwent mandatory sperm examination with morphological indicators of spermatozoa, genetic analysis of sperm (FISH) and DNA fragmentation. The immune system of spouses and their compatibility by the 2<sup>nd</sup> class of HLA genes were also examined.

The exclusion criteria were the females with monogenic diseases and males with significant pathozoospermia. Controlled ovarian hyperstimulation was performed according to the standard antagonist protocol from day 2-3 of the menstrual cycle with preparations of recombinant follicle-stimulating hormone combined with preparations of human menopausal hormone. Ultrasound monitoring of follicle growth was performed by transvaginal ultrasonography 4-5 times during the multifollicular ovarian stimulation. When the maximum follicle of 14-15 mm was reached, a gonadotropin-releasing hormone antagonist was administered at a dose of 0.25 mg.

Oocyte retrieval was performed in 35-36 hours after the administration of ovulation trigger. Immediately after receiving oocytes and spermatozoa, their morphological assessment was performed. Morphological analysis of oocytes and spermatozoa was carried out immediately after retrieval. Mature, immature and degenerative oocytes can be retrieved by puncturing follicles. More thorough assessment of the state of oocytes can be carried out only after purification before ICSI. The first polar cell is determined in mature oocytes ready for fertilization and designated as M II in the embryological protocol [1,13].

Intracytoplasmic sperm injection was performed for all patients (ICSI method). Two pronuclei form in the normal course of fertilization in 18-20 hours after ICSI (on the 1<sup>st</sup> day). In this case, 2pn rating is assigned to them. Further development of embryo cleavage occurs within 5-6 days. The embryo quality was assessed 40-42 hours (on Day 2), 72-74 hours (on Day 3), and 20 hours (on Day 5) after fertilization. Embryo cleavage should be symmetrical and equal.



Embryos of poor quality were not transferred to the uterine cavity. They were left till Day 5 and then frozen or transferred upon normal blastocyst formation [5,10,14].

It is known that embryos form a blastocyst on Day 5. The quality of blastocysts was assessed by their size from 1 to 5; by the state of the inner cell mass - from "A" to "C" and surrounding cells – trophoblast (from "A" to "C"). The best blastocysts for transfer were those of size 3-5 with the multicellular ICM and trophoblast. Further development of the embryo occurs in the uterus after the implantation. For successful implantation, the blastocyst must exit the surrounding pellucid zone. This process is called hatching. In case of change in the pellucid zone and difficulties in the process of self hatching, auxiliary laser hatching is used [10,12,15].

Biopsy of the embryo was performed on Day 3 after the fertilization at phase 6-10 of blastomeres and blastocytes.

The FISH (fluorescence in situ hybridization) method was used to detect numerical and structural chromosomal abnormalities. This method involves DNA-probes which are a limited-size nucleotide sequence complementary to a specific region of nuclear DNA. The probe has a "tag", i.e. it contains a nucleotide linked to fluorophore (a molecule capable of fluorescence).

After the procedure of hybridization with the formation of a hybrid DNA-probe and DNA-target molecule, fluorescence of specific DNA sequences on chromosomes or in nuclei can be observed on the study cytogenetic preparation by means of a fluorescent microscope [9,13].

Statistical data processing was performed using an application software package SPSS statistics 17.0. The Kruskal-Wallis test was used to evaluate the significance of intergroup differences in several independent samples.

In case of two samples the Mann-Whitney U-test was used for unlinked sequences. The inserted parts of genotypes were assessed for compliance with the Hardy-Weinberg principle by the  $X^2$  criterion in comparison with expected genotype frequencies of equilibrium distribution. The significance of differences in the incidence of qualitative characters was determined by the criterion  $X^2$ .

#### Findings of Study

Mean age of females was  $35.5 \pm 1.0$ . Infertility duration was  $7.5 \pm 5$  years. The patients were comparable ( $p > 0.005$ ) in their etiology of infertility, anamnestic data, mass-height index, structure of previous somatic and gynecological diseases, and surgical interventions. All patients had a normal karyotype.

The results of the study on the characteristics of embryos subjected to pre-implantation diagnosis are shown in Table 1. A total of 220 embryos were subjected to pre-implantation diagnosis: 111 embryos in Group A and 109 embryos in Group B. Patients of each study group were divided into subgroups by age: under the age of 35 and over 35. In Group A, among females aged  $<35$ , the number of embryos subjected to pre-implantation diagnosis was 52 and in females aged  $>35$  the number of embryos subjected to pre-implantation diagnosis was 59. In Group B, 48 embryos were subjected to pre-implantation diagnosis in females aged  $<35$  and 61 embryos in females aged  $>35$ .

The study findings showed that no pathology of embryos was observed both in females aged  $<35$  and in females aged  $>35$  in the group with successful IVF in 69.2% and 59.3% of cases respectively. These values are statistically significantly higher than similar values in the group of females with non-effective IVF results, respectively, 41.7% ( $p < 0.01$ ) and 24.6% ( $p < 0.01$ ). Embryos with abnormalities were detected statistically more often in the group with negative IVF results (67.9%) than in the group of successful IVF (36.0%,  $p < 0.01$ ).

Distribution of embryos with abnormalities showed that in the group of non-effective IVF results statistically significant increase in the relative incidence of embryo pathology was observed both in females aged  $<35$  and in females aged  $>35$  (58.3% and 75.4% respectively), as compared with the group of females with positive IVF outcomes in the relevant age group, 30.8% ( $p < 0.001$ ) and 40.7% ( $p < 0.001$ ) respectively (Table 1).

Table 1

Characteristics of embryos subjected to pre-implantation diagnosis

| Value                         | Group A<br>n=42 |          | Group B<br>n=44 |          | Total<br>n=86 |
|-------------------------------|-----------------|----------|-----------------|----------|---------------|
|                               | Age < 35        | Age > 35 | Age < 35        | Age > 35 |               |
|                               | abc %           | abc %    | abc %           | abc %    | abc %         |
| Total embryos subjected to PD | 52              | 59       | 48              | 61       | 220           |

|   |         |         |           |            |     |
|---|---------|---------|-----------|------------|-----|
| Embryo pathologies by chromosomes, No           | 36 69.2 | 35 59.3 | 20 41.7** | 15 24.6*** | 106 |
| Embryo pathologies by chromosomes, Yes          | 16 30.8 | 24 40.7 | 28 58.3** | 46 75.4*** | 114 |
| Embryo pathologies by chromosomes within groups | 40      | 36.0    | 74        | 67.9**     | 114 |

Note: \*- \*\*  $p < 0.05-0.01$  as compared to Group A of the same age

In view of the fact that the frequency of viable embryos formation varies in both groups, studying the frequency and nature of pathologies of viable embryos in these groups is of great interest. Viable embryos reached 35% in the group of females with positive IVF outcomes that was statistically more than in the group of negative IVF result – 20.3% ( $p < 0.01$ ) (Table 2). A detailed study of the frequency of viable embryos in patients of different age subgroups showed statistically significant high values among females aged > 35 with positive IVF outcomes (37.5%) in comparison with females of the same age with negative IVF outcomes (15.2%,  $p < 0.05$ ).

The study of unviable embryos frequency showed a contrary picture. Unviable embryos were observed statistically more often in females aged >35 in the group with the negative IVF outcome (84.8%) as compared to females of the same age with the positive IVF outcome (62.5%,  $p < 0.05$ ). Among females aged <35, there was no relevant difference in the frequency of viable and unviable embryos between the study groups.

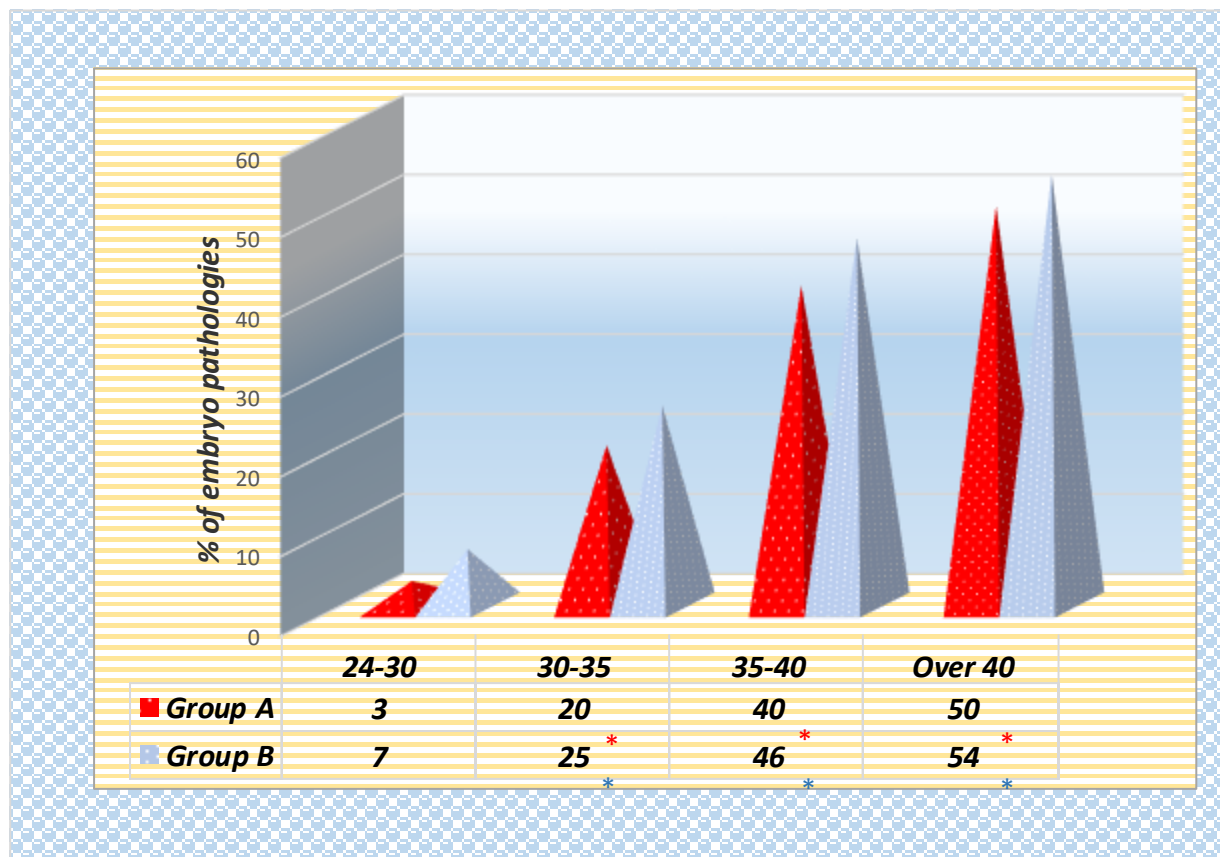
Table 2

| Features of embryos with pathologies detected by pre-implantation diagnosis |                 |          |                 |          |
|---|-----------------|----------|-----------------|----------|
| Value   | Group A<br>n=40 |          | Group B<br>n=74 |          |
|   | Age < 35        | Age > 35 | Age < 35        | Age > 35 |
|   | abc %           | abc %    | abc %           | abc %    |
| Total embryos with pathologies  | 16              | 24       | 28              | 46       |
| Unviable embryos  | 11 68.75        | 15 62.5  | 20 71.4         | 39 84.8* |
| Viable embryos  | 5 31.25         | 9 37.5   | 8 28.6          | 7 15.2*  |
| Total viable embryos within groups  | 14              | 35.0     | 15              | 20.3**   |

Note: \*- \*\*  $p < 0.05-0.01$  as compared to Group A of the same age

The study of the paternal age effect on the embryo pathology incidence revealed a direct dependence between a chromosomal abnormality and the paternal age (Figure 1). In group A, males aged 30-35 had embryo pathology in 20.0% of cases that is statistically higher than in males aged 24-30 years with embryo pathology observed in 3.0% of cases ( $p < 0.01$ ). Abnormalities were observed in 40.0% of males aged 35-40 and in 50.0% of males aged >40. The detected difference in the frequency of embryos with pathologies in different age subgroups for the Group A was statistically significant ( $p < 0.01$ ).

Figure 1. Dependence of the embryo pathology incidence on the paternal age in the comparison groups



$P < 0.01$  as compared to the previous age within each group

A similar trend was observed in group B. The incidence of chromosomal abnormalities in embryos increased with increasing paternal age. The highest relative incidence of chromosomal abnormalities in embryos was observed in males of the older age subgroups. In persons aged >40, 54.0% abnormal embryos were observed, that is statistically more than in males aged 35-40 with the incidence of embryo abnormalities was fixed at the level of 46.0% ( $p < 0.05$ ). In males aged 30-35 this pathology was reported in 25.0% that is statistically less than in males of the older age groups ( $p < 0.01$ ) and in males aged 24-30 ( $p < 0.01$ ) (Figure 1).

Comparative analysis of the embryo pathology incidence among the study groups of similar age didn't show a relevant difference.

The study of the structure of chromosomal pathology of viable embryos in the comparison groups showed the following (Table 3). In Group A, trisomy 21 (Down syndrome) was diagnosed in 41.7% of embryos. In Group B, this syndrome was reported in 40.0% of embryos ( $p > 0.05$ ). Patau syndrome (trisomy 13) and Edwards syndrome (trisomy 18) were diagnosed in 25.0% and 16.7% of viable embryos of Group A that is comparable to the similar data in Group B where the incidence of the above mentioned syndromes diagnosed in embryos was 20.0% and 13.3% respectively ( $p > 0.05$ ). There was no relevant difference between the groups in the incidence of Klinefelter syndrome (XXY) and polysomy Y (YYY) in viable embryos ( $p > 0.05$ ).

Table 3

Nature of chromosomal pathology in the studied pathological viable embryos

| Viable embryos | Group A<br>n=42 | Group B<br>n=44 | Total       |
|----------------|-----------------|-----------------|-------------|
|                | 12<br>abc %     | 15<br>abc %     | 27<br>abc % |

|                               |   |      |   |      |    |      |
|-------------------------------|---|------|---|------|----|------|
| Klinefelter syndrome (XXY)    | 0 | 0    | 1 | 6.7  | 1  | 3.7  |
| Turner syndrome (X0)          | 1 | 8.3  | 1 | 6.7  | 2  | 7.4  |
| Down syndrome (trisomy 21)    | 5 | 41.7 | 6 | 40.0 | 11 | 40.7 |
| Patau syndrome (трисомия 13)  | 3 | 25.0 | 3 | 20.0 | 6  | 22.2 |
| Edwards syndrome (trisomy 18) | 2 | 16.7 | 2 | 13.3 | 4  | 14.8 |
| Polysomy Y (XYY)              | 1 | 8.3  | 2 | 13.3 | 3  | 11.1 |

The study of the structure of chromosomal pathology in females of different age groups (>35 and <35) didn't reveal a relevant difference in the relative incidence of the above mentioned abnormalities (Table 4). Down syndrome was diagnosed in most cases in viable embryos both in females aged <35 and in females aged >35 (38.5% and 42.8% respectively,  $p>0.05$ ). A relevant difference also was not revealed in the incidence of other syndromes in viable embryos with abnormalities in females of the experimental age groups.

Table 4

Nature of chromosomal pathology in pathological viable embryos in females of different age groups

| Viable embryos                | Group A + Group B |      |         |      | Total |      |
|-------------------------------|-------------------|------|---------|------|-------|------|
|                               | Age <35           |      | Age >35 |      |       |      |
|                               | abc               | %    | abc     | %    |       |      |
|                               | 13                |      | 14      |      | 27    | 100  |
| Klinefelter syndrome (XXY)    | 1                 | 7.7  | 0       | 0    | 1     | 3.7  |
| Turner syndrome (X0)          | 1                 | 7.7  | 1       | 7.1  | 2     | 7.4  |
| Down syndrome (trisomy 21)    | 5                 | 38.5 | 6       | 42.8 | 11    | 40.7 |
| Patau syndrome (трисомия 13)  | 3                 | 23.1 | 3       | 21.4 | 6     | 22.2 |
| Edwards syndrome (trisomy 18) | 2                 | 15.4 | 2       | 14.3 | 4     | 14.8 |
| Polysomy Y (XYY)              | 1                 | 7.7  | 2       | 14.3 | 3     | 11.1 |

In summary, the study of pre-implantation embryo characteristics in the IVF program revealed higher indices for embryos without chromosomal abnormalities in the group with positive IVF outcomes and lower indices for the relative frequency of embryos with chromosomal abnormalities as against the group with negative IVF outcomes.

In females aged >35 from the group with positive IVF outcomes viable embryos were found more frequently and unviable embryos were found less frequently. The nature of chromosomal pathology in study females didn't show a relevant difference among the comparison groups.

Large enough quantity of morphologically healthy but genetically abnormal embryos was also detected. With no PGD an embryologist would undoubtedly choose the embryos that reached the blastocyst phase. And this would lead to a negative IVF outcome.

Along with this, there were also the embryos that were genetically healthy but morphologically defective. All these data suggest that the protocols of controlled ovarian hyperstimulation, used medicinal drugs, embryological phase and procedure of PGD itself need to be improved to obtain a high-quality embryo and positive IVF outcome.

So, while there are contradictory data, the analysis of the world literature data and the results obtained by us in the course of the study revealed great advantages of pre-implantation diagnosis. With its wide diagnostic capabilities, PGD as part of the ART program makes it possible to select and transfer embryos with no chromosomal abnormalities into the uterine cavity, to reduce the risk of miscarriage and multiple pregnancies and to improve the chances of successful implantation and the birth of a healthy child.

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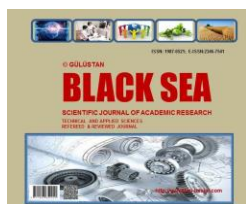
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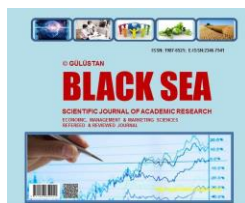


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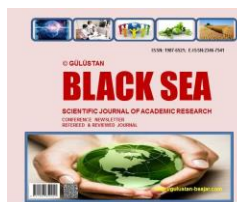
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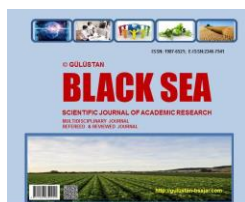
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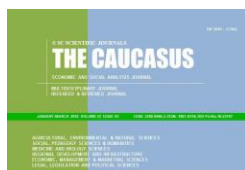


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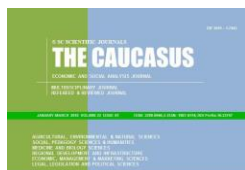


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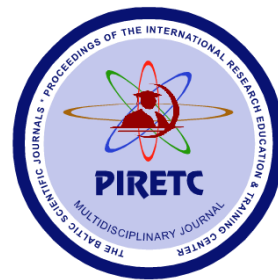
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