

REPUBLIC OF AZERBAIJAN

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ABSTRACT

for the dissertation for the degree of Doctor of Philosophy

**FUNCTIONAL STATUS OF LIVER
AND CYTOKINE STATUS
IN PATIENTS WITH DIABETES MELLITUS**

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Field of science: Medicine
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Baku – 2022

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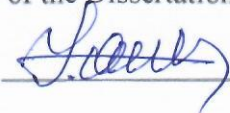
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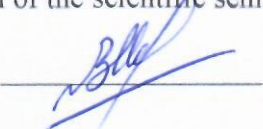
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GENERAL CHARACTERISTICS OF WORK

The actuality of the subject. Diabetes mellitus (DM) is accepted one of the main problems after cardiovascular and oncological diseases, in modern times. The special interest in this problem is explained by the fact that DM is a very common endocrine pathology and has a tendency to increase sharply and is considered a medical and social problem in many economically developed countries. This increase is due to hereditary predisposition, decreased physical activity of the population, excessive consumption of high-calorie foods and, consequently, the development of obesity and insulin resistance^{1,2}.

Metabolic disorders during DM lead to changes in the functional activity of all organs and systems, vision, cardiovascular, digestive systems, as well as the hepatobiliary system. DM is one of the diseases in which the liver is more and more severely damaged and therefore, the study of its functional status is of particular interest, because liver damage significantly affects the course of the disease, the level of compensation, and the prognosis^{3,4,5}.

¹ Егорова, Е.Г., Ильченко, Л.Ю. Печень и метаболический синдром // Гепатология, – 2015. № 4, – с. 28-35.

² Лиходей, Н.В. Анализ факторов, препятствующих формированию приверженности лечению среди больных сахарным диабетом, и стратегий, способствующих ее повышению / Н.В.Лиходей, М.Ф.Калашникова, Е.М.Лиходей [и др.] // Сахарный диабет, – 2018. – Т. 21, № 1. – с. 5-14.

³ Lee, M.K. Changes in metabolic syndrome and its components and the risk of type 2 diabetes: a nationwide cohort study / M.K.Lee, K.Han, M.K.Kim [et al.] // Sci Rep., - 2020.10 (1), - p.2313-2318.

⁴ Wang, S.T. Physical activity intervention for non-diabetic patients with non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials / S.T.Wang, J.Zheng, H.W.Peng [et al.] // BMC Gastroenterol, - 2020,20(1), - p. 66-78.

⁵ American Diabetes Association. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Medical Care in Diabetes-2021 // Diabetes Care, - 2021. 44 (Suppl.1), - p. S40-S52.

The true prevalence level of type 2 DM that is based on references is 2-3 times higher than the recorded morbidity level. In half of the cases of the diseases, type 2 DM is found in the 5-7th year of the onset of the disease therefore, in 20-30% of such patients, complications specific to diabetes occur as soon as diabetes is detected ^{6,7}. All this determines its medical and social significance not only among other forms of diabetes, but also among all chronic non-communicable diseases. In addition to the rapid increase in the incidence of type 2 DM, its characteristics are: the tendency to rejuvenate the debut age, the relatively late detection of both the disease and its complications (due to its long-term asymptomatic course), polymorbidity (especially in combination with cardiovascular disease and obesity) ^{8,9,10}.

The attention of clinicians in different countries is focused on the prevention, diagnosis and treatment of complications of DM that among them injuries of the hepatobiliary system (HBS) occupies an important place. For this reason, the search for new diagnostic methods that expand the possibilities of assessing the degree and nature of its injury during in various diseases of the liver is a very topical issue.

⁶ Eslam, M. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement / M.Eslam, P.N.Newsoms, S.K.Sarin [et al.] // *J. Hepatol.*, – 2020.73(1), – p. 202-209.

⁷ Artasensi, A. Type 2 Diabetes Mellitus: A Review of Multi-Target Drugs / A.Artasensi, A.Pedretti, G.Vistoli [et al.] // *Molecules.* – 2020. 25 (8) – p. 1987-1998.

⁸ Дедов, И.И. Инновационные технологии в лечении и профилактике сахарного диабета и его осложнений // *Сахарный диабет*, – 2013. 3, – с. 4-10.

⁹ Ceriello, A. The burden of type 2 diabetes in Europe: Current and future aspects of insulin treatment from patient and healthcare spending perspectives / A.Ceriello, H.W.deValk, B.Guerci [et al.] // *Diabetes Res. Clin. Pract.*, – 2020. 161 – p. 108053.

¹⁰ Cotter, T.G., Rinella, M. Nonalcoholic Fatty Liver Disease 2020: The State of the Disease. // *Gastroenterology*, - 2020.158 (7), – p. 1851-1864.

In the body, the endocrine and digestive systems are closely linked. The physiological role of the liver, pancreas and intestines in the regulation of glycemia and insulinemia is known to all. In addition, the liver is one of the leading organs involved in the regulation of insulin activity, which plays a major role in the pathogenesis of diabetes. The interaction between the small intestine and the insulin-producing apparatus of the pancreas is called the “entero-insular nucleus.”¹¹

It leads to the formation of active forms of oxygen - cholestasis syndrome, the transition of chronic liver injury, as well as glycolysis of proteins. Along with the formation of this, initiation of lipid peroxidation processes leads to the development of vascular complications, which causes to an increase in the severity of diabetes and disability of patients.¹²

All of the above, laid the groundwork to conduct current research.

Object of the research. Patients with type 1 and type 2 diabetes.

The aim of the study was a comprehensive study of the functional status of the liver in patients with type 1 and type 2 diabetes mellitus (DM) and the development of recommendations for the correction of the detected changes.

¹¹ Dongiovanni, P. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver / P.Dongiovanni, S.Stender, A.Pietrelli [et al.] // J Intern Med., - 2018. 283 (4), – p. 356-370.

¹² Gilani, S.Y. Gender differences of dyslipidemia in type 2 diabetics / S.Y.Gilani, S.Bibi, N.Ahmed [et al.]// J. Aeub. Med. Coll. Abbottabad., – 2010. 22 (3), – p. 146-148.

Objectives of the research:

1. To study the clinical picture of hepatobiliary system injuries in patients with type 1 and type 2 DM.
2. To determine the frequency and character of injury of the liver in patients with type 1 and type 2 DM by laboratory and instrumental methods of examination and assess the severity of major biochemical syndromes (cytolysis, cholestasis, hepatocellular insufficiency, mesenchial-inflammatory).
3. To study the immunological characteristics of patients with type 1 and type 2 DM with liver pathology.
4. To monitor the course of the disease against the background of long-term use of hepatoprotectants in patients with type 1 and type 2 DM.

Methods of the research. Laboratory, immunological and instrumental examination methods.

The main provisions of the dissertation that raised to the defense:

- The nature and frequency of liver damage are related to the type of diabetes, the duration of the disease, and the severity. Timely diagnosis and properly selected treatment can lead to stabilization of the process.
- During diabetes mellitus, liver damage is characterized by diffuse changes in the form of steatosis, accompanied by mixed dystrophy of hepatocytes and this reflects changes which occurring in the metabolism of proteins, lipids and carbohydrates.
- The main role in the development of DM disease belongs to different subpopulations of T-lymphocytes. Total CD3+, helper CD4+, cytotoxic CD8+ T-lymphocyte counts, and NK cells are higher in patients with type 1 DM than in patients with type 2 DM. At the same time in the lysis of the pancreas increases the inflammatory cytokines (IL-1 β , INF- γ , IL-13).
- Examination of the functional status of the liver and gallbladder allows you to detect pathological changes that occur in the activity

of the hepatobiliary system in all patients with type 1 and type 2 DM, Using a set of biochemical methods and US- diagnostics.

The scientific novelty of the work. Clinical-laboratory and instrumental examinations of the liver in DM patients were studied in a complex way, depending on the type, duration and severity of DM.

For the first time, the immunological characteristics of patients with type 1 and type 2 DM were extensively studied. Immunograms were studied in DM patients to identify risk groups for the development of liver pathology, the involvement of immune cells (T-lymphocytes, B-lymphocytes and subpopulations) in the pathogenesis of the disease, their Inflammatory, anti-inflammatory and regulatory cytokines (INF- γ , IL-1 β , IL-10, IL-13), which are their functional immunological markers, have been identified.

The course of the disease was monitored against the background of long-term use of hepatoprotectants in patients with type 1 and type 2 DM.

Practical recommendations have been developed for the timely diagnosis and carrying out of the therapeutic correction of hepatobiliary system injuries based on the functional status of the liver and the results of examinations in patients with type 1 and type 2 DM.

Practical significance of the work. Clarification of the nature and mechanisms of changes in the functional state of the liver in patients with DM (Taking into account the type of DM, duration, severity of the disease, concomitant pathology) and the proposed method for the comprehensive detection of these changes increases the effectiveness of treatment tactics.

Detailed clinical-laboratory and instrumental examination of all patients has been shown to be important for assessing the functional status of the liver in patients with DM, even in the early stages of the process, in the absence of serious clinical symptoms.

When patients with DM are diagnosed with severe immune disorders or secondary immunodeficiency, they should undergo immunorehabilitation under the supervision of an immunologist.

Detection of early signs of liver damage and conducting of targeted clinical and functional examination of patients with DM has

been justified for the purpose of their additional correction.

Approbation of the work. The main provisions of the work were presented at the following scientific-practical conferences: At the scientific conference on “Actual problems of medicine” (Baku, 2018), at the 1st International Conference on “Immunopathological diseases” (Baku, 2019), and at the International Medical Conference “Biomedical Perspectives III”(Sumy, Ukraine, 2021).

Materials of the dissertation at the meeting of the Department of Internal Medicine-2 of AMU (protocol №19 on 17.05.2018), at the scientific seminar of BFD 2.27/1 Dissertation Council operating under AMU (05.10. 2021, protocol № 01) were reported and discussed.

Application of the research results. The results of the research are applied in the teaching process of the II Department of Internal Medicine of AMU, in the practical work of the Training-Therapeutic Clinic of AMU and the Republican Endocrinology Center.

Place of performance of the research. The dissertation work was carried out at the II Department of Internal Medicine of the Azerbaijan Medical University, the Republican Endocrinology Center.

Publication of the main results of the dissertation. Based on the materials of the dissertation, 13 scientific works reflecting the main content of the work - 8 articles (3 abroad) and 5 theses were published.

Volume and structure of the dissertation. The dissertation is written on 166 pages (200522 characters) with computer text, consists of introduction (5 pages, 8514 signs), 5 chapters of the research (120 pages, 159766 signs), final (13 pages, 24977 signs), results (2 page, 2194 signs), practical recommendations (1 page, 684 signs) and a bibliocharity (20 pages) which included 195 bibliocharitic sources and 13 of them refer to the homeland, 182 - to foreign sources. The work is illustrated with 45 tables and 28 charts.

MATERIALS AND METHODS OF THE RESEARCH

The research was carried out at the Department of Internal Medicine-2 of the Azerbaijan Medical University, the Teaching Therapeutic Clinic of AMU and the Republican Endocrinology Center. Clinical and biochemical examinations were carried out on the basis of the Republican Endocrinology Center using the prospective method. Laboratory immunological examinations were carried out in the Scientific-Research Immunology Laboratory of AMU.

156 patients those suffering from diabetes mellitus organized the main group during the research. These patients were divided into the following observation groups according to the diagnostic criteria and classification proposed by the WHO Expert Committee in 1999: To the Group I - 40 patients aged 20 and older out of 99 patients with type 1 DM and hepatobiliary system disorders selected and included in the group, and Group II consisted of 57 patients with type 2 DM and disorders of the hepatobiliary system. All patients were advised by an endocrinologist to determine treatment tactics. The control group consisted of 23 practically healthy individuals.

The functional status of the liver in patients with type 1 and type 2 DM was studied in accordance with the objectives of the study. All patients who participated in the study were examined at the beginning and after 6 months: Dynamics of blood glucose (according to laboratory and self-monitoring data), general examination of blood, general examination of urine, biochemical examination of blood, ultrasound examination of the liver in order to detect pathology of the liver, the nature of the pain in the right subcostal region, their localization (including at the point of projection of the gallbladder) during palpation and presence of positive symptoms of Georgiyevsky-Mussi, Ortner, Kerr, Murphy have been identified and the size of the liver was determined by Kurlov, and most importantly, attention was paid to the results of the functional state of the liver (compared with clinical data).

In order to determine the status of carbohydrate metabolism, to verify the diagnosis of DM, the average daily amount of fasting glucose in the blood serum, the level of glucose in the blood serum

after exercise, the amplitude of mean diurnal changes in glucose levels (AGA) was examined and, the amount of glycosylated hemoglobin (HbA1c), urinary excretion of microalbumins was determined.

During the examination, the main biochemical parameters characterizing the condition of the liver were studied in all patients. The activity of the main enzymes: alanine aminotransferase, aspartate aminotransferase, gamma glutamyltranspeptidase, alkaline phosphates was studied. Levels of AP were assessed using through the Olvex Diagnosticum kit on methods of Bessey and Lowry. Bilirubin levels were assessed using the Vital Diagnostics SPb diagnostic kit using the Van den-Berga method. AlAT and AsAT levels were assessed through the BIO-LA-Test diagnostic kit according to the method of Reitman and Frankel.

Status of lipid metabolism: Total cholesterol was determined using through the "CHOLESTEROL liquicolor" (CHOD - PAP Method) test system. Assessment of liver synthesis function: The total protein was determined using through the biuret method with the set "Obshiy belok Agat" (OOO "Agat-Med"). Blood plasma albumin was determined using through the kit "ALBUMIN FS" (ZAO "DIAKONDS"). The prothrombin ratio was determined using through the Renamplastin (NPO Renam) kit.

The following immunological examinations were performed on the patients:

1. Determination of the number of leukocytes in the blood, the relative and absolute number of lymphocytes.
2. Phenotyping of peripheral blood cells: Determination of total T cells (CD3+), T helpers (CD4+), T cytotoxic cells (CD8+), immune regulatory index (IIT = CD4+/CD8+), B lymphocytes (CD19+), activated T lymphocytes (HLA-DR), apoptosis of cells (CD95+) by the method of flow cytometry on the FaxScan device.
3. Determination of INF- γ , IL-1 β , IL-10, IL-13 from cytokines by the method of immunoenzyme.

Methods of statistical processing. All charts obtained in the course of the study were statistically analyzed. The indicators in the groups are arranged in a variational order and the mean (M), its

standard error (m) were calculated for each variational series. The statistical significance of the difference between the group indicators was based on Wilcoxon (Manna-Whitney) U test on quantitative indicators and on non-parametric method such as Fishers accurate test on qualitative indicators. With these methods, a decision was made on the statistical accuracy of the differences, $p < 0,05$. Statistical processing of data was performed on a computer using Statistica 6.0 and MS Excel programs.

RESEARCH RESULTS AND THEIR DISCUSSION

In order to achieve the goal of the study, 97 patients with DM and hepatobiliary system disorders were examined on the basis of clinical-laboratory criteria developed during 2009-2015 (Group I - 40 (41.0%) patients with type 1 DM; Group II - 57 (59.0%) patients with type 2 DM). Type 1 DM patients were $33.2 \pm 1,6$ years of age, the duration of the disease was up to 15 years. Patients with type 2 DM were 46.4 ± 1.5 years old, and the duration of the disease was up to 15 years. The control group consisted of 23 healthy individuals compared to each other by age and sex, whose detailed examination allowed to exclude any significant changes affecting the condition of the internal organs. In the examination groups of patients, women (53 people) dominance over men (44 people) was noticeable, - 54.5% and 45.5%, respectively. Depending on the duration of DM, patients were divided into 3 groups. Patients with DM lasting up to 5 years accounted for 42.0% of those examined and from 5 to 10 years - 32.0%, those over 10 years - 26.0%.

According to the degree of compensation, the majority of patients with DM were in the subcompensation phase, which was confirmed by high values of glycolysed hemoglobin (from 6.6% to 7.5%). The mean body mass index (BMI) in patients with type 1 DM was 22.4 ± 1.3 kg/m^2 on average and did not differ significantly from the group of healthy individuals (23.5 ± 0.8 kg/m^2) ($p > 0.05$). The BMI has organized 32.5 ± 1.8 kg/m^2 in patients with type 2 DM, which was significant addition to the typical clinical signs of diabetes mellitus, pain syndrome (especially in the right subcostal region) has been observed in patients with any duration of diabetes. The following symptoms

were found in patients with type 1 DM: feeling of heaviness under the right rib - in 14 (35.0±7.5%) patients; pains having a dull character - in 3 (9.0±5.0%) and 5 (71.0±17.1%) patients with moderate and severe disease, respectively; persistent pain syndrome - in 3 (9.0±5.0%) and 1 (14.0±13.2%) patients, periodic pains in the right rib- in moderate and severe disease in 10 (30.0±8.0%) and 2 (29.0±17.1%) patients, respectively. The following symptoms were found in patients with type 2 DM: feeling of heaviness under the right rib - in 36 (63.0±6.4%) patients; pains having a dull character – in 30 (67.0±7.0%) and 7 (58.0±14.2%) patients with moderate and severe disease, respectively; persistent pain syndrome - in 2 (4.0±3.1%) and 8 (67.0±13.6%) patients, periodic pain under the right rib - in 10 (22.0±6.2%) and 3 (25.0±6.0) patients with moderate and severe disease, respectively, periodic pain under the right rib - in 10 (22.0±6.2%) and 3 (25.0±12.0%) patients with moderate and severe disease, respectively.

During the type 1 DM, among the dyspepsia disorders dryness in the mouth was observed more periodically in 20.0±6.3% of patients with moderate to severe disease. Dyspepsia symptomocomplex was observed in patients with type 1 DM in the form of nausea (20.0±6.3%), feeling of bitterness in the mouth (12.0±5.2%), in the form of decreased appetite (18.0±6.0%), in the form of bloating (25.0±6.8%), and in type 2 DM patients in 23.0±5.6%, 30.0±6.1%, 28.0±6.0%, 40.0±6.5% cases, respectively.

Presence of pain, dyspepsia syndrome, enlargement of the liver, positive gallbladder symptoms and signs of intestinal damage indicates from injury to the digestive organs in patients with DM. This situation can cause the development of hypoglycemia inpatients and the labile course of diabetes.

The frequency of dyspepsia complaints has increased along with the rise of the duration and severity of the disease. This can be explained, on the one hand, by the adverse effects of anti-diabetic drugs on the condition of the liver (patients with DM take them for a long time), and on the other hand - by liver damage as a result of long-term disease which leads to the development of dystrophic changes.

The following symptoms were found as a result of carrying out of ultrasound examination of the liver (Tables 1).

Table 1

Data from ultrasound examination of the liver during type 1 and type 2 diabetes mellitus

Parameters	Patients with type 1 DM (n=40)		Patients with type 2 DM (n=57)	
	Abs.	%	Abs.	%
Exogenous:				
Normal	25	62.0±7.6	26	46.0±6.6
High	15	38.0±7.6	31	54.0±6.6
Exostructure:				
homogeneous	9	23.0±6.6	-	-
relatively homogeneous	18	45.0±7.9	26	46.0±6.6
non-homogeneous	13	32.0±7.4	31	54.0±6.6
Density:				
hardened	11	28.0±7.1	18	32.0±6.2
Not hardened	29	72.0±7.1	39	68.0±6.2
Contours and edges:				
precise, smooth	40	100	57	100
notsmooth	-	-	-	-
Dimensions:				
Rightlobe: Enlarged	5	12.0±5.2	15	26.0±5.8
Leftlobe: enlarged	4	10.0±4.7	16	28.0±6.0
Bileducts and veins:				
enlarged	3	7.0±4.2	4	7.0±3.4
Not enlarged	37	93.0±4.2	53	93.0±3.4
Vein image: Saved	33	82.0±6.0	13	23.0±5.6
Decreased	7	18.0±6.0	44	77.0±5.6

During the examination of the exogenous liver, it was found to be normal in the majority of patients with type 1 DM - 62.0% and increased volume was found in 38.0% of patients. In the study of exostructure, its relative homogeneity was more pronounced in 45.0%, and its non-homogeneity in 32.0% of patients. In most cases, according to the data of the exogram, the liver did not hardened in 72.0% of patients, but the liver hardened in 28.0% of patients those examined with type 1 DM.

During the examination of the exogenous liver, in the majority of patients with type 2 DM, 54.0% of its normal volume was found, and in 46.0% of patients its growth was found. During the examination of the exostructure, it was often found to be non-homogeneous - in 54.0% of patients, the relative homogeneity of this parameter was found in 46.0% of patients. In most cases, according to the data of the exogram, the liver was not hardened in 68.0% of patients, hardened liver was observed in 32.0% of patients with type 2 DM. In all patients with both type 1 DM and type 2 DM, smooth contours and edges of the liver were identified.

During the examination of the size of the liver in 22% of patients revealed an enlargement of the sizes of this organ. The transverse vertical dimension (VVS) of the organ was 162.5 ± 1.4 mm. The right lobe of the liver was enlarged in 5 (12.0%) patients. The average size of the right lobe was 14.6 ± 0.5 cm. The size of the left side of the liver also enlarged in 4 (10.0%) patients those examined with type 1 DM. The average size of the left lobe was 7.8 ± 0.2 cm.

Larger changes in liver size were found in our examination compared with other group of patients in the group of 30 to 40 years: right lobe - 15.6 ± 0.4 cm, left lobe - 8.8 ± 0.3 cm. More enlargement in the right lobe was found in the group of patients with DM lasting more than 10 years: The size of the right lobe was 15.2 ± 0.6 cm, the size of the left lobe was 8.6 ± 0.3 cm. In the severe course of the disease, the sizes of the liver have enlarged to a higher degree relative to the average degree. Right lobe - 15.9 ± 0.7 cm, left lobe 8.8 ± 0.4 cm. The bile ducts and veins were dilated in 3 (7.0%) patients with type 1 DM, and not dilated in 37 (93.0%) patients. The mean diameter of the portal

vein was 12.6 ± 0.4 mm. Hepatic veins were not dilated in any patient. During the study of the vascular pattern, a decrease was observed in 7 (18.0%) patients with type 1 DM in the majority of patients examined, the vascular pattern was preserved in 33 (82.0%) patients.

Larger changes of the dimensions of the liver were found in the age group of 50 and older compared with a group of patients aged 40 to 50 years. Right lobe was- 15.8 ± 0.6 cm, left lobe was- 8.8 ± 0.4 cm. More significant changes in the size of both lobes were identified in the group of patients whose duration of disease lasts more than 10 years: the size of the right lobe was 15.9 ± 0.6 cm, the size of the left lobe was 9.3 ± 0.4 cm. During the course of the disease, the sizes of the liver have enlarged to a higher degree than to moderate: right lobe - 16.5 ± 0.6 cm, left lobe - 9.4 ± 0.4 cm.

Thus, as a result of ultrasound examination of the liver, the following symptoms were found in patients with type 1 DM. During the examination of exogenous liver, it was normal in the majority of patients with type 1 DM, and in most patients (72.0%) the liver was not hardened. In all patients in this group, the contours of the liver were smooth and the edges were clearly visible. The organ has not hardened in most cases (68.0%), due to the exostructure of the liver in patients with type 2 DM and smooth contours and edges of the liver were found in all patients.

The bile ducts and veins were dilated in 4 (7.0%) patients with type 2 DM, but not dilated in 53 (93.0%) patients who examined. The mean diameter of the portal vein was 12.6 ± 0.8 mm. Hepatic veins were not dilated in any patient. Its decrease was observed in 44 (77.0%) patients in the majority of patients who examined during the study of the vascular shape, vascular shape was preserved in 13 (23.0%) patients with type 2 DM.

Based on the results of USM of 23 people in the control group, we believe that the following dimensions should be taken as normal liver size, regardless of gender, age and body weight - from 12.2 cm to 13.8 cm of the right lobe, from 6.1 cm to 7.6 cm of the left lobe; thickness of the lobes of the liver - from 126.2 to 137.3 mm of the right lobe, from 62.1 mm to 73.2 m of the left lobe. The changes that

detected were evaluated as signs of fatty hepatosis.

The following changes were detected during conducting USM of the gallbladder in patients with type 1 DM. During examination of the gallbladder revealed an enlargement in its size in 9 (22.0%) patients, and its normal size was found in 31 (78.0%) patients. The average size of the gallbladder was 68.0 x 24.0 mm or 6.8 x 2.4 cm. Hardening of the gallbladder wall was detected in 2 (5.0%) patients, but its wall has not hardened in 38 (95.0%) patients with type 1 DM.

Thickening of the gallbladder wall was found in 19 (47.0%) patients, the wall thickness was normal in 21 (53.0%) patients. The mean thickness of the gallbladder wall was 2.4 ± 0.6 mm. The width of the total bile duct was 4.8 ± 0.4 mm.

During of the examination of the shape of the gallbladder in 6 patients with type 1 DM (15.0%) its curved deformity was found in the neck area. The contents of the gallbladder cavity were recorded in a stagnant manner with different compositions in 37 (93.0%) patients those examined. Concretions standing in second place according to the frequency among between the secretion of the gallbladder were found in 3 (7.0%) patients. Mentioned symptoms, for example enlargement in the size of the gallbladder - in 22.0% of cases, change of its shape (often bent deformation in the neck area) - in 15.0% of cases, thickness of wall - 47.0% of cases and stagnant, different content - 93.0% of cases indicate the presence of development of chronic cholecystitis, as well as dyskinesia of the biliary tract on the hypotonic type.

Enlargement in its size was observed in 38 (67.0%) patients, its normal size was recorded in 19 (33.0%) patients during the examination of the gallbladder of patients with type 2 DM. The average size of the gallbladder was 66.0x25.0 mm or 6.6x2.5 cm. Hardening of the walls of the gallbladder was detected in 4 (7.0%) patients with type 2 DM, but it has not hardened in 53 (93.0%) patients. Thickening of gallbladder was found in 20 (35.0%) patients, the wall thickness was normal in 37 (65.0%) patients. The mean thickness of the gallbladder wall was 2.4 ± 0.3 mm. The width of the total bile duct was 5.6 ± 0.2 mm.

Deformation of the gallbladder was found in 7 (12.0%) patients

during the examination of the shape of the gallbladder: its bent deformity in the neck area- in 2 patients with type 2 DM, bent deformation in the physical area - in 5 patients. Most of the time, in 51 (90.0%) examined patients, the contents of the gallbladder cavity were of various composition, stagnant. Concretions which standing in second place according to the frequency among the secretions of the gallbladder were found in 6 (10.0%) patients. Mentioned symptoms, for example, enlargement in gallbladder size in the case of 67.0%, change of its shape (often its bent deformation in the neck area) in the case of 12.0%, thickness of wall in 35.0%, content of stagnant, of different composition in the case of 90.0% indicate the development of chronic cholecystitis, as well as dyskinesia on hypotonic type of the bile-extracting tracts.

When studying the US landscape in the control group, it is possible to observe the relationship between the intensity of signals (internal echo) reflected in the parenchyma and the age of the examined persons. In addition, it was determined that excess body weight has a significant impact on the characteristics of the exo- image. When the thickness of the subcutaneous fat layer is too thick, it is impossible to accurately visualize not only the liver and gallbladder, but also other internal organs (pancreas, spleen).

The distribution of "internal echo" in the parenchyma of the organ is moderately unequal to the norm, therefore, the exohepatogram has a brighter, more grainy image compared to the unaltered shape of liver exoscan. The reason for the rise in the frequency of external biliary tract damage from the liver, most likely changes that occur in the functional state of hepatocytes are considered- they cause dyscholia, which later causes a change in the colloidal persistence of the bile this leads to the formation of cholecystitis and gallstones.

In such cases, the factors that contribute to gallbladder damage are considered biliary stagnation and dyskinesia of the bile ducts, this forms against the background of stagnant changes which occurs in the gallbladder wall and in turn stimulates the development of trophic disorders and inflammatory changes there which also increases the severity of diabetes.

Thus, it is clear from the results that the volume of gallbladder secretion remained the same in any type of DM. This confirms that patients with type 1 DM have signs of stagnation, however, the ability of the gallbladder to accumulate was maintained, and a sharp decrease in the functional activity of the gallbladder and hypokinesia of biliary excretory system (BES) were found in patients with type 2 DM. Changes in the BES depend on the duration of diabetes. Hypotension of the gallbladder and its accumulation, decreased evacuation function, hypokinesia of the BES, ODMi sphincter insufficiency are more pronounced when DM persists more than 10 years.

Determination of cytolytic activity in the diagnosis of liver injury (ALAT, AsaT, LDH indicators) provides information about changes in the structure of liver cells, primarily hepatocytes. Statistically accurate changes in cytolysis syndrome have been reported in patients with type 1 and type 2 DM ($p < 0.05-0.01$). The symptoms of this syndrome depend on the age of the patients, the duration of the disease and the degree of severity. This dependence was particularly noticeable when comparing clinical levels with the levels of key indicators of the biochemical syndrome. As the severity increased, the number of indicators of cytolysis syndrome was higher ($p < 0.001$). An increase of the level of indicators of this syndrome in the short-term course of the disease can be explained by the involvement of the liver in the pathological process in the early stages of DM.

Statistically significant changes in cholestasis syndrome have been reported in patients with DM those examined. A rise of total bilirubin indicators in patients with type 1 DM was observed in people aged 30-40 years and if the disease lasted more than 10 years ($p < 0.001$). A more rise of AP levels was observed when the disease lasted more than 5 years ($p < 0.001$) and in the severe course of diabetes mellitus. The highest level of GGTP was in the same tendency in patients with type 1 DM ($p < 0.001$).

In the group of patients with type 2 DM, these indicators were GGTP, which differed from the control group with high accuracy. Its increase is in the group of patients aged 50 years and older, if the disease lasts from 5 to 10 years, observed in the severe course of

disease ($p < 0.001$). As patients age, duration, and severity of the disease, the number of indicators of cholestasis syndrome has also increased.

During type 1 and type 2 DM, total cholesterol, albumin and cholinesterase levels were studied in order to assess hepatocellular insufficiency syndrome depending on the age of the patients. Cholesterol and albumin levels in both types of DM patients were not significantly different from the control group. Only in older patients in the type 1 and type 2 DM group of cholinesterase indicator, cholinesterase levels decreased as the duration and severity of the disease increased ($p < 0.001$).

Immunological parameters of patients with DM, especially cellular immunity and cytokine status, were examined in order to determine the role of the immune system in the development of diabetes mellitus.

It was found during the determination of immune cells in the peripheral blood that the total number of T-lymphocytes (CD3+), T-helper (CD4+) and T-suppressor (CD8+) populations in both groups of diabetic patients was significantly higher than in the control group ($p < 0.001$).

As for the B-lymphocyte population the absolute number of B-lymphocytes was significantly higher than in the control group during type 1 DM ($p < 0.001$) and was practically indistinguishable from type 2 DM. At the same time it was determined that the number of natural killer (NK-CD16+/56+) cells was moderately higher than control values in all patients ($p < 0.01$) (Chart 1).

During the study of immunological examination data of patients with type 2 DM, it was determined that significant changes were found in this group compared to the data in the control group ($p < 0.001$).

It was found that during type 1 DM, the amount of all inflammatory cytokines, as well as anti-inflammatory cytokines, increased. This confirms their active participation in the pathogenesis of immune regulation.

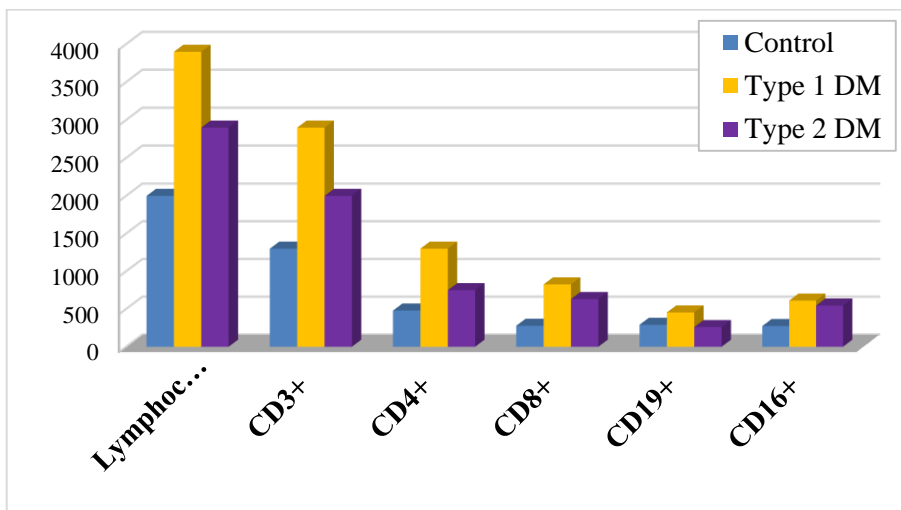


Chart 1. Absolute indicators of cellular immunity in patients with type 1 and type 2 diabetes mellitus

It has been shown that IL-1 β and INF- γ play an important role in the developmental mechanism of type 1 DM, they are involved in the activation of antigenic cells in the autoimmune process lesion of the pancreas and create conditions for intensification of apoptosis processes and necrosis of insulin-synthesizing cells (Charts 2, 3).

Cytokines which has different directional effects - in the amount of IL-13 and IL-1 β particularly high indicators were found. A greater increase in the amount of IL-1 β from inflammatory cytokines was observed ($p < 0.001$) in both type 1 DM and type 2 DM, however, the amount of INF- γ only increased significantly during type 1 DM.

Significant differences in the amount of IL-10 anti-inflammatory cytokines were also found. In patients with type 1 DM, the mean level was 45 pg / ml, in some patients in this group it was between 34 and 89 pg / ml, and the control value was up to 5 pg / ml. The increase of this cytokine did not take on a significant character compared to control values ($p > 0.05$). It can be assumed that a significant increase in serum levels of IL-13, which has a regulatory effect on the balance of the immune system, has a certain anti-diabetic protective effect.

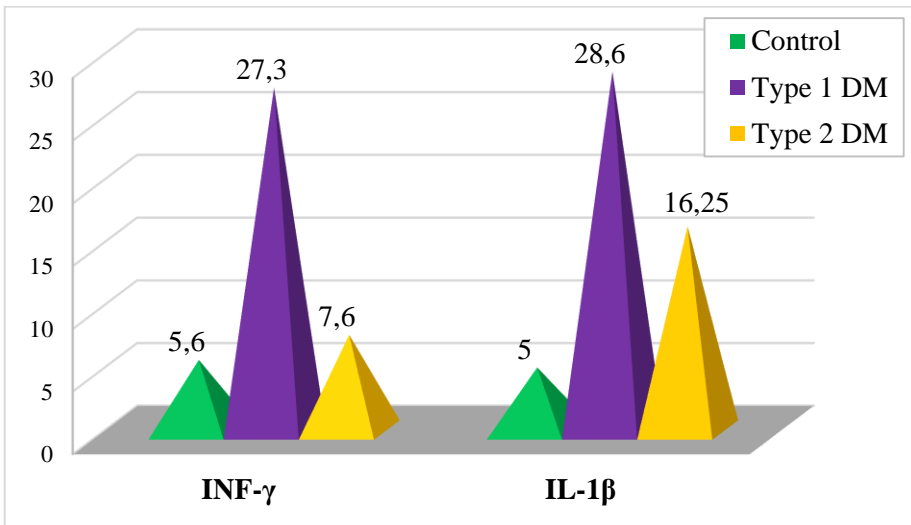


Chart 2. Characteristics of cytokine status in patients with diabetes mellitus

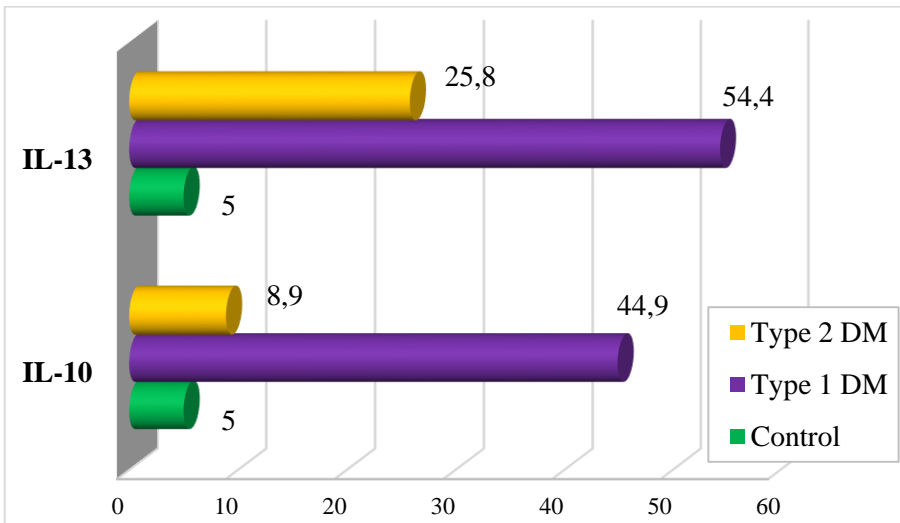


Chart 3. Indicators of IL-10 and IL-13 cytokines in type 1 DM and type 2 DM patients

In order to achieve a positive result during the treatment of patients with DM, therapeutic modulation should focus not only on the correction of carbohydrate, lipid and protein metabolism, but also on the fight against secondary immunodeficiency. That's why ursodeoxycholic acid was the drug of choice during the course of treatment of patients with DM.

The effectiveness of treatment which conducting with ursodeoxycholic acid is shown in the table below (Table 2).

Table 2

Biochemical parameters in the blood serum of DM patients after a course of treatment conducting with ursodeoxycholic acid

Indicators	Healthy persons (n=23)	Before treatment (n=78)	After treatment (n=78)
AIAT (mmol/hour.ml)	0,80±0,12	0,92±0,08	0,66±0,05
AsAT (mmol/hour.ml)	0,72±0,18	0,86±0,01	0,57±0,04
LDH (U/l)	188,0±7,4	328,0±29,4	242,0±20,4
GGT (U/l)	26,8±7,8	48,2±4,5	34,3±5,2
AP (U/l)	92,5±2,3	146,2±6,8	96,5±7,5
Total cholesterol (mmol/l)	4,1±0,18	6,1±0,57	4,3±0,8
Triglycerin (mmol/l)	1,5±0,12	3,1±0,53	2,4±0,34
LDLP (mmol/l)	1,02±0,09	0,84±0,16	1,1±0,41
HDLP (mmol/l)	2,8±0,23	4,9±1,2	3,2±0,2
Total bilirubin (µmol/l)	12,6±0,42	15,6±0,62	14,2±0,43
Combined bilirubin(µmol/l)	3,2±0,58	3,8±0,72	3,3±0,58
Total protein (g/l)	75,4±5,6	78,2±5,7	77,4±5,2
Albumins (%)	56,3±2,4	54,7±3,8	56,0±3,5
Globulins a1 (%)	6,1±0,23	4,8±0,32	5,5±0,38
Globulins a2 (%)	8,3±0,22	7,6±0,48	6,4±0,42
Globulins β (%)	10,8±0,6	12,7±1,3	11,9±1,2
Globulins γ (%)	18,5±0,6	22,5±1,8	18,2±1,3
Globulins γ (abs., g/l)	12,5±4,2	16,8±4,6	14,1±4,3
Thymol turbidity test (U)	2,7±0,28	3,15±0,32	1,84±0,13
β-lipoproteins (U)	44,5±5,2	52,3±2,5	46,8±2,3

After a course of treatment conducting with ursodeoxycholic acid due to increased albumin synthesis ($p < 0.05$) and decreased synthesis of globulins, especially β - and γ -globulins ($p < 0.01$), which are responsible for the inflammatory process in the liver parenchyma, significant improvement in liver protein synthesis was found during the examination of biochemical parameters of liver functional tests. Normalization of both total and conjugated bilirubin levels was observed ($p < 0.01$), this indicates the assimilation and conjugation of bilirubin and the extinction of the early inflammatory response of reticulendotelial and parenchymal cells responsible for excretion into the bile ducts.

At this time, a decrease in alkaline phosphatase and GGT was found and this suggests the prevention of symptoms of intrahepatic cholestasis in the background of hepatosis of fat. Cytolytic syndrome is also reduced due to the normalization of aminotransferase activity. Improvement of lipid metabolism was observed. In this case, the levels of cholesterol, LDLP, HDLP and triglycerides did not change significantly in the background of treatment, but the tendency to normalize these indicators was observed.

Thus, a complex clinical, laboratory and instrumental examination of patients with type 1 and type 2 diabetes mellitus was conducted in the dissertation, the results obtained revealed certain characteristics and ursodeoxycholic acid medication have been confirmed to be effective.

CONCLUSIONS

1. The study of clinical and anamnestic data on damage to the hepatobiliary system in patients with type 1 and type 2 DM revealed that the severity degree of injuries of the hepatobiliary system also increases as the duration of the disease increases. This increase was particularly pronounced in patients with type 2 DM in clinical signs ($p < 0.001$) [7] such as pain in the right subcostal space ($p < 0.001$) during palpation, pain along the large intestine ($p < 0.001$), and covering of the tongue with scum [6].

2. Although US examination revealed a high frequency of liver damage in patients with type 1 and type 2 DM, this was more common in patients with type 2 DM ($p < 0.001$). Cytolysis syndrome depending on the severity degree of the disease during the analysis of biochemical changes (in type 1 DM - elevation of AsAT, AlAT, LDH ($p < 0.001$) in patients with diabetes mellitus in both groups, as well as an increase in the level of all indicators during type 2 DM ($p < 0.001$)); cholestasis syndrome (elevated levels of alkaline phosphatase and gamma-glutamyltranspeptidase in both types of DM ($p < 0.001$)); hepatocellular insufficiency syndrome (decrease in the level of XE in type 1 and type 2 DM ($p < 0.001$)), as well as mesenchymal-inflammatory syndrome (increased levels of γ -globulins in type 1 DM ($p < 0.001$), also a statistically significant correlation was found between elevated levels of β - and γ -globulins ($p < 0.001$) during type 2 DM [2,4,5].
3. It was found that higher changes in the peripheral blood of patients with type 1 DM were found in the populations of T- lymphocytes CD3 +, CD4 + and CD8 +, their absolute values were higher than normal values ($p < 0.001$). An increase in the absolute number of B-lymphocytes was also found ($p < 0.001$). Immunological markers from inflammatory cytokines INF- γ ($p < 0.001$) and IL-1 β ($p < 0.01$) were found to be several times higher in patients with type 1 DM than in both type 2 and control groups. Although the anti-inflammatory cytokine IL-10 did not differ significantly from the control group during type 2 DM, this indicator in patients with type 1 DM was significantly higher than in the control group ($p < 0.001$) [1, 7, 8, 9, 10].
4. Cytolysis, cholestasis syndrome, indicators of lipid metabolism after 6 months were statistically significant decrease compared to pre-treatment values ($p < 0.01$) as a result of treatment with the applied drug ursodeoxycholic acid based on the functional status of the liver and the results of examinations [3, 11, 12, 13].

PRACTICAL RECOMMENDATIONS

1. Normalization of carbohydrate metabolism is necessary to reduce the frequency of damage to the hepatobiliary system in patients with DM. In addition to the determination of USM and biochemical syndromes (cytolysis, cholestasis, hepatocellular insufficiency), it is recommended to assess the indicators of lipid metabolism and immune status.
2. When severe immunodeficiency or secondary immunodeficiency is detected in patients with DM, they are advised to consult an immunologist.
3. It is recommended to prescribe a course of treatment with ursodeoxycholic acid preparation if patients with DM have presence of injury to the hepatobiliary system: The course dose of the drug is 10 mg / kg per 1 kg of body weight – taken 1 times a day for 6 months.

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LIST OF ABBREVIATIONS

AlAT	– alanine aminotransferase
AP	– alkaline phosphatase
AsAT	– aspartate aminotransferase
DM	– diabetes mellitus
GGT	– gamma glutamyl transpeptidase
HDLP	– high density lipoproteins
IL	– interleukin
INF	– interferon
LDH	– lactate dehydrogenase
LDLP	– low density lipoproteins
US	– ultrasound examination

The defense will be held on 03.03. 2022 at 14.00 at the meeting of the Dissertation council BFD 2.27/1 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical University

Address: AZ 1022, Baku, A.Gasimzade str, 14

Dissertation is accessible at the Library of Azerbaijan Medical University

Electronic versions of dissertation and its abstract are available on the official website of Azerbaijan Medical University (<https://www.amu.edu.az>)

Abstract was sent to the required addresses on 28.01. 2022

Signed for print: 25.01.2022

Paper format: 60 x 84 1/16

Volume: 37707

Number of hard copies: 30