REPUBLIC OF AZERBAIJAN

On the rights of the manuscript

ABSTRACT

of the dissertation for the degree of Doctor of Science

GENETIC, IMMUNE AND CLINICAL ASPECTS OF DIABETES IN CHILDREN AND FEATURES OF THE THERAPEUTIC APPROACH

Speciality: 3220.01- Pediatrics

3216.01 - Endocrinology

Field of science: Medicine

Applicant: **Gunduz Ahmad oglu Ahmadov**

Baku – 2021

The work was performed at Azerbaijan Medical Univercity on the department of "Children's diseases II"

Scientific consultant:	Honored Scientist, Doctor of Medical Sciences, prof. **Amalia Abdulla kizi Eubova**
Scientific consultant:	Doctor of Medical Sciences **Valeh Agasafa oglu Mirzazade**
Official opponents:	Doctor of Medical Sciences, prof. **Nargiz Mir Bagir kizi Huseynova**
	Doctor of Medical Sciences, ass.-prof. **Adila Sabir kizi Haciyeva**
	Doctor of Medical Sciences, prof. **Gulnora Nishanovna Rakhimova**
	Doctor of Medical Sciences **Tofiq Vahid oglu Mehdiyev**

Dissertation council BED 2.27/2 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical Univercity

Chairman of the Dissertation council:	Doctor of Medical Sciences, prof. **Yaqub Ziyaddin oglu Qurbanov**
Scientific secretary of the Dissertation council:	Doctor of Medical Sciences, ass.-prof. **Gulnaz Mahir kizi Dadashova**
Chairman of the scientific seminar:	Doctor of Medical Sciences, ass.-prof **Alekper Qazanfer oglu Hasanov**

İMZANI TƏSDİQ EDİRƏM
Azərbaycan Tibb Universitetinin
ELMİ KATİBİ
Tibb elmləri doktoru, professor
Nazim Adil oğlu Panahov
17.05.2021

GENERAL DESCRIPTION OF WORK

Relevance of the problem. Diabetes mellitus ranks first among endocrine pathology in both adults and children, and in recent years the number of cases of this disease has been steadily growing[1]. This is reflected in the annual reports of the International Diabetes Federation, the World Health Organization and other important international organizations[2]. The relevance of the pathology is determined by the annual increase in the incidence of diabetes mellitus, which may be due to various environmental and genetic factors[3]. The studies carried out in the field of identifying the etiology of the disease did not completely clarify the question of the influence of some factors on the development of pathology. The role of various bacteria and viruses has been established[4]. There is an opinion about the possibility of diabetes mellitus development as a result of the influence of viruses on the immune system[5]. However, this process is also influenced by genetic factors, which may be different in each population[6]. Although the role of as the genes HLA system, CTLA-4, the insulin gene -23HphI, PTPN22 and other genes has been studied among the peoples of Europe and Asia.

---

1. Əliyev, T.Ə., Mirzəzadə A.Ə. "Endokrinologiya". Dərslik /-Bakı: "Maarif" nəşriyyatı, -1993. -328 s.
2. International Diabetes Federation. IDF Diabetes Atlas (9th ed.) / -Brussels: International Diabetes Federation, -2019, -168 p.
3. Steck, A.K. Type 1 Diabetes Genetics Consortium. Stepwise or linear decrease in penetrance of type 1 diabetes with lower-risk HLA genotypes over the past 40 years / A.K.Steck, T.K.Armstrong, S.R.Babu [et al.] // Diabetes, -2011. Mar; №60(3), -p.1045-1049.
4. Huang, Y. Gut microbiota profiling in Han Chinese with type 1 diabetes / Y. Huang , S. Li , J. Hu , H. Ruan //Diabetes Res Clin Pract [et all], -2018. Jul; 141: -p.256-263.
5. Qurbanov Y.Z., Qanyaradıcı və endokrin sistemi xəstəliklərinin propedevtikası. Dərslik. /-Bakı: -Azərbaycan Respublikası Səhiyyə Nazirliyi, ATU, CBS Nəşriyyatı, -2016. Cild 4, -325 s.
6. Hummel, S., Ziegler, A.G. Early determinants of type 1 diabetes: experience from the BABYDIAB and BABYDIET studies // Am. J. Clin. Nutr., -2011. Dec; № 94 (6 Suppl), -p.1821S-1823S.

These studies have not been carried out in our population. Each population has its own group of specific alleles and haplotypes, and their detailed study in our population is of scientific interest as well as practical importance. Analysis of these genetic factors will allow in the future to identify alleles that create a high risk of diabetes mellitus in our population and will create conditions for successful prevention of the disease. The identification of these genetic factors, undoubtedly, has both theoretical and practical value. The study of various bacteria and viruses that play the role of triggers in the development of diabetes mellitus among children is also of interest: in recent years, the number of studies in this area has been growing. The incidence of diabetes mellitus and mortality from it in different countries are not the same[7]. Differences can take place even within the same country[8]. Differences in the incidence of clinical forms of the disease in different countries are still not completely clear from a scientific point of view. The clinical forms of diabetes mellitus (DM) in children also differ. Among this contingent, the autoimmune form of type 1 diabetes mellitus (T1DM) prevails[9]. Diabetes mellitus is considered one of the major health problems in all countries. The increase in the incidence of this pathology requires the health care of each country to organize standardized care[10]. Currently, the number of cases of type 1 diabetes mellitus is rapidly growing both in developed and developing countries[11].

---

7. Алиева, Т.Т. Забоеваемость сахарным диабетом Азербайджане, странах СНГ и странах Европы //-Bakı:Metabolizm jurnalı,-2008.cild 9, № 2,-s.10-12.
8. Алиева, Т.Т. Распространеность сахарного диабета среди районах Азербайджана по данным официальной статистики //-Bakı: Azərbaycan təbabətinin müasir naliyyətləri, -2008. № 3, -c.182-185.
9. Məmmədhəsənov, R.M. Şəkərli diabet. Dərslik. / R.M. Məmmədhəsənov. -Bakı; Təbib, -2017. -319 s.
10. Науменко, С.А., Динамика заболеваемости сахарным диабетом 1 типа и ее прогноз в разных возрастных группах детей Калининградской области / С.Л. Науменко, Т.Л. Кураева [и др.] //-Москва: Сахарный диабет, -2005. № 4, -с. 52-55.
11. Demirbilek, H., Ozbek, M.N., Baran, R.T. Incidence of type 1 diabetes mellitus in Turkish children from the south-eastern region of the country: a regional report // J. Clin. Res. Pediatr. Endocrinol., -2013. 5, -p. 98-103.

Clinical forms of diabetes mellitus has a different character[12]. The most common types of the disease are found even among young children[13]. According to the data of the IDF for 2019, around the world among people under the age of 19, 1,110,100 people suffer from diabetes mellitus, T1DM is diagnosed in 128,900 children in 2019 year[2]. In recent years, the rate of increase in cases of diabetes mellitus in children has become global, the annual growth rate is 3%[2]. Despite the extensive study of the clinical manifestations of T1DM, there is very little information about other types of this disease. There is no data on the types of pathology in the state register. The number of children with diabetes mellitus in the Republic of Azerbaijan at the end of 2015 was 1100 (0-14 years). Most of them live in the city of Baku, the rest in the districts. T1DM prevails among them[9]. According of statistics for Republic of Azerbaijan by the end of 2019, the number of newly diagnosed T1DM was 1295 people[14]. The increase in the number of children with T1DM, along with differences in the frequency of occurrence in different regions, is of interest. Various clinical forms of diabetes mellitus in the Republic of Azerbaijan, as well as the genetic aspects of the disease in this population and their relationship with the immune system, have not been completely studied. It is obvious, that after the identification of various clinical forms of diabetes mellitus, the therapeutic tactics in relation to them will also be different. In this regard, research work is of undoubted interest.

Aim of the study

The purpose of the research was to study the genetic, immunological and clinical aspects of diabetes mellitus in children and to determine the characteristics of the therapeutic approach.

---

12. Green, A. EURODIAB (EURope and DIABetes): The EURODIAB studies on childhood diabetes 1988–1999. Europe and Diabetes // Diabetologia, -2001. № 44, suppl 3, -p.B1–B2
13. EURODIAB ACE Study Group: Variation and trends in incidence of childhood diabetes in Europe: [Electronic resource] / Lancet, -2000. № 355, -p.873–876.
14. Healthcare, social protection and housing conditions in Azerbaijan / State Statistical Committee of the Republic of Azerbaijan, Statistical yearbook, -Baku, -2020, -260 p.

Tasks set during the study

1. The study of the genes PTPN22, the insulin gene -23HphI, CTLA-4 +49 A/G, DRB1, DQB1, DQA1 class II HLA system for diabetes mellitus in children of the Azeri population.
2. Detection of alleles and haplotypes prone to diabetes in the Azerbaijani population for prognostic purposes.
3. To study the relationship between genes predisposing to diabetes and chronic complications of the disease.
4. To study the relationship between the immune system and the genes of the HLA system in children with diabetes mellitus.
5. To determine the role of intestinal microflora as a risk factor for children with newly diagnosed diabetes mellitus.
6. To study the characteristics of the lipid profile in children with diabetes mellitus and their relationship with the alleles of the genes of the HLA system.
7. To study the frequency of diabetes mellitus, identify its various clinical forms and improve therapeutic approaches to this pathology.

Key Points to Protect

1. The study of the genetic and immunological features of diabetes mellitus in children of the Azeri population brings clarity to the clinical course of the disease.
2. The study of the incidence of diabetes allows us to predict the prevalence of diabetes in the Republic of Azerbaijan.
3. The study of the relationship between the HLA system and the immune system in diabetes mellitus has important diagnostic value.
4. The study of intestinal microflora in patients with diabetes mellitus in the Azeri population allows us to identify microorganisms that play a role in the development of this disease as a risk factor.

Scientific novelty of research

- For the first time, alleles that increase the risk of diabetes in the Azeri population have been identified by conducting molecular genetic testing in a healthy group and children with diabetes. This will undoubtedly be important in predicting diabetes in the future.

- Through genetic and other research methods, clinical forms of diabetes mellitus in children have been identified.
- The relationship between genes and changes in the immunological and lipid profile in diabetes mellitus was studied.
- The role of intestinal microflora as a risk factor in the development of diabetes was studied, and its relationship with genes was also investigated.
- For the first time in the Baku city and Absheron, the incidence of type 1 diabetes mellitus was revealed.
- After identifying the various clinical forms of diabetes mellitus, the therapeutic approach to this pathology was improved.

Practical significance of scientific work

- The study of class II genes of the HLA system and other genes involved in the formation of diabetes in the Azeri population can be a prognostic criterion for early detection of the disease.
- The study of the incidence of type 1 diabetes mellitus allows predicting its spread in the Republic of Azerbaijan.
- The identification of a certain composition of the intestinal flora, which contributes to the development of diabetes mellitus, may be considered in the future as a risk factor in healthy children.
- The combined study of the genetic and laboratory parameters of diabetes mellitus can help in identifying various clinical forms of this pathology.
- The study of the relationship between the lipid profile and HLA genes in children with diabetes mellitus has important diagnostic value.

Application of research results in practice

The research was carried out in the Department of "Children's Diseases II" of the Educational and Therapeutic Clinic of AMU and in the endocrinology department of the Children's Clinical Hospital No. 6 of Baku. The results obtained were used in the practical work of the doctors of the department “Pulmonology, Allergology, Pediatric Endocrinology” of the Educational and Therapeutic Clinic AMU and the endocrinology department of Children's Clinical Hospital No. 6.

The theoretical foundations and practical recommendations of the dissertation are used in the educational process by residents and students of the Department of "Children's Diseases II" of AMU.

Testing of dissertation materials. The dissertation materials was presented at the 38th Annual Meeting of the International Society for Pediatric and Adolescent Diabetes taking place in Istanbul, Turkey (Turkey, Istanbul, 2012, October 10-13), The 19TH ESPE Winter school (Georgia, Kakereti, 2014, February 20-26), 53rd Annual Meeting of the European Society for Paediatric Endocrinology, Dublin (Ireland, Dublin, 2014, September 18-20), and at meetings of "The Society of Endocrinologists of Azerbaijan (Baku, 2014, October 31) and "The Society of Pediatricians-endocrinologists of Azerbaijan" (Baku, January 24, 2017). Preliminary discussion of the dissertation at the interdepartmental meeting on 03.07.2018 (meeting No. 16), and discussion of the scientific seminar on 13.04.2021 at the scientific seminar of the Dissertation Council BED 2.27/2 at AMU (protocol № 01) held.

Name of the organization where the dissertation work is performed. The dissertation work was carried out at the of Educational and Therapeutic Clinic of the AMU and the endocrinology department of the Children's Clinical Hospital No. 6 in Baku.

Publications. 42 scientific works (30 articles and 12 abstracts), 1 textbook, 2 methodical textbook were published on the basis of dissertation materials. 14 articles on the topic of the dissertation were published in foreign journals included in the international indexed citation database (SCIE, Scorpus, PubMed/Medline, Thomson Reuters, Biosis Previews). 7 of them included in the list of Web of Science Group.

The structure and scope of the dissertation. The work consists of 260 pages compiled on a computer, introduction, literature review, research materials and methods, and 3 chapters of personal research, conclusions, results, practical recommendations, and literature indexes of 36 domestic and 381 foreign scholars. The research work is illustrated with 68 tables and 17 figures. The volume of the structural sections of the dissertation consists of introduction 10473 Chapter I 114245, Chapter II 29202, Chapter III 49841, Chapter IV 32363,

Chapter V 84966, final part 48258, results 3647, and practical recommendations 970, except for pictures, tables and bibliography with separate symbols. The total volume of the dissertation is 373965 characters.

MATERIALS AND RESEARCH METHODS

The research was carried out at the Department of " Children's Diseases II " of the Azerbaijan Medical University in different years. 1162 people were examined as the object of research. Of these, 644 children with diabetes, and the control group was 518 people. In 104 children (primary patients), the DRB1 gene was studied (autoantibodies in 104, CD in 48 examined children), HLA and other genes were determined in 160 children, CD in 30 children who have been ill for several years, intestinal microflora in 19 children, insulin determination in 215 children, 21 of them using a pump, 26 of them were from Ganja, 27 - from Baku, lipids were determined in 40 children (in 30 children with joint determination of CD). In the control group, in 200 students identified the DRB1 genes, in 271 - HLA and other genes, in 15 practically healthy children from the College of Medicine - CD, and in 32 - intestinal microflora. The examined patients were between the ages of 0-18 years old and underwent inpatient treatment at the Educational and Therapeutic Clinic of AMU and the Children's Clinical Hospital No. 6 in Baku. The diagnosis of diabetes was established on the basis of a carefully collected medical history, clinical, laboratory and instrumental methods of research. The basis of the research program was the analysis of anamnestic data, clinical manifestations of the disease, the results of laboratory and instrumental methods of research.

DRB1 gene in 104 sick children (patients with early detection of diabetes), as well as autoantibodies in these children (104 children and 2 in addition), CD markers in 48 of them, in addition HLA DQB1, DQA1 genes in 160 sick children in different years , CTLA-4, insulin gene -23HpHI, PTPN22, CDs were identified in 30 children with diabetes for several years, intestinal microflora in 19 children, 215 children used new insulin preparations and 21 children used insulin pump.

The control group included children whose health status was established after going to the hospital, as well as students of the secondary school No. 47 of the Narimanov district of Baku and students of the Medical College No. 1 and 2. A special questionnaire was used when diagnosing diabetes.

Research methods. The diagnosis of diabetes was made on the basis of the criteria of the International Society for Pediatric and Adolescent Diabetes was used[15]. The examined patients with diabetes mellitus had characteristic signs of this disease. The main characteristic clinical symptoms for diagnosing diabetes were excessive fluid intake, frequent and plentiful urination, nocturnal enuresis, sudden weight loss for several days, a sharp smell of acetone in the exhaled air, as well as the manifestation of these symptoms for several days. The examined patients were selected according to the following criteria: age from 0-18 years, first identified, ill for several years, the diagnosis of diabetes mellitus, which was confirmed by clinical and laboratory signs. Using the classification of diabetes mellitus, the types of diabetes, as well as the forms of diabetes associated with the genetic syndromes, were identified according to the clinical picture.

Genetic research. Characterization of the clinical material of the examined patients. For genetic testing, only persons of Azerbaijani nationality were selected. For this purpose, a specially designed questionnaire was used. Venous blood and saliva were used to isolate the DNA. Genetic testing was conducted in genetic laboratories at the Motol University Clinic in Prague, Czech Republic, and at the genetic laboratory of the Auckland Children's Research Clinic in California, USA. Analyzes were mailed to laboratory data. HLA DQA1, DQB1, DRB1 (HLA), CTLA-4 and the insulin gene HphI were studied in patients and healthy children. The applied immunological tests were carried out in the immunological laboratory of the course "Clinical Allergology and Immunology" of Azerbaijan Medical University.

15. Craig, M.E. Definition, epidemiology, and classification of diabetes in children and adolescents / M.E.Craig, C.Jefferies, D.Dabelea, [et al.] // Pediatric Diabetes, -2014. 15 (Suppl. 20), -p.4–17.

Venous blood was used for genetic testing in patients with diabetes, in some healthy children from the control group was used venous blood, and in another group was used saliva. For genetic testing, only persons of Azerbaijani nationality were selected. For this purpose, a specially designed questionnaire was used, a personal card was filled out for each child. The symptoms of the disease, the time of onset of the disease, the blood sugar level at the first detection of the disease, where and by whom the diagnosis was first made, the patient's passport data and the results of laboratory tests were noted in the patient's personal card. All examined children underwent laboratory tests.

Methods for the study of immune status (membrane markers of CD). Monoclonal antibodies of identified membrane markers of immunocompetent cells (indicators of cellular immunity T-lymphocytes ($CD3^+$), T-helpers ($CD\ 4^+$), T-suppressors ($CD8^+$), natural killer cells ($CD16^+/CD56^+$) and indicators of humoral immunity B-lymphocytes ($CD19^+$) was determined by flow cytometry on an Epics XL instrument (Bekmon Coulter, France).

Determination of antibodies against the pancreas. Anti-pancreatic autoantibodies such as GAD 65 and IA-2 autoantibodies have been identified. Antibodies GAD 65 against glutamate dehydrogenase, IA-2 antibodies against tyrosine phosphatase were determined by enzyme-linked immunosorbent assay on an enzyme-linked immunosorbent analyzer StatFax 2100 (Awareness Technology, Inc., USA) and ChemWell 2910 analyzer (Awareness Technology, Inc., USA). Patients were also tested for blood glucose, glycogemoglobin, and C-peptide.

Microbial diversity analysis. After collecting samples of fresh feces, they are immediately placed in the freezer of the refrigerator at -20 ° C and stored for later examination. Microorganism DNA was determined using an E.Z.N.A.® laboratory kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocol attached to the kit. All the results obtained were calculated using the special microbiological computer program QIIME, version 1.17.

Taxonomic units were grouped using the (OTUS) UPARSE program (version 7.1).

RESEARCH RESULTS AND DISCUSSION

The genetics of diabetes is one of the most widely studied. In 40-50% of cases, it is associated with HLA system. The main genetic determinants of this disease are DQ and DR. Haplotypes DR3 (DRB1*03:01-DQA1* 05:01-DQB1*02:01) and DR4 or haplotypes (DRB1*04:01 /02/04 /05/08-DQA1*03:01-DQB1*03:02/04 or DQB1 *02) pose a high risk of developing diabetes. For both haplotypes, the heterozygous form (OR=16.59; 95%, CI 13.7-20.1) is a greater risk than the homozygous form (DR3/DR3, OR=6.32; 95% CI 5,12-7.80; DR4/DR4, OR=5.68; 95% CI 3.91). A number of haplotypes, for example, DRB1*15:01-DQA1*01:02-DQB1* 06:02 (also called "DR2"; OR=0.03; 95% CI 0.01-0.07) are protective haplotypes. Alleles DPB1*04:02, DPB1*03:0 and DPB1*02: 02 are also diabetes sensitive alleles. Allele of class I HLA B*39:06 (OR=10.31; 95% CI 4.21-25.1) is also a high risk[16]. The aim of our study was to investigate the relationship between diabetes and HLA genes in six different populations.n In populations, alleles of polymorphic HLA genes are found in various ways. Studying HLA in diabetes allows us to identify the relationship between HLA and this disease, and also creates opportunities for predicting, differential diagnosis and treatment tactics. In many countries, studies have been conducted to investigate the genetic causes of diabetes. In studies conducted in Azerbaijan (104 patients, 200 healthy), Bangladesh (100 patients, 155 healthy), Mali (100 patients, 200 healthy), Pakistan (100 patients, 200 healthy), Haiti (80 patients, 20 healthy) and Sudan (60 patients, 206 healthy) in children was determined the gene DRB1of HLA. In the control group, saliva was used for analysis, and in the group of patients, blood was used. Genetic testing was conducted at the Children's Hospital at Oakland Research Institute (Oakland, California, USA). Analyzes were taken in each country and sent to the same institute for genetic testing.

16. Noble, J.A., Valdes, A.M. Genetics of the HLA region in the prediction of type 1 diabetes // Curr. Diab. Rep., -2011. Dec; №11(6), -p. 533-542.

Azerbaijan: the results of the analysis showed that our population contains alleles characteristic of both Europe and Asia. The high risk of developing diabetes is characteristic for the DRB1*03: 01, DRB1*04: 02, DRB1*04: 05, DRB1*09: 01 alleles, and the DRB1 *15:01 (Europe) and DRB1*15:02 (Asia), DRB1*11: 01 alleles have protective properties. Bangladesh: There are both Asian and European alleles here. Risk factors for developing diabetes are the alleles DRB1*04:01 and DRB1 *11:01. Unlike the Azeri population, the DR B1*03:01 allele here does not predispose to diabetes. Interestingly, not one of the alleles identified here possessed protective properties. Despite the detection of 5 different alleles of the DR2 gene, none of them has anything to do with diabetes. Haiti: In this population, 80 patients and 20 children in the control group were examined. A small number of samples did not allow to obtain certain results. However, the DRB1*09:01 allele has been identified as a risk of developing diabetes in this population. Mali: In this population, only the DRB1*04:05 allele posed a risk for diabetes. Pakistan: In this population, there are both Asian and European alleles. As in our population in the Pakistani population, the DRR1*03:01 and DRB1*04:01 alleles are associated with diabetes mellitus. Allele DRB1 *04:03 has protective properties. Alleles DRB1*15:01 (Europe), DRB1 *15:02 (Asia), DRB1*11:01 also have protective properties. In contrast to the protective DRB1*14:01 allele found in Europeans, the DRB1*14:04 allele is relatively susceptible to diabetes. When comparing the Azeri population with the populations of the countries surveyed, it is clear that the populations of Sudan and Pakistan are closest to ours. In the Sudanese population, the alleles DRB1*03:01 and DRB1*04:05, as in our population, are alleles predisposing to diabetes. It should be noted that both Sudan and Pakistan are Muslim countries. As the table shows, there is a strong relationship between the HLA DRB1 gene and the disease in patients from Azerbaijan ($p=1.16x10-25$) and Pakistan ($p=8.55x10-11$), while among patients from Mali ($p=0.0002$) and Bangladesh ($p=0.028$), this correlation was weak. Alleles DR2 DRB1*1501 and DRB1*1502 have strong protective properties for both Azerbaijan and Pakistan.

(The allelic group DR2 includes the alleles *1501,*1502, *1503, *1504,*1505,*1507,*1601, *1602,*1604). When comparing our population with Azerbaijanis living in Iran, it was found that the frequent detection of HLA DR3-DQ2 haplotypes increases the risk of T1D[18]. Azerbaijanis living in Iran compared with healthy people have the DRB1*0301 alleles (82.5 with 11.3%), DQA1*0501 (82.5 with 36.3%), DQB1*0201 (81.3 with 35%) have a higher risk[17]. One study examining the role of HLA in the formation of T1D among Arabs showed that the HLA alleles DRB1*03:01 and *04:05 (OR=7.76 and 7.52, respectively) represent a risk. At the same time, the DRB1*04:01 and *04:02 alleles predispose to diabetes. Alleles DRB1 *10:01, *13:01, *15:02 and *16:01 have a protective effect. As can be seen, in the Azeri population, the DRB1*03:01 and *04:05 alleles predisposing to diabetes were also found in Arabs[18]. Thus, the DRB1 alleles are very different in the studied populations. The haplotypes DR3 and DR4 are associated with diabetes, and are widespread. Some alleles (for example, DRB1*11:01) have different characteristics in different populations. Further full study of the DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, DPB1, A, B, C genes in the future will make it possible to put forward hypotheses about haplotypes. It should be borne in mind that the presence of different nationalities in small geographical areas can lead to incorrect results. In our study, we tried to collect in two groups of patients of the same nationality, but despite this, people of different nationalities could also be randomly examined. The genes of Azerbaijanis, like those of the people that were formerly part of the Soviet Union, are likely to have mixed with the genes of other Soviet peoples. This is more true for individuals selected for a healthy group. This group can also be mixed with Russian nationality.

17. Rabbani, A. HLA DRB, DQA, and DQB alleles and haplotypes in Iranian patients with diabetes mellitus type I / A.Rabbani, F.Abbasi, M.Taghvaei [et al.] // Pediatr Diabetes, -2013. Aug; №14 (5), -p.366-371.
18. Hamzeh, A.R., Nair, P., Al Ali, M.T. The profile of HLA DRB1 alleles in Arabs with type 1 diabetes;meta-analyses//Tissue Antigens,-2015.Dec; №12,-p.12-19.

However, based on the results, the HLA DQB1*0304 allele is less common in the control group. In Russian Slavs, the DQB1*0304 allele (2.4%) is more common than in the population of Azerbaijan (0.7%). It is also not associated with the CTLA-4 gene. This once again proves that the control group consisted only of Azerbaijanis. In order to study the alleles of HLA DQ gene and DRB1*04 subtypes in the Azerbaijani population, 160 children with diabetes and 271 healthy individuals were examined. Of 160 patients, 50.6% (n=81) were boys, and 49.4% (n=79) were girls. The study included children with diabetes under the age of 18 years. The average age of patients was 9.1 years. They were divided into age groups 0-4 (n=18), 5-9 (n=63), 10-14 (n=72), 15-18 (n=7). DQB1, DQA1 and DRB1 *04 HLA were determined by polymerase chain reaction. Alleles of HLA *01-06, DQA1 and *02, *0301, *0303, *0304, *0401, *0402, *0501, *0502, *0503, * 0601, *0602, * 0603, *0604- were determined 9 alleles of HLA DQB1. The alleles DRB1*0401-0408 of the subtype HLA DRB1*04 were also investigated. The frequency of the DQB1 and DQA1 alleles in patients with diabetes mellitus and healthy children is presented in table 1.

Table 1.

Phenotypic frequency of HLA DQB1 and HLA DQA1 alleles in children with T1DM and healthy children

Allele	Patients (%) n=160	The control group (%) n=271	Odd ratio (confidence intervals)	p
HLA -DQB1*				
02	116 (72,5)	76 (28,0)	6,8 (4,4-10,5)	$<10^{-10}$
0301	14 (8,8)	121 (44,6)	0,12 (0,07-0,22)	$<10^{-10}$
0302	86 (53,8)	63 (23,2)	3,8 (2,5-5,8)	$3x10^{-9}$
0303	2 (1,3)	11 (4,1)	0,30 (0,07-1,37)	0,88
0304	12 (7,5)	2 (0,7)	10,9 (2,4-49)	0,005
0401	2 (1,3)	1 (0,4)	3,4 (0,31-38,0)	1,00
0402	0 (0,0)	11 (4,1)	0,14 (0,02-1,05)	0,11
0501	14 (8,8)	38 (14,0)	0,59 (0,31-1,1)	0,88
0502	11 (6,9)	30 (11,1)	0,59 (0,29-1,2)	0,96

Continued from Table 1.

0503	0 (0,0)	19 (7,0)	0,08 (0,01-0,59)	0,002
0601	7 (4,4)	40 (14,8)	0,26 (0,12-0,61)	0,020
0602	1 (0,6)	31 (11,4)	0,05 (0,01-0,36)	0,0011
0603	9 (5,6)	26 (9,6)	0,56 (0,26-1,2)	0,96
0604	5 (3,1)	20 (7,4)	0,40 (0,15-1,1)	0,79
HLA - DQA1				
01	43 (26,9)	170 (62,7)	0,22 (0,14-0,33)	$<10^{-10}$
02	16 (10,0)	38 (14,0)	0,68 (0,37-1,3)	0,87
03	105 (65,6)	97 (35,8)	3,4 (2,3-5,2)	$2x<10^{-8}$
04	0 (0,0)	2 (0,7)	0,56 (0,06-5,42)	0,99
05	105 (65,6)	144 (53,1)	1,7 (1,1-2,5)	0,086
06	0 (0,0)	2 (0,7)	0,56 (0,06-5,4)	0,99

As a result of our study, the DQB1 02, *0302 and *0304 alleles of the DQB1 gene were identified as diabetes-prone alleles, while the DQB1* 0301, *0503, *0601 and *0602 alleles were protective alleles. For the DQA1 gene, the DQA1*3 allele was identified as a risk of developing diabetes, and the DQA1*01 allele was identified as a protective allele. At the next stage, haplotype DQB1*02-DQA1*05 (haplotype DQ 2.5) and haplotype DQB1*0302-DQA1*03 (haplotype DQ8) were studied as a risk of developing diabetes mellitus. The odds ratio for the DQB1*02-DQA1 *05 haplotype is 6.64 (95% confidence interval 4.28-10.31), and for the DQB1*0302-DQA1*03 haplotype, the odds ratio is 3.92 (95% confidence interval 2.57-5.397). The odd ratio for heterozygosity DQB1*02-DQA1 *05/DQB1*0302-DQA1 *03 (DQ2.5/DQ8) was 15.38 (95% confidence interval 7.06-33.5). The absence of DQ 2.5 and DQ8 haplotypes is a protective property, and the odds ratio here is 0.13 (95% confidence interval 0.08-0.22). With the simultaneous detection of the DQB1*02 allele with the DQA1*05 allele (haplotype DQB1*02, DQA1*05 or haplotype DQ 2.5), the risk of developing diabetes is identified. This haplotype is a common risk factor for the European population. Haplotype DQ 2.5 was found on the chromosomes of 74% of patients and 17% of the healthy group (odds ratio 6.3, 95% confidence interval 4.3–9.2). However, according to the haplotype DQB1*02-DQA1*02 (haplotype

DQ2.2), no signifycant difference was found between the two groups (9.4% of patients and 11% in the healthy group) with an odds ratio of 0.84, and 95% confidence interval was 0.44-1.6. The odds ratio for the haplotype DQB1*02-DQA1*03 (haplotype DQ2.3) was 2.3, and the 95% confidence interval was 0.79-6.7. Haplotype DQB1*02-DQA1 *03 was found in 5.0% of patients and 2.2% in a healthy group. For comparison, 86 children with type 1 diabetes mellitus and 62 healthy children with the haplotype DQB1*0302-DQA1*03 were taken. As a result of the study, it was found that the 0401 allele of the DRB1*04 subtype was detected in 9 patients (5.6%) and 4 healthy children (1.5%), the 0402 allele in 59 patients (36.9%) and 34 healthy children (12.5%), the 0403 allele in 1 patient (0.6%) and 13 healthy children (4.8%), the 0404 allele in 7 patients (4.4%) and 10 healthy children (3.7%), the 0405 allele in 13 patients (8.1%) and 2 healthy (0.7%), the 0406 allele was not found in any patient and was found in 3 healthy (1.1%), the 0407 allele in 1 patient (0.6%) and not one healthy (0.7%), allele 0408 in 3 patients (1.9%) and not one healthy. The DRB1*0403 allele has a protective character (odds ratio 0.04, 95% confidence interval 0.01-0.35). Although the alleles DRB1*0402 and DRB1 *0405 are more common in patients with diabetes mellitus, no significant difference was found. No link was found between these genes and age. However, for heterozygosity, DQB1*0302 p=0.21, DQB1 *02 p=0.28 and DQB1*02/DQB1*0302 p = 0.28. Thus, for the first time in the Azeri population, the relationship between class II HLA genes and diabetes was studied, and it was found that the DQ2 HLA haplotype is also a risk of developing diabetes in Azerbaijanis. Unusual for this population, the HLA allele DQB1*0304, unlike other populations, also increases the risk of developing diabetes (odds ratio 10.9, 95% confidence interval 2.4-49). Alleles DQB1*0602, DQB1 *0301, DQB1*0503, DQB1*0601 were identified as protective alleles for type 1 diabetes mellitus. The DQB1*0603 allele was less common and was identified as a neutral allele. Alleles DQB1*02, DQB1*0302 and DQB1*0304 were identified as a risk for diabetes. When analyzing the DQA1 gene, it was found that DQA1*01 was protective, and DQA1*03 was a risk for developing diabetes. Allele DQA1*05

was neutral. The HLA haplotype DQB1*02-DQA1*05 (haplotype DQ2.3) is closely associated with diabetes for a number of European populations. Different nationalities have different frequencies of the DQB1 allele. However, for the Azeri population, the characteristics of some DQB1 alleles are unique; thus, DQB1 *0304 is a risk factor for developing diabetes mellitus. DQB1 *0601 allele has protective properties. Allele DQB1 * 0601 is found in 15% of healthy people and is typical for a number of peoples of Asia and Iran. A study of the DRB1*04 subtypes in individuals with the DQ8 haplotype showed that the DRB1*0403 alleles have a protective effect against diabetes mellitus. The presence of the DRB1*0403 allele along with the DRB1*0406 allele enhances the protective effect. Thus, in the Azeri population, the haplotype DQB1 *0302-DQA1*03/DQB1*02-DQA1 *05 (DQ8/DQ2.5) is a high risk of developing diabetes. Given all of the above, one of the goals of the our study was to determinate the risk of diabetes for the DQ2 and DQ8 haplotype in children in the Azeri population. For genetic testing, 160 children with diabetes were recruited. The control group consisted of 271 individuals of various ages. Patients were aged 1-18 years, the average age was 9.1 years. All patients were diagnosed T1D. Diabetic retinopathy was detected in 1.9% (n=3) of the examined patients, nephropathy - in 2.5% (n=4), and neuropathy - in 4.4% (n = 7). According to questionnaire data, 7.5% (n=12) of the families of patients had T1D and 31.2% (n=50) of type 2 diabetes (T2D) in the age group from 5 to 4 years old were 11.0% (n = 7), “negative” - 11.0% (n =11), in the age group of 5–9 years, DQ2- “positive” 48.0% (n=30), “negative” - 34.0% (n=33), in the age group 10-14 years old DQ2-“positive” 38.0% (n=24), “negative” - 50% (n = 48), in the age group 15-18 years old DQ2-“positive” 3.0% (n = 2) and “negative” 5.0% (n=5). Overall, DQ2 was “positive” in 39.4% (n = 63) and “negative” in 60.0% (n=97). The DQ2 haplotype was more often found at the age of 5-9 years. It should be noted that the manifestation of diabetes occurs precisely during this period. Thus, the relationship between HLA-DQ and type 1 diabetes mellitus in children has been investigated. The DQ2 haplotype was found in 18.8% (n=51) of healthy children, and in patients with

diabetes mellitus, the DQ2 haplotype was found in 39.4% (n=63) and there was a significant difference between these parameters ($p<0.02$). In most patients, the alleles DQA1*0501 and DQB1 *0201 were identified ($p <0.05$). The correlation between different types of diabetes in the family with DQ alleles, manifestations of diabetes, and the age of patients was studied. It was found that there is a positive correlation between the 2 DQA1 allele, the manifestation of diabetes and age ($r=+0.18$, $p<0.05$). A negative correlation was found between the presence of type 1 diabetes mellitus in the family and the period of diabetes ($r=-0.21$, $p <0.05$). This once again indicates that diabetes occurs at a younger age. Haplotype DQ8 HLA was present in 53.7% (n=86) of the examined patients, in 22.4% (n=62) in the control group ($p<0.0001$), the haplotype DQ2 / 8 HLA was found in 31.9 % (n=51) of patients and 2.8% (n=8) of children in the control group ($p<0.0001$). To study the HLA DQ8 haplotype, boys and girls with diabetes were divided into the following age groups: 0 - 4 years, 5-9, 10-14, 15-18 years. DQ8- “positive” in the age group of 0- 4 years were 66.6% (n=12), “negative” - 33.4% (n = 6), in the age group of 5-9 years, DQ8- “positive” were 55.5% (n=35)," negative "- 44.56% (n=28), DQ8-" positive "in the age group of 10-14 years were 50.0% (n=36)," negative ”- 50.0% (n=36), in the age group 15-18 DQ8- “positive” were 42.8% (n=3), and “negative” 57.2% (n=4). Thus, no significance was found between age and the DQ8 haplotype; at all ages, the frequency of occurrence was the same. In the age group from 0- 4 years, DQ2/8 - “positive” were 50.0% (n=9), “negative” - 50.0% (n=9), aged 5–9 years DQ2/8 - “positive” 71.4% (n=45), 28.6% (n=18) - “negative”, in the age group of 10-14 years DQ2/8 - “positive” 69.4% (n=50), “negative” 30.6% (n=22), DQ2 / 8- “positive” in the age group 15–18 years old were 72.4% (n=5), “negative” - 27.6% (n=2). As can be seen from the table, in contrast to the DQ8 haplotype, in this case, the significant difference in the ages of 5–9 and 10–14 years is significantly higher ($p<0,0002$). DQ8 was found in 53.8% of patients (n=160) and in 22.9% (n=271) of healthy people. DQ 2/8 was observed in 31.9% of patients (n=160) and in 3.0% of healthy people (n=271). A comparative analysis of the haplotypes DQ8 and DQ2/8

was performed. There were 86 patients with the DQ8 haplotype (n = 160) (53.8%), 62 (22.9%) in the healthy group (n=271), and 51 patients(31.9%) with the DQ 2/8 haplotype (n=160), in the healthy group (n=271) - 8 (3.0%), there were 28 (17.5%) patients without haplotypes DQ2 and DQ8 (n=160), healthy group (n=271) - 166 times (61.3%). As can be seen from the table, between all 3 indicators there was a high significance of differences ($p < 0.0001$). So, as you can see, while in our population the haplotypes DQ8 and DQ2/8 HLA are more common, other haplotypes predominate in other populations. One of the goals of the research work was the study of CTLA-4 gene polymorphism in children of the Azeri population. CTLA-4 gene polymorphism was studied in 160 children with type 1 diabetes mellitus and in 271 healthy children. Exon 1 at the 49th position of the CTLA-4 gene was determined by polymerase chain reaction-PCR. In the patients, the frequencies of genotypes A/A, A/G, G/G, the frequencies of phenotypes A, G, and the frequencies of alleles A and G of CTLA-4 were compared. Thus, our study shows that no correlation was found between CTLA-4 +49 A/G polymorphism and T1D in the Azeri population. However, other studies have shown that in some countries there is a relationship between CTLA-4 polymorphism and diabetes mellitus. This polymorphism is observed in countries adjacent to each other. For example, this polymorphism was found among Russian and Iranian peoples. A negative result in the Azeri population does not exclude a positive result of other CTLA-4 variants. The purpose of the research was to study the insulin gene in children of the Azeri population. The insulin gene is located at the 15.5th position of the short arm of the 11th chromosome. This gene takes part in the synthesis of an insulin molecule. The insulin gene was studied in 160 children with type 1 diabetes mellitus and in 271 healthy children. In the examined patients, the frequencies of genotypes, phenotypes and alleles of the -23HphI insulin gene were compared (table 2). With the homozygous form of the insulin gene−23HphI A/A, the odds ratio was 3.6 (2.2-5.9, $p<0.0001$), with the heterozygous form A/T, the odds ratio was 0.28 (0.17-0.46, $p<0.0001$), the reliability indicator was high, with a homozygous T/T form, the odds

Table 2.
Frequencies of genotypes, phenotypes and alleles of the insulin gene

Genetic marker −23HphI	Patients n=160 (%)	Control group n=271 (%)	Odds ratio	p
Genotype frequencies				
−23*Hph*I "+/+" A/A	135 (84,4)	163 (60,1)	3,6 (2,2-5,9)	$<10^{-6}$
−23*Hph*I "+/-" A/T	22 (13,8)	99 (36,5)	0,28 (0,17-0,46)	$<10^{-6}$
−23*Hph*I "-/-" T/T	3 (1,9)	9 (3,3)	0,56 (0,15-2,0)	0,56
Fenotype frequencies				0,56
−23*Hph*I "+" A	157 (98,1)	262 (96,7)	1,8 (0,48-6,7)	0,56
−23*Hph*I "-" T	25 (15,6)	108 (39,9)	0,28 (0,17-0,46)	$<10^{-6}$
Allele frequencies n=320		n=542		
−23*Hph*I "+" A	292 (91,3)	425 (78,4)	2,9 (1,9-4,4)	$<10^{-5}$
−23*Hph*I "-" T	28 (8,8)	117 (21,6)	0,35 (0,22-0,54)	$<10^{-5}$

ratio was 0.56 (0.15-2.0, p>0.05), no reliability was established. Separately, according to the T allele of the −23HphI gene marker, the odds ratio of phenotypic frequencies was 0.28 (0.17-0.46, p<0.0001), the confidence indicator was quite high, however, the odds ratio for the phenotypic frequencies of allele A was 0.28 (0.17-0.46, p>0.05), but no reliability was noted. For the alleles A and T of the marker of

the −23HphI gene compared with healthy ones, the odds ratio was 2.9 (1.9-4.4, p<0.0001) and 0.35 (0.22-0.54), respectively, confidence indicators were high. All calculations show that the -23HphI insulin gene in the Azeri population is not important for the formation of diabetes mellitus. The PTPN22 gene was studied in 160 children with type 1 diabetes mellitus and 271 healthy children. Polymorphisms of the PTPN22 gene are presented in table 3. Three polymorphisms of the PTPN22 gene were studied in the Azerbaijani population: -1123 (rs2488457), +1848 (rs2476601 or R620W), +2740 (rs1217412).

Table 3.
PTPN22 gene polymorphism in the Azeri population

Genotype PTPN22	Children with diabetes (n=160)	Control group (n=271)	
Polymorphism -1123			
G/G	106 (66%)	164 (61%)	
G/C	47 (29%)	101 (37%)	
C/C	7 (4,4%)	6 (2,2%)	
No positivity for allele C minor OR=0,78, 95% CI:0,52-1,2			
Polymorphism +1848 C/T (or R620W)			
R/R		152 (95%)	269 (99%)
R/W		7 (4,4%)	2 (0,74%)
W/W		1 (0,63%)	0
There is positivity for the W minor allele OR=7,1, 95% CI:1,5-34			
Polymorphism -2740			
A/A		93 (54%)	140 (52%)
A/G		55 (35%)	119 (44%)
G/G	12 (7,5%)	12 (4,4%)	
No positivity for G minor allele OR=0,77, 95% CI:0,52-1,1			

The control group was tested using the statistical method of Hard-Veinberq (P-adjusted> 0.10). The Hard-Veinberq statistical method is provided for checking genetic frequencies. Between polymorphisms -

1123 C/G and +2740 A/G there is an imbalance (Lewontine D`= 0.99, $r^2$=0.72, p <$10^{-3}$). However, the -1123 C/G and +2740 A / G polymorphisms have no imbalance with R620W (D`= 0.14, r^2 =0.16, p> 0.9). Only between the R620W polymorphism and type 1 diabetes mellitus there was a connection. As can be seen, the minor allele (W) was found in 8 (5%) patients with diabetes mellitus and only in 2 healthy (0.74%) patients (OR =7.1, 95%, CI: 1.5-34), and it is reliable. No correlation was found between polymorphisms -1123 C/G and +2740 A/G and diabetes mellitus. The results of our study allow us to conclude that among the Azeri population, children with type 1 diabetes mellitus are dominated by patients with the minor allele "T" w620 (or T1858) or "W" (dominant), tryptophan (W) (odds ratio = 14.8 , 95% confidence interval 2.0–651). The R allele (arginine) (translated wild-type means "mutant") is considered a mutant allele. Regarding polymorphism 1858 C>T, -1123 G> C, +2740 A> G of the PTPN22 gene in healthy and sick children, no reliability was obtained (p> 0.05). The R allele of codon 620 and the minor alllels of codons -1123, +2740 are neutral in nature and constitute a risk of autoimmune conditions in the population. This can also explain the low prevalence of diabetes in the Azeri population. In the Azeri population, the odds ratio of the haplotype -1123C W620+2740G is higher (odds ratio=14.8, 95%, CI: 2.0-651). No correlation between the sexes was found. At the same time, the trend was also not revealed. One of the objectives of our study was to study the relationship between genes predisposing to diabetes. According to the purpose of the study, 160 children with diabetes and 271 healthy individuals were examined. In both groups, HLA, insulin and CTLA4 genes, haplotypes DQ2 and DQ8 were determined. It was found that diabetes was detected on average at 9.03±0.27 years. The average age of the children was 10.8±0.31 years. Remission was detected in 31.1% of patients, and decompensation period - in 68.9%. The correlation between the age of diabetes, the period of remission and the type of diabetes in the family was studied. It was found that there is a relationship between these parameters. No correlation was found between complications of diabetes mellitus and the HLA genes, the insulin gene, and the DQ2 and DQ8 haplotypes.

There was only a tendency towards a positive correlation between diabetic nephropathy and HLA DQB1 (r=0.66, p=0.052). Thus, although there was no correlation between the complications of diabetes mellitus and the HLA, CTLA-4+ 49A/G, -23HphI, haplotypes DQ2 and DQ8 in the Azeri population[26]. In our study, we studied the characteristics of the immune system and its relationship with the HLA DRB1 genes in children with initially diagnosed diabetes. For this purpose, 48 newly ill children and 15 healthy children were examined. There was no correlation between CD and the age of patients with primarily diagnosed diabetes mellitus, only between CD19 + (r =-0.40, p <0.05) and lymphocytes (r=-0.43, p <0.05) and age negative correlation detected. A correlation was found between glycolgemoglobin HbA1c and CD3+ (r=-0.28, p<0.05) and CD8+ (r=-0.40, p <0.05) in children with initially diagnosed diabetes. We studied the normal distribution of CD indicators in both groups. As it became clear from the study, there is a negative correlation between the HLA genotype DRB1 and lymphocytes (r=-0.29, p<0.05). One of the interesting results was the detection of a negative correlation between CD4 + and GAD65 antibodies (r =-0.38, p<0.007). An increase in the level of GAD 65 antibodies leads to a decrease in the level of CD4 + cells. Thus, our results show that the immune system is actively involved in damage to the pancreatic beta cells. On the other hand, there was a strong association between the HLA DR B1_2 genotype and lymphocytes. The correlation between various indicators in patients was also studied. A positive correlation was found between the parameters of autoantibodies themselves (r =+0.38, p<0.05), between glycogemoglobin and blood glucose (r =+0.33, p <0.05) and the patient's age (r=+0.28, p<0.05). There is a positive association between glycogemoglobin and the duration of the disease and age. The relationship between CD values in patients was studied. A positive correlation was found between CD3+ and CD4+ (r =+0.83), CD8+ (r =+0.60). A negative relationship was observed between CD16+/56+ and CD3+ (r=-0.59), CD4+ (r=-0.46), CD8+ (r=-0.47). A negative relationship was also observed between leukocytes and IRI, CD4+ /CD8+ (r=-0.58). Thus, a close relationship was found between the CD

indices in children with diabetes mellitus. A relationship has also been studied between indicators of cellular immunity in children with type 1 diabetes mellitus. When studying the interrelation between the indicators, it turned out that if in the control group there was a relationship between CD3+, CD4+ and CD19+, then in the initially diagnosed patients and in patients ill for several years this relationship was not observed. On the contrary, a new correlation is created between CD3+ and CD4+. CD4+, i.e. T-helpers are protective and protect the pancreas from autoimmune damage. This feature manifests itself both in the case of a newly diagnosed disease, and in patients with diabetes for a long time. In this group of patients, the relationship between CD4+ and the immunoregulatory index and CD4+ /CD8+ is also disrupted. A negative correlation is observed between CD4+ and CD16+ / 56+, T-helpers and T-killers in both newly diagnosed patients and those who have been ill for several years. In each of the 3 groups (the control group, the group of children with newly diagnosed diabetes and the group of children who have been ill for several years), no reliability was observed between the average indicators of CD markers ($p>0.05$), only between B-lymphocytes and CD19+ in the first diagnosed the significance of differences was observed in patients and the control group ($p<0.05$). Thus, despite the lack of reliability between the average values of CD markers in patients with diabetes, significant changes were observed between the indicators themselves. It was found that with a long course of the disease, along with CD8+ and CD19+, statistical significance was also found in CD16/56+ and IRI in patients who had been ill for several years. We studied the statistical reliability between the average values of CD markers in both newly diagnosed patients and long-ill patients. From the study it became known that there is a correlation between the marker CD8+ ($r=-0.76$, $p<0.05$) and IRI ($r=+0.72$, $p<0.05$) in newly diagnosed patients and children in the control groups. There is also a positive correlation between the CD8+ and CD3+ marker in long-ill patients ($r=+0.60$, $p<0.05$) and a positive correlation between CD8+ markers in long-ill patients and CD19+ in newly diagnosed patients ($r=+0.66$, $p<0.05$). Another relationship was found between the markers

CD16/56+ and IRI among patients for a long time (r=+0.67, p<0.05). Thus, it is obvious that there are significant relationships and statistical significance between the CD markers. Another objective of our study was to identify various types of diabetes mellitus based on the detection of autoantibodies, C-peptide, HLA genotype DRB1 in children with initially diagnosed diabetes mellitus. For this purpose, GAD 65 and IA-2 autoantibodies were determined in 106 children with diabetes mellitus. GAD 65 and IA-2 are used to diagnose autoimmune diabetes in children. Other autoantibodies (islet cell autoantibodies - ICA), to insulin (IAA), and autoantibodies to zinc transporter 8 (ZnT8Ab) can be determined in addition to them. However, their clinical significance is usually low. GAD 65 autoantibodies are usually found in 70-80% of patients, IA-2 in 60% of patients, while other autoantibodies are less common in people with diabetes. When studying the relationship between GAD 65 and IA-2 autoantibodies, a lack of correlation was found (r = 0.067, p = 0.46). No correlation was observed between the GAD 65 antibodies and the C-peptide (r=+0.025, p=0.011). The relationship between antibodies and HLA DRB1 in 106 children was also studied. The relationship between autoantibodies and glycohemoglobin has also been investigated. No association between HbA1c and antibodies GAD 65 and IA-2 (n=106) was noted. However, a positive correlation was observed between blood glucose and GAD 65 (r=+0.21, p=0.02). A comparison was made between indicators of autoantibodies in children with a ketoacidotic state and in children without a ketoacidotic state. A negative correlation was observed between HbA1 and İA-2 (r=-0.79, p<0.05) in children without a ketoacidotic state, but with a high level of İA-2 (n=9). In children with severe ketoacidosis (n = 9), a positive correlation was observed between blood glucose and children with high levels of İA-2 autoantibodies (r =-0.67, p<0.05) and GAD 65 (r=-0,72, p<0.05). In children with a high level of C-peptide and indices of İA-2 in the blood (n=9), a positive correlation was observed between these indices (r=+0.75, p<0.05). The following alleles of the HLA DRB1 gene were identified in patients with autoimmune diabetes as diabetes risk alleles: *03:01, *04:02, *04:05, *04:08,

*09:01. Thus, as we see, there are different relationships between autoantibodies and other indicators in children with diabetes. One of the main conclusions is that a high antibody content in patients with ketoacidosis is not associated with a ketoacidotic state. An increase in the level of autoantibodies has a damaging effect on beta cells.

The purpose of this study was to study the role of microorganisms in the occurrence of the disease in populations located in geographically remote areas. For work for 3-14 days, feces were collected in children 3-18 years old with newly diagnosed diabetes mellitus. For one patient, 2 additional samples were taken from children comparable in age and lifestyle. Samples for control were collected within 1 month, which allowed the determination of microorganisms of the same family. Analyzes were not taken in children with obesity, chronic intestinal injection, active tuberculosis, high fever, and in patients who received antibiotics in the last month. In total, 73 children with diabetes were examined (19 from Azerbaijan, 20 from Jordan, 14 from Nigeria and 20 children from Sudan). The control group included 103 children. In addition to studying microflora, in all the examined patients, the HLA genes DQB1 and DQA1 were determined in the stool analysis for each population. The following polymorphisms: rs1990760 in the IFIH1 gene, rs2476601 in the PTHN22 gene, rs689 in the İNS gene of the DRB1*04 allele, are at high risk for developing diabetes. These genes were found in the examined children. In various populations, the role of bacteria and viruses in the occurrence of T1D has been studied. The study revealed that there is a positive relationship between Escherichia and T1D. Compared with the control group, a difference of 1.8 times was noted. The conditional logistic regression model showed that the risk of diabetes was 41%. At the same time, the difference between Gammaproteobacteria (class) and Proteobacteria (type) was 1.88 and 1.71 times, respectively (P_{corr}= 0.024 and P_{corr}=0.054, respectively). A link was also found between diabetes mellitus and Eubacterium (P_{corr}=0.041), and Roseburia (Pcorr =0.018) (Figure 1.). Both relationships were valid according to the logistic regression model. During the study of the remaining 4 stool

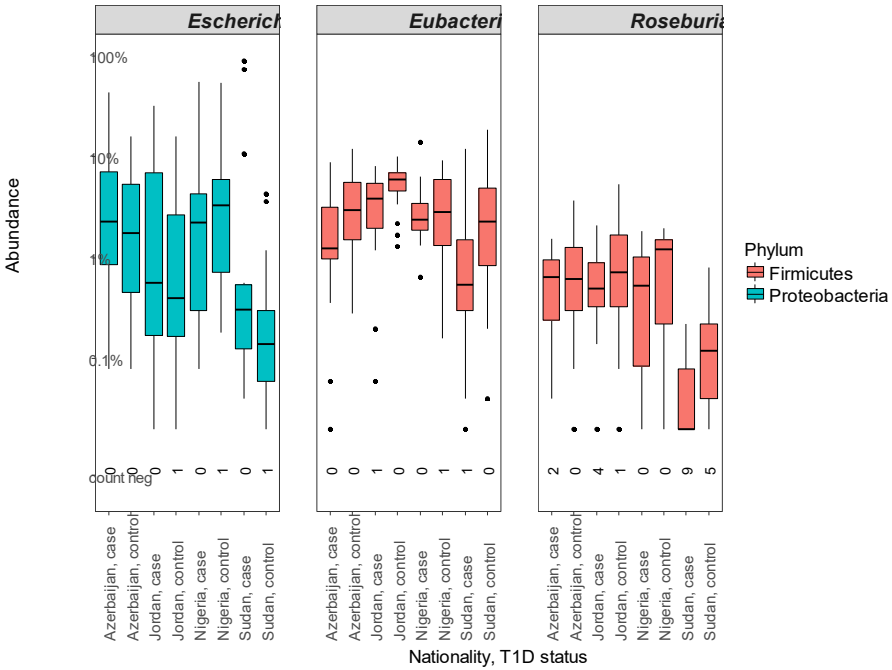

Figure 1. Abundance of the three genera associated with T1DM by nationality and case-control status

samples, it turned out that there is a close strong relationship between diabetes mellitus and Haemophilus. The same relationship was found with Clostridium clusters IV or XIVa. The odds ratio in the simple logistic regression model was 0.59 (p=0.004). HLA DQ2 or DQ8, or both were found in 75% of patients with T1DM and 38% of the examined in the control group 38%, and HLA alleles DQB1 *0602 in 5.9% and 14%, respectively, the protective allele İNS rs689 (-23 HphI) - in 79% and 91%, with, respectively, the İFİH1 rs1990760, 946Thr allele in 68% and 53%, respectively. When conducting genetic analysis, it was found that patients were more likely to have HLA DQ8, -DQ2 alleles, or both simultaneously ($p=3\times10^{-4}$ model of conditional logistic regression) or the 946Thr IFIH1 allele rs1990760 (p=0.015). The relationship between the HLA-DQB1*0602 allele and

the insulin gene SNP rs689 was not strong. In the literature, there are few studies devoted to the study of the relationship between T1D and the gastrointestinal tract. In our work, we analyzed this kind of connection in children representing 4 geographical zones. A connection was established between 3 types of bacteria (Escherichia, Eubacterium and Roseburia) and T1D[28]. In all 4 populations, Escherichia were found 2 times more often than in the control group. Escherichia are the most famous representatives of the Enterobacteriaceae family of the Gammaproteo-bacteria class. Patients with T1D showed a negative association for Clostridia (type Firmicutes), Pseudobutyrivibrio, Eubacterium, and Roseburia. Researchers who studied this problem before us took 1 sample for study, but in our work we examined 2 samples. A study in the city of Denver, Colorado (USA) showed that bacteria with the species Alistipes, Staphylococcus, Thalassospira and Bacteroidetes are more common in patients with T1D. However, in our work, no relationship was found between diabetes and microorganisms of the species Alistipes, Staphylococcus, Thalassospira, and Bacteroidetes. However, in a sample from Sudan, the relationship of Staphylococcus with T1D is established. When comparing our results with data on European and non-European populations, a difference is noticeable. This may be due to various reasons. The final result is influenced by the methods of analysis collection, the need to send over long distances, different research methods, as well as methods of statistical data processing.

The strength of our study was that the samples for analysis were taken from 2 continents and 4 different populations, were evaluated synergistically, but despite the difference in nutrition, climatic conditions, food habits, the results were identical in all 4 populations. The control group was composed of representatives of different families, since it is known that the different microbial spectrum of feces is almost identical for different members of the same family. The analytical approach in our study is conservative. Information is given only on statistically significant results, a model of negative binomial regression is used, moreover, the number of examined is more than in previous works. For the reliability of the diagnosis of T1D, a genetic

analysis was performed. It was found that polymorphisms of the HLA DQ gene, insulin gene, İFİH1 and PTPN22 genes were found in the monitored patients, which suggests that these children have T1D. At the same time, a weakening of the HLA-DRB1*15: 01-DQB1*06:02 allele, protective for non-European populations, was revealed. As a result of the analysis of the data of 4 populations, it was found that 3 species of microorganisms have a connection with T1D, and the revealed connection was not found in previous works. Primary and secondary changes in the gastrointestinal tract are characteristic of T1D. In addition, it is likely that these microorganisms are involved in the progression of the disease. For the study, 19 children with primary diabetes mellitus and 32 healthy children were examined as a control group (a total of 20 girls and 31 boys). The age of the examined ranged from 3-18 years. In children, the following types of microorganisms were determined: bacteria (Prevotella, Bacteroidia, Prevotellamassilia, Parabacteroides, Alistipes, Parabacteroides, Alistipes, etc., a total of 10 species), firmicoots, which are a special type of bacteria with a small number of nucleotides in the composition (Anaerococcus, Anaerotusr, Anaerostusrus, Anaerostrusus, Anaerotusrussia , Blautia, Butyrivibrio, Catenibacterium, etc., total 41 species), actinobacteria (Bifidobacterium, Collinsella, Enterorhabdus, total 3 species), proteobacteria (Escherichia, Klebsiella, Succinivibrio, etc., total 6 species), verucobacteria (Akkermansia), euryarchaeota (Methanosphaera, Methanobrevibacter), cyanobacteria (4c0d-2). Viruses from the group Tymovirales, Caudovirales, Picornavirales, a total of 85 types of viruses were determined. In both groups, a genetic study of the HLA system, which characterizes the tendency to diabetes, was carried out. According to the results of the study, it became apparent that microorganisms associated with T1DM include Escherichia (class Gammaproteobacteria, type Proteobacteria), Prevotellamassilia (class Bacteroidia, type Bacteroidetes) and Megasphaera (class Clostridia, type Firmicutes). Negative association was observed for Clostridia (type Firmicutes), Pseudobutyrivibrio, Eubacterium, and Roseburia. No relationship was found with any of the viruses. HLA haplotypes DQ2 and / or DQ8 were considered indicators of an increased risk of

diabetes. The connection of microorganisms with T1D along with children of Azerbaijani nationality was also studied by representatives of other nationalities. This included 20 patients with diabetes from Jordan, 14 from Nigeria and 20 from Sudan, 103 children were examined as a control group. The results obtained almost coincided. Thus, for the first time in our work, we studied the microorganisms involved in the development of type 1 diabetes mellitus in children of the Azerbaijani population. This is a confirmation that these bacteria are involved in autoimmune processes. At the same time, there was no association of diabetes mellitus with viruses.

The incidence of diabetes among children under the age of 15 years was analyzed, since it is the age of 15 years that is proposed in the reports of the International Diabetes Federation to evaluate this indicator. 36.0% (n=38) of the patients were from Baku, 4.7% (n=5) from the Absheron region, 4.7% (n=5) from Ganja, 4.7% (n=5) from Shamkir, 3.7% (n=4) from Sumgait, 3.7% (n=4) from Guba, 2.8% (n=3) from Khachmaz. 39.7% (n=42) of patients came from different regions of Azerbaijan (28 districts, 1-2 people from each region). The results are shown in Figure 2. In children, different types of diabetes can be detected. type 1 diabetes mellitus is more common, but type 2 diabetes, monogenic diabetes mellitus and other types are also detected. The incidence of type 1 diabetes mellitus globally varies from 1 to 60 per 100,000 children in different regions (for example, Finland and Venezuela). This may be due to both genetic factors and environmental factors, the reasons are still not fully understood. The condition of the patients was evaluated clinically. Ketoacidosis was diagnosed both clinically and by the level of ketones in the urine. Body mass index was evaluated according to WHO standards for <5 years and >5-18 years old using a special program. Blood glucose, glycohemoglobin, GAD 65, autoantibodies IA-2, C-peptide, alleles of the HLA system (n=104) were determined in patients.

HLA alleles were identified in only 104 children. As a result of observation over one year (01/03/2014-05/ 03/2015), the incidence of

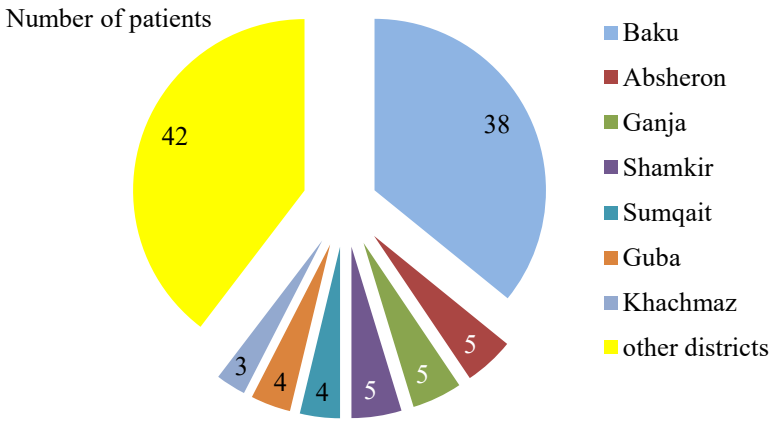

Figure 2. Number of primary diagnosed patients

diabetes was established. 91 (91%) children were under the age of 15 years. 43 of them were from Baku and Absheron. The incidence of diabetes in Baku and Absheron was calculated, and the incidence rate was 7.05 per 100,000 people. Observations made during the year showed that 96.3% of children (n=102) had type 1 diabetes mellitus, 0.94% (n=1) type 2 diabetes mellitus and 2.8% (n=3) others forms of diabetes mellitus (atypical diabetes in 1 patient and Wolcott-Ralisson genetic syndromes in 1 patient and Koolen di Vriessinder 17q21.31 in 1 patient). All patients were from the Azerbaijani population. The average age of patients with diabetes was 8.9±4.4 years (range from 1.0 to 17.3 years). The median age for detecting the disease was 9.3 years. Peak age accounts for 10 years. T1D was detected at the age of 1 year - 4 years in 25%, at the age of 5-9 years - at 32.7%, at the age of 10-14 years - at 33.7% and at the age of 15-18 years - at 8,7% of patients. In most patients (n=106), polyuria, polydipsia, and weight loss were clinically detected. Diabetic ketoacidosis (DKA) was observed in 58% of patients. DKA was 42.3% at the age of 1-4 years, 84.8% at the age of 5-9 years, 52.8% at the age of 10-14 years and 33.3% at the age of 15-19 years. Among children from Baku, DKA

was found in 61.0%, and among children from remote areas - in 54.4% of cases. The average body mass index was 15.5±2.7 kg / m2 (in the range of 9.9-30.7 kg / m2). The standard tendency for BMI was 6.10–2.61 (average=-1.06). In 3 people, the standard BMI propensity was> 2 (interval 2.07–2.61). In one of these three people, one of the autoantibodies was positive. In 2 people, BMI was <5.0 (interval 5.53-6.10). And these 2 children had autoantibodies positive. The average blood glucose value was 24.0±8.0 mmol/L (range 9.4-44.4 mmol/L), the average HbA1c value was 12.2±1.7% (108.7±19.1 mmol/mol) range 5.5-14.0% (range 36.6-129.5 mmol/mol). 3 patients (3%) had a concomitant disease. In one of the patients aged 2 years and 3 months was diagnosed diabetes insipidus. In this patient, GAD 65 was positive, but no atrophy of the optic nerve and deafness was detected. This patient was diagnosed with Wolfram syndrome. Another patient was diagnosed with Coulin de Vries syndrome. In a third patient, glucose-6-phosphate dehydrogenase deficiency was detected. Among close relatives of 7 patients (7%), T1D was detected: 2 maternal aunts, 2 sisters, 1 father, 1 uncle, and 1 case of paternal cousins. Among other forms of diabetes, atypical diabetes was diagnosed in 1 child, genetic syndrome Koolen di Vriessindr 17q21.31 in 1 child, and Wolcott-Ralisson syndrome in 1 child.All patients received insulin treatment. 60 patients (58%) used long-acting insulin, 61 patients (59%) used short-acting insulin, and 44 patients (42%) used insulin analogues. 51 patients (49%) received 5 injections of insulin per day, 45 patients (43%) received four injections and 8% received 3 injections. 1 child with a type 2 diabetes mellitus (BMI (z=+3.36)) received metformin tablets and long-acting insulin tablets once a day. A patient with atypical diabetes had a BMI (z=-1.13). This patient was taking a hypoglycemic tablet due to severe hypoglycemic events, even when using small doses of insulin injections. The average C-peptide value in 104 patients with T1D was 0.11±0.10 nmol / L (0.32±0.31 ng/ml). In 40 patients (38.5%), the C-peptide was ≤0.07 nmol / L (≤0.20 ng / ml), in 89 patients (85.6%)<0.13 nmol / L (<0,40 ng/ml), in 8 patients (7.7%) 0.13-0.26 nmol/L (0.40-0.80 ng/ml) and in 7 patients (6.7%) 0,26-0.56 nmol/L (0.80-1.70) ng/ml) (the maximum value of the C-peptide is

0.56 nmol/L (1.70 ng/ml)). The level of C-peptides was less than 0.07 nmol/L (≤0.20 ng/ml) in 59 children aged 0–9 years (18.6%) and 45 children aged 10–18 years (64.4%). The C-peptide was 0.53 nmol/L (1.60 ng/ml) in children with T2D and 0.06 nmol/L (0.18 ng /ml) in children with atypical diabetes. Of 104 patients with T1D, 41 (39%) were IA-2 positive, 64 (62%) GAD 65 positive, 77 children (74%) showed one or both of these two antibodies, and 28 (27%) both antibodies were positive. GAD 65 was positive in 59 children aged 0–9 years (74.6%) and in 45 children aged 10–18 years (46.7%). The average value of IA-2 was 112.3±186.9 IU / ml, for GAD65 - 138.6 ± 168.5 IU / ml. No autoantibodies were found in children with T2D, and only one child with atypical diabetes was IA-2 positive. The HLA DRB1 genotype was determined in 200 healthy and 104 children with diabetes. DRB1*03:01+DRB1*04:02 were heterozygous (24 detected; 15 expected; p=3.4E-03), DRB1 *4:05 were homozygous (3 found; 0.5 expected; p=7,1E-03), DRB1 *09:01+DRB1*07:01 were heterozygous (3 detected; 0.25 expected; p=6.0E-04). In addition, there was a significant absence of DRB1*07: 01+DRB1*03: 01 heterozygous (0 detected; expected 3.75; p=1.48E-02). A study of the DDRB1 locus showed that 38 alleles exist in the population. Analysis of the association with alleles in the human genetic database showed that 14 of these alleles are quite common in people with diabetes. The other 24 alleles were combined for association. At the level of this locus, significant heterogeneity was found in patients with diabetes mellitus and in the healthy group (p<2.22E-16). DRB1*03:01 and DRB1*04:02 were positively associated with diabetes mellitus (OR=5.06 and 4.47; p=7.77E-13 and 2.27E-10). In addition, DRB1 * 04:05 was positive (OR=3.53; p=1.90E-03). Six of the 11 alleles were negatively associated with the disease, i.e. were protective. These included the alleles of the DR2, DRB1*15:01 and DRB1*15:02 groups. However, in the children's population of Baku city and Absheron region this indicator is 7.05 per 100,000 people. In some cases with diabetes, deaths are possible. However, since the children examined in Baku are part of the urban population, the number of deaths will be lower due to the timely assistance provided to these

patients. In this study, 96.3% of children were diagnosed with T1DM. The structure of T1DM in Azerbaijan is similar to the European and Turkish populations in terms of the peak incidence and classic symptoms of the disease. In our study, unlike the study conducted in Turkey, the seasonal nature of diabetes was not confirmed. In Turkey, the disease is seasonal and more common in winter and autumn. If in Turkey and Uzbekistan T1DM is more common among girls, then in Azerbaijan, on the contrary, this disease is more common among boys. While the frequency of occurrence of diabetic ketoacidosis in the world is at the level of 12-80%, in Azerbaijan this indicator is 57.7%. This figure is quite high, but in Uzbekistan, diabetic ketoacidosis occurs with a frequency of 50.8%, and in Turkey - 65.9%. The high frequency of diabetic ketoacidosis increases the risk of death among patients and creates the conditions for the development of chronic complications. 48% of patients arrived in Baku from remote areas, and among them, the frequency of diabetic ketoacidosis was identical to that in Baku. In the study, 61.5% of patients with T1D showed auto-antibodies to GAD65, 39.4% showed autoantibodies to IA-2, and only 26.9% showed both autoantibodies. C-peptide was below normal in most patients (86%<0.13 nmol / L [<0.40 ng / ml]). There is very little such information about other Turkic peoples. Only one study in Turkey indicated that 63% of children were positive for GAD 65, and for 27.6% of Azerbaijanis living in Iran, these autoantibodies were also positive. In Iranian Azerbaijanis, C-peptide values were below normal in 94.1% of cases (<0.17 nmol / L (<0.50 ng / ml)). There is a similarity between the results for GAD 65 and the levels of C-peptide in our study and the results of an extensive study in the United States. In this study, antibodies to GAD 65 were positive in 61.4% of cases, and the C-peptide was below normal in 38.4% of cases (≤0.07 nmol / L [≤0.20 ng/ml]). Alleles of DRB1, characteristic of both the population of Europe and Asia, are present in the Azeri population. The results are consistent with data on the European population. In the Azeri population, unlike the European one, the allele predisposing to diabetes is not DRB1*04:01, but the DRB1*04:02 allele. The Azeri population is considered heterozygous for the DRB1*03:01 and

DRB1*04:02 alleles and homozygous for the DRB1*04:05 allele. These combinations of alleles are important in the development of diabetes in our population. Our population has the DR2 DRB1*15:01 allele, found in Europeans, and the DR2 DRB1 *15:02 allele, found in the peoples of Asia. Both alleles are considered protective alleles for diabetes. A study of the HLA loci DQA1 and DQB1 showed that the alleles for diabetes are DQB1*02 (mainly found in the haplotype DRB1*03:01), DQA1*03, DQB1*03:02 and DQB1 *03:04 (all found in the haplotype DRB1*04). Alleles DQB1 *06:02, DQB1 *05:03 and DQB1*06:01, found in the haplotype DRB1 - 15:xx or 16:xx, are usually protective for the European population. A study in southwestern Turkey showed that the alleles predisposing to diabetes were DQB1 *02 and the haplotype DRB1 *03 -DQB1*02. It was also found that the DQB1*03 allele is a protective allele, but a low-resolution genotype study showed that the DQB1 *03:01 allele (usually considered a diabetic protector) is different from the DQB1*03:02 allele (usually a diabetes-prone allele). In our country, mainly T1D occurs among children, and in rare cases, other types of diabetes. In the Republic of Azerbaijan, the incidence of diabetes, as in most countries, is considered average. Clinical features, C-peptide values and HLA DRB1 status are similar to European populations. Thus, as a result of a one-year observation in our population, various clinical forms of newly diagnosed diabetes mellitus and predisposing and protective alleles of the DRB1 gene were revealed. To date, a comprehensive assessment of the use of insulin analogues in children under the age of 2 years has not been conducted. All this once again proves the relevance of the work and allows you to optimize the treatment of diabetes. For this purpose, children used Aspart, ultra-short-acting insulin, and Detemir, prolonged-acting insulin. To evaluate the effectiveness of treatment, HbA1c (Clover A1c, Infopia Co Ltd), fasting blood glucose, and glycemic profile 4 times a day were determined. The number of hypoglycemia, body mass index, and clinical condition of the patients were also evaluated. Several groups were followed up for 11 months at the Children's Clinical Hospital No. 6 in Baku. The study included children with newly diagnosed diabetes

mellitus and patients with a disease duration of 4.2±2.8 years. The initial glycogemoglobin value averaged 10.1±2.49%. The age of the patients was from 0 to 18 years, the average age was 12.3±1.97 years. Patients divided according to the degree of compensation according to the criteria of the International Glycohemoglobin Guidelines (ISPAD Consensus Guidelines, 2014). The following classification was used: (HbA1c compensation ≤7.5%; HbA1c subcompensation 7.6–9.0%; HbA1c decompensation ≥9.1%). Patients with a glycohemoglobin level below 7.5% were 12%, children with a rate of 7.6-9.0% - 21%, and among those with a glycohemoglobin level of more than 9.1% - 67% were prevalent in patients with decompensated diabetes mellitus. Diabetes mellitus was stable in 45% of patients, in 55% laxative with serious metabolic changes and the need for dose adjustment of insulin. A certain group of children was treated in a hospital. At the beginning of the study, all children used long-acting human isophane (NPH) insulin and short-acting human insulin (rDNA). Later, the children were transferred to Aspart and Detemir, which are new analogues of insulin. Children with newly diagnosed diabetes mellitus achieved a faster state of compensation than children using human (rDNA) and human isophane (NPH) insulins. The dose of the long-acting insulin analogue for 11 months did not change significantly, fluctuating on average from 19±7.6 units to 20±6.9 units ($p>0.05$). There was no significant increase in the dose of Aspart: from 21±8.1 units to 22±8.4 units. In 6 patients, due to the high glucose level in the morning, Aspart was additionally applied at 6 a.m. In the general group, glycohemoglobin was 10.1±2.49% at the beginning of the study and 7.2 ±1.97% after 11 months ($p<0.001$). The number of hypoglycemia in patients decreased mainly at night ($p<0.001$). 4 patients had short-term redness, swelling, itching at the injection sites of insulin, which later disappeared. In the group receiving Aspart/Detemir, stabilization was observed in HbA1c values. This indicator was 10.1±2.49% at the beginning of treatment and 7.2±1.97% at the end of treatment ($p< 0.05$). Best results were obtained in children taking Detemir twice a day. In terms of carbohydrate metabolism, children were divided into 3

groups: compensation (12%), subcompensation (21%) and decompensation (67%). The best results were obtained in the group of children with decompensation. So, at the beginning of the study, the level of HbA1c was high in 67% of children, but after 11 months only 30% of children found decompensation ($p<0.01$). In the subcompensation group, HbA1c improved by 32%. Positive dynamics of HbA1c indices was also recorded in the compensated group. The effectiveness of basal insulin is assessed by blood glucose in the morning. Patients who received Detemir were more likely to have normal blood glucose levels in the morning than those who received human isophane (NPH) insulin, and did not need additional injections. The initial basal dose was 0.44 units /kg /day, but was later increased to 0.61 units/kg /day ($p<0.001$). Thus, as a result, the dose of Detemir was increased. There were no changes in the dose of insulin of ultrashort action. Thus, our observation showed that long-acting insulin is necessary to adjust the dose of insulin. When correcting carbohydrate metabolism, in addition to glycemic control, the amount of hypoglycemia should also be considered. It is known that the number of hypoglycemia increases three times with intensive insulin therapy. According to published data, the use of insulin analogues reduces the number of hypoglycemia. The incidence of hypoglycemia decreased from 35% to 5% in patients receiving Detemir ($p<0.001$). At the same time, Detemir does not affect the body mass index. Within 11 months in children receiving Detemir, against the background of normalization of carbohydrate metabolism, there was no increase in BMI ($p>0.05$). Another group consisted of 21 children aged 1-2 years.

The results of the examination of young children are shown in figure 3 below. As can be seen from figure 3, the combined use of human isophane (NPH) insulin and human insulin (rDNA) is more effective in children under 2 years of age than the new insulin analogues Detemir and Aspart. When using human insulin (rDNA) and human isophane (NPH) insulins, the average glycohemoglobin values were 8.9±1.24%, while for those receiving insulin analogues this indicator was 11.5±2.22% ($p<0.001$). Despite the fact that the total doses of insulin analogues were increased, compensation was not achieved.

Thus, an 11-month observation showed that positive dynamics of glycohemoglobin was observed when using insulin analogues.

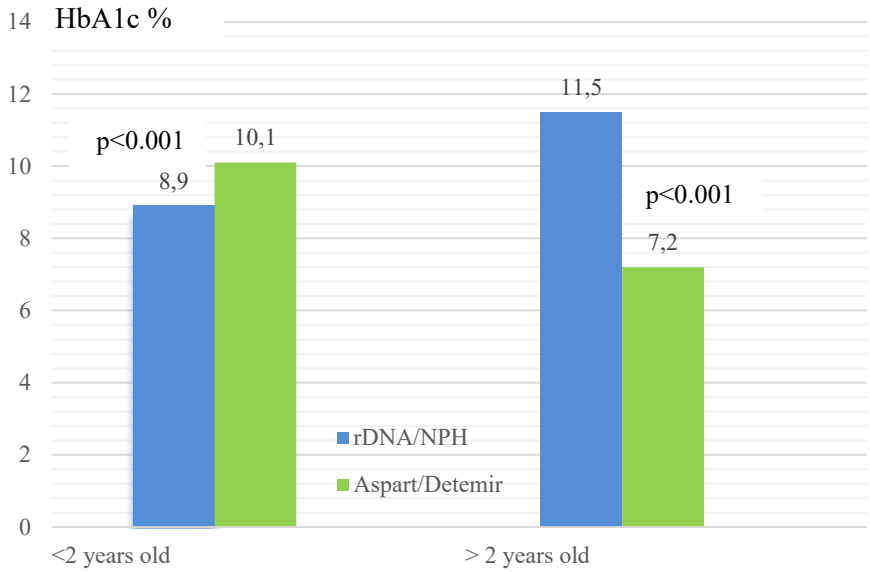

Figure 3. HbA1c values of the children

The main reason for decompensation is the lack of dose adjustment of basal insulin. The use of basal analogues made it possible to reduce the number of hypoglycemia and improve the quality of life of patients. In 2014, 21 children with diabetes on an insulin pump were monitored, in 2015-11 children and -in 2016-8 children. All children used the DANA Diab-care Remote System insulin pump (Seoul, Korea, SOOIL Development). For 3 years, the same children participated in the study. But in the following years, some of them could not participate further. The examination was conducted at the Children's Clinical Hospital No. 6 in Baku. All children were allowed to use the insulin pump after careful training in the rules for its use. When comparing the data obtained for 2014 and 2015, it was found that the HbA1c in 2014 was 9.4%, and in 2015-8.7%. Although there

was no reliability between these indicators, a negative correlation was observed between the duration of the disease and HbA1c. This indicates that the use of an insulin pump had a positive effect on the compensation of the disease. Another goal of our study was to compare the clinical and laboratory parameters of children with diabetes living in several regions of Azerbaijan. For this, children with diabetes living in the cities of Baku and Ganja were examined. 26 children registered in the endocrinological hospital in Ganja were examined in 2007, 20 children in 2009 and 27 children were examined in Baku in 2010. 7.4% (n=2) of patients living in Baku had diabetic nephropathy at the stage of microalbuminuria, and 7.4% (n=2) were diagnosed with chronic kidney diseases not associated with diabetes mellitus. In Ganja, in 2007, 7.7% of children (n=2) were diagnosed with diabetic cataracts, 3.8% of children (n=1) had diabetic retinopathy, and 3.8% of children (n=1) had diabetes nephropathy at the stage of microalbuminuria. In 2009, in Ganja, 5% had (n=1) cataracts, 10% (n=2) had diabetic hyropathy, and 5% (n=1) had a diabetic foot (Charcot's foot). It should be noted that Charcot's foot is one of the rare complications of the joints in diabetes[31]. The features of the lipid profile in children with diabetes were studied. During 2014–2015, 63 children with primary diabetes and 70 children with diabetes for several years were examined. Patients with newly diagnosed diabetes were found to have positive correlations between blood glucose and triglycerides, and those who have been ill for several years had positive correlations between the duration of the disease and cholesterol, triglycerides, LDLP. Thus, an increase in blood glucose leads to an increase in cholesterol, triglycerides and LDLP. Lipids were studied in comparison in boys and girls separately. Another interesting correlation is the negative correlation between triglyceride and the age of the boys ($r=-0.42$, $p=0.026$). High triglycerides are more common in young children.

RESULTS

1. As a result of our study, it was revealed that in the Azeri population, the alleles DQB1*02, *0302, *0304 and DQA1 predisposed to the development of diabetes, while the alleles DQB1 *0301, *0501, *0601, *0602 and DQA1*3 were identified as protective alleles. The genetic risk of developing type 1 diabetes mellitus in the Azeri population is mainly the haplotype HLA DQ2, then HLA DQ8. The odds ratio for heterozygosity of DQB1*02-DQA1 *05/DQB1 *0302-DQA1*03 (DQ 2.5/DQ8) was 15.38 (95% confidence interval: 7.06-33.5). In the 5–9-year-old age group, the HLA DQ2 haplotype (48%, $p<0.05$) and the HLA DQ2/DQ8 heterozygosity (71.4%, $p<0.0002$) were detected more often. Studies of the DRB1 locus showed that 38 alleles are characteristic for the Azeri population, of which 14 of them occur during T1DM [2, 3, 7, 10, 13, 19, 20, 23, 24, 36, 42].
2. The genes CTLA-4 + 49 A/G, insulin -23 HphI and polymorphisms of the PTPN22 -1123 C/G and +2740 A / G genes do not pose a risk for T1DM in the Azeri population. There is only a connection between the T1DM and the R620W polymorphism of the PTPN22 gene [26, 27, 33].
3. There is a positive correlation between diabetic nephropathy and HLA DQB1 ($r=0.66$, p 0.052) [29].
4. In children with primarily diagnosed diabetes mellitus compared with healthy children among the CD indices, a significant difference was found only in the CD19+ indices ($p<0.05$). In these patients, a negative correlation was observed between the HLA DRB1 genotype and lymphocytes ($r=-0.29$, $p<0.05$) [17, 18, 25].
5. The results of the studies showed that in the Azeri population there is a relationship between T1DM and microorganisms Escherichia (class Gammaproteobacteria, type Proteobacteria), Prevotella-massilia (c.Bacteroidia, p.Bacteroidetes) and Megasphaera (c. Clostridia, p.Firmicutes). No association between viruses and this disease was detected. In all age groups of children, a connection was also observed between T1D and Haemophilus bacteria, while

in older children only between Clostridium clusters IV, XIV [34, 38].

6. In children with diabetes, there was no association between the lipid profile and the alleles of the DRB1 gene [9, 12].
7. The incidence of T1D in the Baku and Absheron region among children under the age of 15 is 7.05 per 100,000 people. Within one year of observation, T1DM was detected in 96.3% (n=102) of children, T2DM was detected in 0.94% (n=1) ($p<0.0001$), in 2, 8% (n=3) ($p<0.0001$) other forms of diabetes were found. Among other forms of diabetes, atypical diabetes was diagnosed in 1 child, genetic syndrome Koolen di Vriessindr 17q21.31 in 1 child, and Wolcott-Ralisson syndrome in 1 child. The disease is prone to more frequent development in boys. The incidence of diabetes is not seasonal. In patients with autoimmune diabetes mellitus, alleles *03:01, *04:02, *04:05, *04:08, *09:01of the DRB1 HLA gene were found. In a study in patients with T1D in 61.5% of cases were detected GAD 65 autoantibodies, in 39.4% of cases - IA-2, and only 26.9% of patients had both autoantibodies positive. Diabetic ketoacidosis in most cases was detected at the age of 5-9 years (84.8%) [22, 31, 39, 41].
8. In children under 2 years of age, the combined use of human isophane (NPH) insulin and human insulin (rDNA) is more effective than the use of the new Detemir and Aspart insulin analogues, the average glycohemogobine in these patients is 8.9±1.24%, while in patients taking new insulin analogues, this indicator is 11.5±2.22%. ($p<0.001$). When using Aspart/Detemir in children older than 2 years, stabilization of HbA1c was observed. This indicator was 10.1±2.49% at the beginning of treatment and 7.2±1.97% at the end of treatment ($p<0.001$). Despite the absence of a significant difference between HbA1c values in children using an insulin pump, a negative correlation was observed between the duration of the disease and HbA1c ($r=-0.69$, $p<0.05$), which is a positive result. In children living in Ganja, in comparison with children living in Baku, chronic complications of T1DM were more common ($p<0.05$) [6, 28].

PRACTICAL RECOMMENDATIONS

1. By studying the alleles of class II genes of the HLA DRB1, DQA1, DQB1 systems, it is recommended to identify the risk of diabetes in the Azeri population at an early stage.
2. The study of the incidence of type 1 diabetes mellitus in Baku city and Absheron allows predicting its prevalence in the Republic of Azerbaijan.
3. Genetic research methods is recommended used along with routine ones create conditions for identifying various clinical forms of diabetes in children.
4. The identification of Escherichia (class Gammaproteobacteria, type Proteobacteria), Prevotelmassilia (c.Bacteroidia, p.Bacteroidetes) and Megasphaera (c.Clostridia, p.Firmicutes) which contribute to the development of diabetes mellitus, can be considered as a risk factor for the development of this disease in healthy children.
5. It is recommended the use of insulin human isophane (NPH) insulin and human insulin (rDNA) in the treatment of diabetes in children under 2 years of age.

LIST OF WORKS PUBLISHED ON THE THEME OF THE DISSERTATION

1. Əhmədov G.Ə. 1-ci tip şəkərli diabetin genetikası //-Bakı: Azərbaycan Metabolizm Jurnalı, -2004. Yanvar-İyun; №1, -s.2-6.
2. Ahmedov, G.A. Genetic association of type 1 diabetes in an Azerbaijanian population: the HLA DQ, -DRBI*04, the insulin gene, and CTLA-4 / G.A.Ahmedov, L.Ahmedova, P.Sedlakova [et al.] //Pediatric Diabetes, -2006.7, -p.88-93.
3. Cinek, O. No independent role of the -1123G>C and +2740A>G variants in the association of PTPN22 with type 1 diabetes and juvenile idiopathic arthritis in two Caucasian populations / O.Cinek, O.Hradsky, G.Ahmedov [et al.] // Diabetes Res. Clin. Pract., -2007. 76, -p.297–303.
4. Əhmədov G.Ə. Uşaq və yeniyetmələr arasında şəkərli diabetin epidemiologiyası //-Bakı: Allerqologiya, İmmunologiya və immunoreabilitasiya üzrə IV Azərbaycan Milli Konqresinin materialları -2012, 19-20 Oktyabr; -s.321-328.
5. Ахмедов Г.А. Анализ состояния детей по основному информационному листу в течении 3 лет // -Bakı: Allerqologiya, immunologiya və immunoreabilitasiya üzrə IV Azərbaycan Milli Konqresinin materialları, -2012. 19-20 Oktyabr; -s. 320.
6. Ахмедов, Г.А.Сравнительная оценка эффективности базисболюсных режимов терапии с инсулинами Левемир и Новорапид на гликемический контроль у детей и подростков с диабетом 1 типа //-г. Алмата: Журнал «Медицина»,- 2014. №11/149, -с.32-37.
7. Noble, J.A. Type 1 diabetes: around the world with HLA / J.A. Noble, J.A.Lane, G. Ahmadov [et al] // -Denver, Colorado, USA, 40th Annual Meeting of the American Society for Histocompatibility and Immunogenetics, Human Immunology: -2015. -p. 230 (abstract LBP225).
8. Ahmadov, G. Incidence of diabetes mellitus and associated complications among children: results from Azerbaijan diabetes registry 1997-2007 //-Dublin, European Society for Paediatric

Endocrinology (ESPE), 53rd Annual Meeting, Horm. Res. Paediatr. -2014. September; 82 (1):-507, -p.363
9. Əhmədov, G.Ə. Şəkərli diabeti olan uşaqlarda lipid mübadiləsinin xüsusiyyətləri/-Bakı:Azərbaycan Tibb Jurnalı,-2015. №2,-s.21-25.
10. Ahmadov, G, Immunogenetics of type 1 diabetes in developing countries / G.Ahmadov, J.Noble, J.Lane [et al] //-Vancouver, Canada: IDF World Diabetes Congress, -2015. 30 November- 4 December; abstract –p. 0387.
11. Əhmədov, G.Ə. Uşaqlarda dislipidemiya //-Bakı, Azərbaycan təbabətinin müasir naliyyətləri, -2015. №3, -s.119-128.
12. Əhmədov, G.Ə., İnsulindənasılı şəkərli diabeti olan uşaqlarda insan leykositar sistemin DRB1 geni ilə lipidlər arasında əlaqənin öyrənilməsi / G.Ə.Əhmədov, A.A.Əyyubova, V.A.Mirzəzadə [və b.] // -Bakı: Azərbaycan Perinatologiya və Pediatriya Jurnalı, -2015. (1) 4,-s.10-16.
13. Noble, J.A. HLA-associated susceptibility to type 1 diabetes in the developing world reveals differences among populations; implications for prediction and intervention / J.A.Noble, J.A.Lane, G.Ahmadov //-Geneva, Switzerland, 29th European Immunogenetics and Histocompatibility Conference, -2015. April; abstract 3262.
14. Əhmədov, G. Uşaqlarda insulindənasılı şəkərli diabetə genetik meyilliyin öyrənilməsi / G.Əhmədov., J.Noble., M.Atkinson [və b.] // -Bakı: Sağlamlıq Jurnalı -2016. №1, -s.140-144.
15. Əhmədov, G. Uşaqlarda şəkərli diabetin müxtəlif klinik formalarının aşkar edilməsi //-Bakı, Azərbaycan Tibb Jurnalı, -2016. №3, -s. 9-12.
16. Əhmədov, G.Ə. İnsulindənasılı şəkərli diabeti olan uşaqlarda hüceyrə immun sisteminin göstəriciləri arasındakı əlaqənin öyrənilməsi //-Bakı: Azərbaycan Metabolizm Jurnalı, -2016, cild 13, Aprel-İyun. №2, -s.34-40.
17. Əhmədov, G.Ə. Uzun müddət şəkərli diabetlə xəstə olan uşaqlarda immun sistemin öyrənilməsi / G.Ə.Əhmədov, G.MNəsrullayeva,

V.R. Məmmədova [və b.] //-Bakı: Azərbaycan Allerqologiya və Klinik İmmunologiya Jurnalı, -2016. (4)2, -s.18-21.
18. Əhmədov, G.Ə., Süleymanlı, A.A. Uşaqlarda insulindənasılı şəkərli diabetdə hüceyrə immunitetinin rolu // -Bakı: Azərbaycan təbabətinin müasir naliyyətləri, -2016. №3, -s.79-82.
19. Əhmədov, G.Ə. Uşaqlarda insan leykositar anticisim sisteminin DR-DQ2 serotipi və insulindənası şəkərli diabetə risk //-Bakı: Sağlamlıq Jurnalı,- 2016. №6, - s. 119-123.
20. Ахмедов, Г.А., Особенности иммунной системы у больных детей с впервые выявленным сахарным диабетом и его взаимосвязь с геномом HLA DRB1 /Г.А.Ахмедов, Г.М.Насруллаева, В.Р.Мамедова [и др.] //-г. Киев: журнал "Вестник проблем биологии и медицины", -2016. (4) 2,134, -с.52-55.
21. Ogle, G.D. Costs to governments of type 1 diabetes care in youth in less-resourced countries-three scenarious for differenr income levels / G.D.Ogle, J. R. Fernandes, G.Ahmadov [et al.] // -Valencia, Spain: International Society for Pediatric and Adolescent Diabetes meeting, Pediatric Diabetes, -2016. 26-29 October; 17 (Suppl.24) 10, -p.35.
22. Ogle, G.D. IDF life for Child six-country epidemiology study-preliminary results from Azerbaijan, Bangladesh, Mali and Pakistan / G.D.Ogle, G.Ahmadov, B.Zabeen [et al.] // -Valencia, Spain: International Society for Pediatric and Adolescent Diabetes meeting, Pediatric Diabetes, -2016. 26-29 October; 17 (Suppl.24) 10, -p.34.
23. Noble, J.A. Differences in HLA DRB1 association with Type 1 Diabetes among Populations in Developing Countries / J.A.Noble, J.A.Lane, G.Ahmadov [et al.] // -New Orleans: American Diabetes Association 76th Scientific Sessions. Diabetes, -2016. June 10-14; 65(1), Abstract number 1596-P, -p, A416-A417.
24. Noble, J.A. HLA DRB1 association with type 1 diabetes differs among populations in developing nations / J.A.Noble, J.A.Lane, G.Ahmadov [et al.]//-Kos Island, Greece: 30th European Immunogenetics & Histocompatibility Conference on Kos island. -2016. 11-14 May;-p. 232. Əhmədov, G.Ə., Süleymanlı, A.A. Şəkərli diabeti olan uşaqalarda hüceyrə immun sisteminin öyrənilməsi //-

Bakı: Allerqologiya, immunologiya və immunoreabilitasiya üzrə V Azərbaycan Milli Konqresinin materialları, -2016. 21 Oktyabr; -s.47-48.
25. Ахмедов, Г.А. Значение гена CTLA-4 у детей с инсулинозависимым сахарным диабетом в азербайджанской популяции //-г. Минск: Журнал "Медицинские новости ", -2017. № 5 (272), -с. 76-78.
26. Ахмедов, Г.А. Изучение гена инсулина у детей с инсулинозависимым сахарным диабетом в азербайджанской популяции // -г. Киев: Семейная медицина. Научно-практический журнал. -2017. №02 (70), -с. 120-123.
27. Əhmədov, G.Ə. Azərbaycanın müxtəlif ərazilərində yaşayan şəkərli diabeti olan uşaqlarda klinik və laborator dəyişikliklərin qiymətləndirilməsi //-Bakı: Azərbaycan Təbabətinin Müasir Naliyyətləri, -2017, №2, -s. 49-52.
28. Əhmədov, G.Ə. Şəkərli diabetin ağırlaşmaları ilə risk genləri arasında əlaqələr //-Bakı: Azərbaycan Tibb Jurnalı , -2017. №2, -s.5-8.
29. Ахмедов, Г.А. Значимость аутоантител при инсулинзависимом сахарном диабете //-г. Казань: Казанский Медицинский Журнал, -2017. (98)4, -с.519-521.
30. Ахмедов, Г.А. Сравнительный анализ генов DQB1 и DQA1 II класса системы человеческих лейкоцитарных антигенов в Азербайджанской популяции // -г. Казань: Казанский Медицинский Журнал, -2017. (98)5, -с.704-708.
31. Əhmədov, G.Ə. İnsulin pompası ilə müalicə alan şəkərli diabetli xəstələrin vəziyyətlərinin qiymətləndirilməsi //-Bakı: Azərbaycan Perinatalogiya və Pediatriya Jurnalı, -2017. (3) 1, -s.70-75.
32. Ахмедов, Г.А. Изучение гена PTPN22 у детей с сахарным диабетом типа 1 в Азербайджанской популяции //-г.Тбилиси: Georgian Medical News, 2017. №10 (271) Октябрь, -с. 45-49.
33. Əhmədov, G.Ə. Uşaqlarda şəkərli diabetin yaranmasında bağırsaq mikroflorasının rolu //-Bakı: Azərbaycan Təbabətinin Müasir Naliyyətləri, -2017. №4, -s.103-106.

34. Əhmədov, G.Ə. Azərbaycan populyasiyasında insulindənasılı şəkərli diabeti olan uşaqlarda İnsan Leykositar Antigen sisteminin II sinifinin DRB1 geninin təhlili / G.Ə.Əhmədov, A.N.Janelle, M.Atkinson [və b.] // Azərbaycan Metabolizm Jurnalı, - Bakı: - 2017. cild 14, yanvar-mart, №1,-s.18-24
35. Noble, J.A. Extreme variability of risk for HLA "DR3" (DRB1 *03:01-DQA1*05:01-DQB1*02:01) Among World Populations / J.A.Noble, S.J.Mack, G.Ahmadov [et al.] // - San Diego, California, USA:American Diabetes Association. -2017. June 9-13;-p. A-6198.
36. Cinek, O. Quantitative CrAssphage real-time PCR assay derived from data of multiple geographically distant populations / O. Cinek, K. Mazankova, G.Ahmadov [et al.] // Journal of Medical Virology, -2018. №1, -p.1-5.
37. Cinek, O. The bacteriome at the onset of type 1 diabetes: A study from four geographically distant African and Asian countries / O.Cinek, L.Kramna, K.Mazankova [et al.] // Diabetes Res. Clin. Pract. -2018. Oct; 144, -p.51-62.
38. Ahmadov, G. A. Epidemiology of childhood-onset type 1 diabetes in Azerbaijan: Incidence, clinical features, biochemistry, and HLA DRB1 status / G.A. Ahmadov, D.Govender, A.M.Atkinson [et al.] // Diabetes Res. Clin. Pract. -2018. Oct; 144, -p.252-259.
39. Edwards, R. A. Global phylogeography and ancient evolution of the widespread human gut virus crAssphage /R.A.Edwards, A.V.Alejandro, G.A.Ahmadov [et al.] // Nature Microbiology,-2019. October; 4, -p.1727–1736.
40. Ondrej Cinek, Eukaryotic viruses in the faecal virome at the onset of type 1 diabetes: a study from four geographically distant African and Asian countries" /Lenka Kramna, Karla Mazankova, Rasha Odeh, Abeer Alassaf, MaryAnn U. G.A.Ahmadov [at.all] //Pediatic Diabetes, 2021
41. Ahmadov, G.A., Noble, J., Ogle, G. Comprehensive Analysis of HLA System Class II DRB1 in children with Insulin Dependent Diabetes Mellitus in the North Azerbaijan and Iranian Azerbaijan // Horm. Res. Paediatr., -2019. 91(suppl 1):1-682, -p.538.

Conditional Abbreviations

IA-2	Protein tyrosine phosphatase autoantibody
IAA	Insulin autoantibodies
IRI	Immunoregulatory index
GAD 65	Glutamate decarboxylase autoantibody
CI	Confidence interval
DKA	Diabetic ketoacidosis
DNA	Deoxyribonucleic acid
BMI	Body mass index
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
LDL	Low density lipoproteins
HLA	Human Leukocyte Antigen
CD	Monoclonal antibodies
$CD3^+$	T-lymphocyte
$CD4^+$	T – lymphocyte helper
$CD8^+$	T - lymphocyte – supressor
$CD19^+$	B-lymphocyte
DRB1	Gene of surface cell membrane β1-chains
DQA1	Gene of surface cell membrane α1-chains
PTPN22	Non-receptor lymphoid protein gene tyrosine phosphatase type 22
DQ2	Allele group, haplotype
DQ8	Allele group, haplotype
HbA1c	Glycogemoglobin
-23HphI	Marker for polymorphism of the insulin gene

The defense will be held on "23" "june" 2021 year at 14^{00} the meeting of the Dissertation council BED 2.27/2 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical University

Address: AZ 1022, Baku city, Str., Anver Gasimzade, 14
(Administrative building of the AMU. 2nd floor, Assembly hall of the meeting of the Academic Council)

Dissertation is accessible at the Azerbaijan Medical Univercity Library

Electronic versions of dissertation and its abstract are available on the official website of the Azerbaijan Medical Univercity:
www.amu.edu.az

Abstract was sent to the required addresses on "18" "may" 2021

Signed for print: 05.05.2021

Paper format: A5

Volume: 79394 sign

Number of hard copies: 20