

EVALUATION OF MORPHOFUNCTIONAL CHANGES OF THE LIVER IN DIABETIC HEPATOPATHY

Ibrahimova G.H.^{1*}, Talishinskaya M.B.¹, Sultanova G.M.¹, Ibrahimova Sh.H.², Seyidova İ.Z.³, Ahmadova R.R.⁴, Mehtiyeva A.Y.⁵

Abstract

The article presents information about the changes in markers of impaired metabolism in the liver (alanine aminotransferase – ALT, aspartate aminotransferase – AST, gamma-glutamyltransferase – GGT, lactate dehydrogenase – LDH) in blood and lymph, lymph flow rate (LFR), as well as histomorphological structures of the liver in experimental diabetes mellitus induced in rabbits by alloxan. Experiments were carried out on 14 rabbits of both sexes. The rabbits used in the study were divided into two groups: the control group consisted of 6 rabbits, and the experimental group consisted of 8 rabbits. Diabetic symptoms were detected 5 days after alloxan injection. For analysis, blood samples were taken from the heart cavity, and lymph from the thoracic duct. The operation was performed under the anesthesia. In both the control and experimental groups of rabbits, blood and lymph were taken to detect glucose level, markers of metabolic disturbances in the liver, LFR, as well as the histomorphological characteristics of the liver were studied. The studied parameters were measured in the 8 rabbits of the second experimental group on the 5th, 15th, and 30th days after alloxan administration. It was determined that the increase in markers of impaired metabolism in the liver in both blood and lymph, as well as the histomorphological changes developing in the liver, were accompanied by a decrease in lymph flow rate.

Keywords: *Diabetic hepatopathy, ALT, AST, GGT, and LDH, lymph.*

INTRODUCTION

Diabetes mellitus (DM) is a rapidly spreading disease worldwide, characterized by long-term complications, and remains one of the serious medical and social problems. According to the World Health Organization, in 2021, the number of people with diabetes worldwide exceeded 500 million, and it is projected that this indicator will reach 700 million by 2030 [1, 2, 3]. Diabetes is also spreading rapidly in Azerbaijan. In 2024, the total number of registered diabetic patients in the unified

electronic registry of medical institutions under the Administration of the Regional Medical Divisions was reported to be 322,353 [4, 5].

One of the organs most affected by DM is the liver. The liver plays a key role in regulating glucose homeostasis. During persistent hyperglycemia, end-products of non-enzymatic glycosylation accumulate in the body, including hepatocytes. This leads to damage of both intracellular and extracellular structures, a decrease in NADPH levels,



an acceleration of oxidative stress, and increased conversion of glucose to sorbitol [6, 7, 8, 9, 10, 11]. As a result of the disruption of the integrity of hepatocyte membranes, enzymes such as ALT, AST, GGT, and LDH are released into the blood and lymph. This is considered an indicator of liver damage. Although significant progress has been made in studying the histomorphological changes in the liver and the alterations in the levels of enzymes considered indicators of liver damage in the blood in diabetes mellitus, however, there is limited information about such changes in the lymph under conditions of persistent hyperglycemia [12, 13, 14, 15]. It should be noted that a significant portion of the lymph in the thoracic duct originates from the liver, and lymphatic fluid can provide more detailed information about metabolic processes occurring in tissues [6, 17, 18]. Although the role of the lymphatic system in the pathogenesis of liver failure is of great importance, its role during diabetes mellitus has not been sufficiently investigated [19, 20, 21, 22].

experimentally induced diabetes mellitus in rabbits using alloxan injection, to determine the lymph flow rate, and to investigate histomorphological changes in the liver.

The aim of this research was to comparatively study the levels of markers impaired metabolism in the liver (ALT, AST, GGT, LDH) in the blood and lymph during

MATERIALS AND METHODS

The study was carried out on 14 rabbits of the "Chinchilla" breed, weighing between 1.8 and 2.3 kg. The rabbits used in the experiments were divided into two groups: the control group included 6 animals, and the experimental group included 8. In the experimental group, diabetes mellitus was induced in rabbits by intraperitoneal administration of 5% alloxan monohydrate solution at a dose of 100 mg/kg. Diabetic symptoms were observed 5 days after the injection. Blood samples were obtained from the cardiac cavity and lymph samples from the thoracic duct. All procedures were carried out under anesthesia. For this purpose, 8 mg/kg of Calypsol and 0.15 ml/kg of 1% Dimedrol solution were injected intravenously. In the control group, blood and lymph samples were collected from six rabbits, and the levels of liver function-related enzymes (ALT, AST, γ -GT, LDH) as well as LFR were determined. In addition, the histomorphological structures of the liver were studied. The same parameters were assessed in the experimental group of 8 rabbits on 5th, 15th, and 30th days after alloxan administration. For histomorphological evaluation, liver tissues were fixed using 10% neutral buffered formalin or Bouin's solution. Subsequently, the specimens were dehydrated through a graded ethanol series, embedded in paraffin, sectioned, and stained for histological examination.

Yazışma üçün əlaqə:

İbrahimova G.H.^{1*}, Talışinskaya M.B.¹, Sultanova G.M.¹, İbrahimova Sh.H.², Seyidova İ.Z.³, Ahmadova R.R.⁴, Mehtiyeva A.Y.⁵

¹ Department of Pathological Physiology, Azerbaijan Medical University, Baku, Azerbaijan

² Department of Allergology and immunology, Azerbaijan Medical University, Baku, Azerbaijan

³ Department of Physiotherapy and rehabilitation, Azerbaijan Medical University, Baku, Azerbaijan

⁴ Research Institute of Living Systems of the Ministry of Science and Education of the Republic of Azerbaijan

⁵ Department of Radiology, Azerbaijan Medical University, Baku, Azerbaijan

*For correspondence E-mail:

doctor.ibrahimova@gmail.com

The numerical data obtained during the course of the study were statistically analyzed in accordance with current recommendations. Statistical analyses were carried out using the method of variation analysis and with the help of the 'IBM Statistics SPSS-26' software. For the comparison of variation series, the non-parametric Mann-Whitney U test was also used.

RESULTS AND DISCUSSION

During the study, the levels of enzymes characterized the diabetic hepatopathy (ALT, AST, γ -GT, LDH), LFR, and the histomorphological structures of the liver were investigated in the blood and lymph of rabbits with alloxan-induced diabetes mellitus over a period of 30 days and compared with control values. The obtained results are presented in the tables below (Table 1).

Table 1. Dynamics of changes in the indicators determined in the blood of animals during alloxan-induced experimental diabetes

Parameters	Control group	Days after administration of alloxan monohydrate solution M (min-max)		
		5	15	30
Glucose mmol/l	4,79 (4,60-5,01)	6,25 (4,91-6,87)*	10,15 (9,48-11,05)*	12,11 (11,45-13,03)*
ALT IU/L	40,43 (39,20-41,64)	46,01 (45,38-46,66)	53,1 (52,02-54,03)	60,41 (59,02-61,03)
AST IU/L	23,84 (23,64-24,07)	27,40 (26,39-28,41)*	35,51 (34,73-36,38)*	43,57 (42,68-44,57)*
QQT IU/L	5,6 (5,3-6,3)	7,5 (6,1-8,1)*	8,8 (7,5-9,0)*	12,2 (11,5-13,1)
LDH IU/L	170,6 (169,1-171,0)	190,9 (189,0-192,1)	210,1 (209,6-211,6)*	230,5 (229,7-231,4)*

*Note: The * symbol indicates the statistical significance of the difference between the experimental and control group parameters.*

As seen from Table 1, after alloxan administration, the levels of blood glucose and the liver function-assessing enzymes (ALT, AST, GGT, LDH) gradually increased throughout the experiment ($p < 0,001$).

The dynamics of changes in the parameters determined in the lymph are in the same direction as in the blood (Table 2).

Table 2. Dynamics of changes in the parameters determined in the lymph of animals with alloxan-induced diabetes mellitus

Parameters	Control group	Days after administration of alloxan monohydrate solution M (min-max)		
		5	15	30
Glucose level mmol/l	5,2 (4,2-6,2)	7,4 (5,9-8,7) *	11,5 (10,7-12,6)*	13,3 (12,4-14,4)*
ALT IU/L	45,3 (44,8-46,8)	57,8 (56,2-58,5)	63,3 (62,5-64,1)*	69,7 (68,8-70,9)*
AST IU/L	27,9 (11,1-12,5)	36,8 (35,9-37,8)	45,3 (44,8-46,8)*	51,8 (50,7-52,0)
GGT IU/L	8,7 (7,8-9,0)	13,4 (12,4-14,4)	17,9 (16,7-18,1)*	24,1 (23,8-25,8)
LDH IU/L	190,8 (189,8-191,5)	222,4 (221,5-223,6)	240,3 (239,3-241,5)*	263,9 (262,1-263,4)*
LFR (ml.min/kg)	1,224 (1,180-1,270)	1,203 (1,160-1,240)	1,013 (0,850-1,150)	0,813 (0,650-0,950)

*Note: The * symbol indicates the statistical significance of the difference between the experimental and control group parameters.*

As seen from Table 2, after alloxan administration, the levels of glucose and liver function-assessing enzymes (ALT, AST, GGT, LDH) in the lymph gradually increased ($p < 0.001$), and the lymph flow rate decreased throughout the experiment (Table 2).

As seen from the presented tables, in animals with induced diabetes mellitus, an increase in the activity of ALT, AST, GGT, and LDH in blood and lymph was observed throughout the experiment compared to the control values. This tendency was more pronounced in the lymph than in the blood. In rabbits with alloxan-induced diabetes

mellitus, the increased markers of metabolic disorders in the liver (ALT, AST, GGT, LDH) in blood and lymph are explained by the acceleration of hepatocyte cytolysis under persistent hyperglycemia [23, 24, 25].

During the experiment, histomorphological changes developing in the liver were studied on the 5th, 15th, and 30th days after alloxan administration. On the 5th day of alloxan-induced diabetes mellitus, dystrophic changes were observed in hepatocytes around the periportal vessels (Figure 1).



Figure 1. Histomorphological changes in the liver on the 5th day of alloxan-induced diabetes mellitus. Hematoxylin and eosin (H&E) staining. Magnification: $\times 206$.

On the 15th day of diabetes mellitus, edema of the connective tissue in the periportal area of the liver is observed (Figure 2).

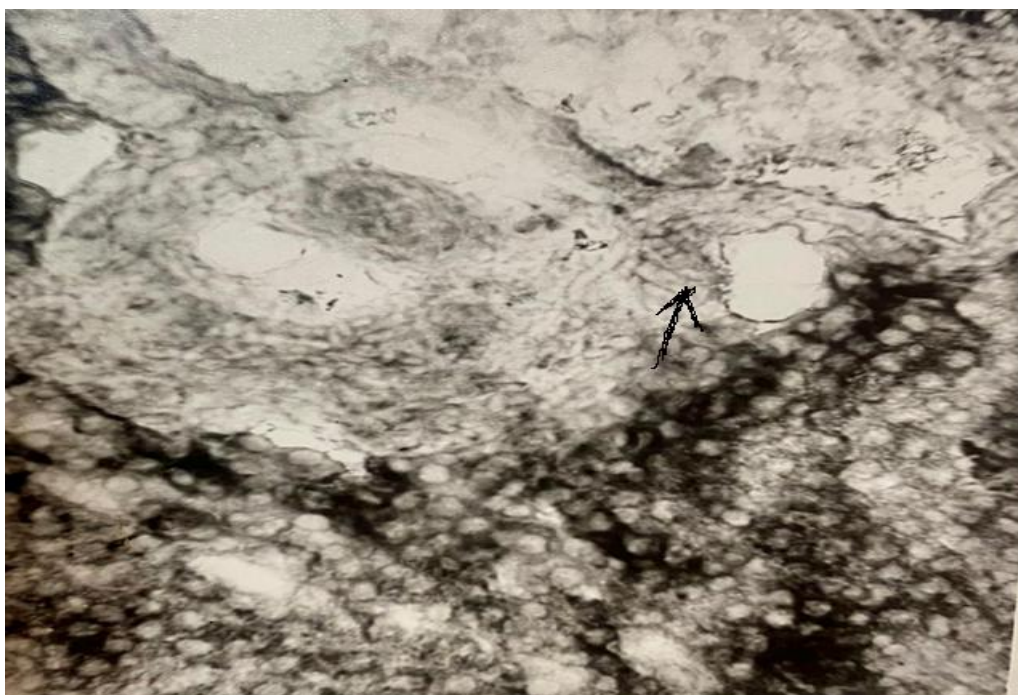


Figure 2. Histomorphological changes in the liver on the 15th day of alloxan-induced diabetes mellitus. Hematoxylin and eosin (H&E) staining. Magnification: $\times 206$.

On the 30th day of alloxan-induced diabetes mellitus, destructive and necrotic changes developed in the liver parenchyma (Figure 3).

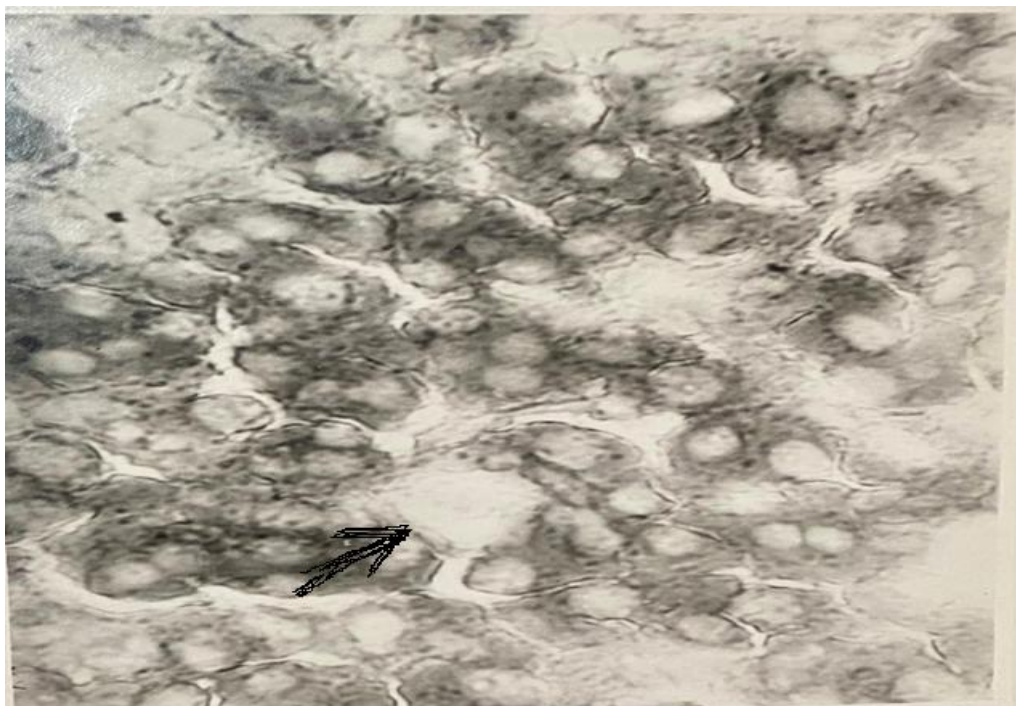


Figure 3. Histomorphological changes in the liver on the 30th day of alloxan-induced diabetes mellitus. Hematoxylin and eosin (H&E) staining. Magnification: $\times 206$.

Based on the information presented above, we conclude that during diabetes mellitus, enzymes released from damaged hepatocytes enter the bloodstream and lymphatic system. The increase of these enzymes in both blood and lymph, along with the histomorphological changes occurring in the liver, is considered the development of diabetic hepatopathy, one of the long-term complications of diabetes mellitus [26, 27, 28, 29]. The decrease in lymph flow rate slows the removal of accumulated toxic products from tissues, further deepening the histomorphological changes that are developing in the liver [30, 31, 32, 33].

CONCLUSION.

In alloxan-induced diabetes mellitus, the increased markers of metabolic disorders in the liver (LDH, AST, ALT, GGT) in both blood and lymph, along with the progressive deepening of histomorphological changes in the liver, were accompanied by a decrease in lymph flow rate.

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DIABETİK HEPATOPATİYA ZAMANI QARACİYƏRİN MORFOFUNKSIONAL DƏYİŞİKLİKLƏRİNİN QIYMƏTLƏNDİRİLMƏSİ

İbrahimova G.H.^{1*}, Talışinskaya M.B.¹, Sultanova G. M.¹, İbrahimova Ş.H.², Seyidova İ.Z.³ Əhmədova R.R.⁴, Mehtiyeva A. Y.⁵

¹ Azərbaycan Tibb Universitetinin, Patoloji fiziologiya kafedrası, Bakı, Azərbaycan

² Azərbaycan Tibb Universitetinin, Allergologiya və immunologiya kafedrası, Bakı, Azərbaycan

³ Azərbaycan Tibb Universitetinin, Fizioterapiya və reabilitasiya kafedrası, Bakı, Azərbaycan

⁴ *Azərbaycan Respublikası Elm və Təhsil Nazirliyinin Canlı Sistemlərin Tədqiqi İnstitutu*⁵ *Azərbaycan Tibb Universitetinin, Radiologiya kafedrası, Bakı, Azərbaycan** *Yazışma üçün əlaqə E-mail: doctor.ibrahimova@gmail.com*

Xülasə

Məqalədə dovşanlarda alloksan vasitəsilə modelləşdirilmiş şəkərli diabet zamanı qaraciyərdə metabolizmin pozulmasının markerlərinin (alaninaminotransferaza – ALT, aspartataminotransferaza – AST, qamma qlutamilttransferaza – QGT, laktatdehidrogenaza – LDH) qanda və limfada dəyişiklikləri, limfanın axın sürəti (LAS), eləcə də qaraciyərin histomorfoloji xüsusiyyətləri haqqında məlumat verilmişdir. Eksperimental tədqiqatlar hər iki cinsə aid olan 14 baş dovşan üzərində aparılmışdır. Tədqiqatda istifadə edilmiş dovşanlar 2 qrupa bölünmüşdür. Kontrol qrupuna 6 və təcrübə qrupuna 8 dovşan daxil edilmişdir. Dovşanlarda alloksan inyeksiyasından 5 gün sonra diabetik simptomlar aşkarlanmışdır. Analiz üçün qan ürək boşluğundan, limfa isə döş limfa axacağından alınmışdır. Əməliyyat anesteziya altında aparılmışdır. Həm kontrol, həm də təcrübə qruplarında olan dovşanlardan qan və limfa alınmış şəkərin səviyyəsi, qaraciyərdə metabolizmin pozulmasının markerləri, LAS, eləcə də qaraciyərin histomorfoloji xüsusiyyətləri öyrənilmişdir. Öyrənilən göstəricilər ikinci təcrübə qrupuna daxil edilmiş 8 dovşanda alloksan yeritdikdən sonra 5-ci, 15-ci və 30-cu gün təyin edilmişdir. Müəyyən olunmuşdur ki, həm qanda, həm də limfada qaraciyərdə metabolizmin pozulmasının markerlərinin artması və qaraciyərdə inkişaf edən histomorfoloji dəyişikliklər limfanın axın sürətinin azalması ilə müşayiət olunmuşdur

Açar sözlər: *diabetik hepatopatiyası və onun markerləri, limfa*

ОЦЕНКА МОРФОФУНКЦИОНАЛЬНЫХ ИЗМЕНЕНИЙ ПЕЧЕНИ ПРИ ДИАБЕТИЧЕСКОЙ ГЕПАТОПАТИИ

Ибрагимова Г.Г.^{1*}, Талышинская М.Б.¹, Султанова Г.М.¹, Ибрагимова Ш.Г.², Сеидова И.З.³, Ахмадова Р.Р.⁴, Мехтиева А.Ю.⁵

¹*Кафедра патологической физиологии Азербайджанского медицинского университета, Баку, Азербайджан*

²*Кафедра аллергологии и иммунологии Азербайджанского медицинского университета, Баку, Азербайджан*

³*Кафедра физиотерапии и реабилитации Азербайджанского медицинского университета, Баку, Азербайджан*

⁴*Институт Исследования Живых Систем Министерства Науки и Образования Азербайджанской Республики, Баку*

⁵*Кафедра Радиологии Азербайджанского медицинского университета, Баку, Азербайджан*

* *Для корреспонденции E-mail: doctor.ibrahimova@gmail.com*

Резюме

В статье представлены данные об изменениях маркеров нарушения метаболизма в печени (аланинаминотрансфераза — АЛТ, аспартатаминотрансфераза — АСТ, гамма-глутамилтрансфераза — ГГТ, лактатдегидрогеназа — ЛДГ) в крови и лимфе, о скорости

лимфотока (СЛТ), а также о гистоморфологических особенностях печени при аллоксан-индуцированном сахарном диабете у кроликов. Экспериментальные исследования были проведены на 14 кроликах обоего пола. Животные были разделены на 2 группы: контрольную (6 кроликов) и экспериментальную (8 кроликов). Диабетические симптомы были выявлены на 5-й день после инъекции аллоксана. Для анализа брали кровь из полости сердца, а лимфу — из грудного лимфатического протока. Операции проводились под анестезией. У кроликов обеих групп исследовали уровень глюкозы, маркеры нарушения метаболизма печени, скорость лимфотока, а также гистоморфологические особенности печени. У животных экспериментальной группы показатели определялись на 5-й, 15-й и 30-й дни после введения аллоксана. Установлено, что повышение маркеров нарушения метаболизма печени как в крови, так и в лимфе, а также развивающиеся гистоморфологические изменения в печени сопровождалось снижением скорости лимфотока.

Ключевые слова: *диабетическая гепатопатия и её маркеры, лимфа*