DOI: 10.17816/KMJ2020-47

Endothelial dysfunction and impaired lymphatic drainage of the heart in the pathogenesis of cardiovascular complications in diabetes

M.Kh. Aliyev, A.I. Mamedzade, U.Dzh. Agamalieva, H.G. Zhakhverdiyev, Dzh.T. Aliyeva, S.I. Hadzhiyeva

Azerbaijan Medical University, Baku, Azerbaijan

Abstract

Aim. To study the role of activation of lipid peroxidation and endothelial dysfunction in disorders of lymphatic coagulation and lymphatic drainage of the heart in streptozotocin-induced diabetes mellitus.

Methods. The experiments were performed on 25 rabbits. To simulate diabetes mellitus, animals were injected intraperitoneally with streptozotocin at a dose of 50 mg/kg. Indicators of lipid peroxidation, coagulation, and endothelial dysfunction were examined in lymph obtained by draining the thoracic lymphatic duct. We also examined the state of lymphatic drainage of tissues at the level of the thoracic lymphatic duct and at the level of the heart, before and after inducing diabetes.

Results. On the 5th day after inducing diabetes mellitus, the concentration of diene conjugates in lymph exceeded the initial level by 66.6% ($p < 0.001$), and the concentration of malondialdehyde increased by more than 2.6 times ($p < 0.001$); 30 min later these indicators of diene conjugates and malondialdehyde exceeded the initial values by 3.2 and 2.2 times, respectively ($p < 0.001$), and the concentration of reduced glutathione decreased by 73.8% ($p < 0.001$). At the same time, the indicators of lymph coagulation, activated partial thromboplastin time and thrombin time, were shortened by 42.2 and 32.9%, respectively ($p < 0.01$). The rate of lymphatic drainage from the thoracic duct decreased by 81.8% ($p < 0.01$). Such dynamics persisted throughout the experiment. The duration of the removal of a lymphotropic dye from the heart at stage I was increased on the 30th and 60th days of the study by 28.1% ($p < 0.05$) and 57.9% ($p < 0.001$), respectively. At stage II, this indicator decreased, starting from the 2nd month of the experiment it exceeded the initial level by 22.2% ($p < 0.05$), and subsequently by 32.7% ($p \leq 0.001$)

Conclusion. The activation of lipid peroxidation and intravascular coagulation of lymph, followed by inhibition of lymphatic drainage of tissues at the level of the thoracic lymphatic duct, especially the heart, creates favourable conditions for the accumulation of toxic products of impaired metabolism in the myocardium, contributing to the development of cardiovascular complications.

Keywords: diabetes mellitus, lipid peroxidation, lymphatic drainage of the heart, cardiovascular complications, lymphatic coagulation.

For citation: Aliyev M.Kh., Mamedzade A.I., Agamalieva U.Dzh. et al. Endothelial dysfunction and impaired lymphatic drainage of the heart in the pathogenesis of cardiovascular complications in diabetes. *Kazan medical journal*. 2020; 101 (1): 47–52. DOI: 10.17816/KMJ2020-47.

Worldwide, the number of people with diabetes mellitus (DM) is growing steadily and is expected to exceed 400 million by 2030. Owing to the rapid increase in prevalence, DM is recognized by experts of the World Health Organization as a non-infectious epidemic. The mortality is 2–3 times higher in patients with DM than in those without DM [1].

Currently, high-quality insulin preparations and other hypoglycemic agents have been developed and are being widely used for DM patients to significantly increase their life expectancy of patients. However, these drugs cannot completely compensate for the metabolic disorders and are insufficient to prevent the development of DM complications, such as cardiovascular disorder, the leading DM-related complication [2].

Total 80%–100% patients with DM demonstrate angiopathy. Numerous studies in this field have shown that the damaging effect of hyper-

For correspondence: rjafarova@bk.ru Received 11.12.2019; accepted 27.12.2019.

glycemia on the vascular wall is mediated by free radicals, the formation of which increases in chronic hyperglycemia via an increase in the glucose autoxidation rate. All this ultimately leads to activation of lipid peroxidation (LPO) and endothelial dysfunction that are accompanied by increased release of von Willebrand factor, prostacyclin, plasminogen activator, thromboglobulin, etc. into the blood flow [3]. A prothrombotic state develops with a change in all three components that constitute the hemostatic system: platelet function and structure, coagulation factors, and vascular wall integrity [4].

This kind of disturbance leads to changes in the heart blood vessels and causes microangiopathies that are accompanied by microcirculation disorders with morphological and functional changes in the myocardium. Microangiopathies are characteristic of DM and are generalized in nature [5]; they affect the entire microcirculation system with impaired metabolism of the heart muscle. At this point, angiography shows normal coronary arteries [6].

Thus, in DM, favorable conditions are created for the accumulation of potentially toxic products of intermediate oxidation links of free fatty acids in the intercellular space, particularly inside the cardiomyocytes, resulting in a harmful effect on the myocardial cells [7]. The transport of toxic metabolites, large-molecular particles, and the remains of destroyed cells from the intercellular spaces is known to occur mainly through the lymphatic system [7–9]. However, thus far, the state of lymphatic drainage of the heart in DM has not been investigated.

Several studies on this subject have been conducted; a technique has been developed for studying cardiac lymphatic drainage under conditions of experimental DM [10]. Considering this, this study was designed to investigate the role of LPO activation and endothelial dysfunction in disorders of lymphatic coagulation and lymphatic drainage of the heart in experimental DM.

According to the technique developed at the Department of Pathological Physiology of the Azerbaijan Medical University [10], experiments were performed on 25 male and female chinchilla rabbits weighing 2.5–3.0 kg. The animals were feed a standard vivarium diet throughout the experimental period. The experiments were conducted as per the requirements of the European Convention for the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes (March 1986). The research topic was approved by the local ethics committee at the Azerbaijan Medical University (protocol No. 3 dated April 11, 2017, Chairman R.O. Beglyarov).

To simulate DM, streptozotocin (Malakoff, France, Keocyt) dissolved in 1 mL of a 0.9% sodium chloride solution was injected intraperitoneally to animals at a dose of 50 mg/kg. The animals did not receive food during the night. Control group rabbits (n = 6) received injections of 0.9% sodium chloride solution.

The blood glucose level was determined on an empty stomach under conditions of food deprivation 14 h before blood sampling on day 5, 15, 30, 60, and 90 after administration of streptozotocin. Lymph samples were obtained from the drained thoracic duct. Drainage of the thoracic lymphatic duct was performed under general anesthesia with ketamine (8 mg/kg) and diphenhydramine (0.15 mg/kg 1% solution) injected into the rabbits' auricular vein.

The lymph outflow rate was determined using the volume of lymph flowing from the drained thoracic duct per unit of time. The severity of LPO in the lymph was determined on the basis of the level of diene conjugates in the lymph as per the method described by V. B. Gavrilov et al. [11]; the level of malondialdehyde was determined as per the method given by L. I. Andreev et al. [12]; the level of reduced glutathione was determined as per the method of G. H. Ellman [13].

The condition of the lymphatic coagulation, anticoagulation, and fibrinolysis system was evaluated using a set of generally accepted tests, such as activated partial thromboplastin time, prothrombin time, von Willebrand factor, thrombin time, fibrinogen concentration, soluble fibrin-monomer complexes, fibrinogen degradation products, antithrombin-III, and fibrinolytic activity on a semiautomatic coagulometer Humaclot-Duo (Germany) using ready-made reagent kits manufactured by Human (Germany) and Coagulotest (Russia).

The condition of the drainage function of the lymphatic system of the heart was studied with the administration of a lymphotropic stain [0.25% Evans blue solution (T-1824) at a rate of 0.1 mg/100 g of heart weight] with a tuberculin needle, subepicardial to the posterolateral wall of the left ventricle in the region of the heart apex [10]. The rate of the lymphotropic stain excretion was determined at the level of the "supracardiac" lymphatic trunk in the department adjacent to the cardiac lymph node. In this case, the time from injection to stain appearance in the lymph flowing along the "supracardiac" lymphatic trunk (I is the stage of excretion) and the time of complete excretion of the lymphotropic stain from the heart were recorded (II is the stage of excretion) [10].

Statistical analyses of the data were performed using the program Statistica 6.0 in the editor of

Table 1. Indicators of lipid peroxidation in lymph with experimental DM ($M \pm m$; $n = 19$)

Indices	Initial state	Time after starting streptozotocin administration, day			
		5	15	30	60
N	4	3	3	3	3
Diene conjugates, μmol/L	1.5±0.2	2.5±0.2***	3.9±0.3***	4.8±0.2***	3.7±0.4***
Malondialdehyde, μmol/L	3.1±0.5	4.4±0.3**	6.5±0.8***	6.9±0.5***	5.8±0.5***
Reduced glutathione, μmol/L	4.2±0.4	4.0±0.2	3.3±0.19**	3.1±0.4**	3.0±0.3**

Note: statistically significant difference with the initial indicators was *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Excel spreadsheets. All the data are presented as arithmetic mean and standard deviation ($M \pm m$) values. Student's *t*-test was used for data with normal distribution. To compare the relative indicators, Fisher's exact test was used. For comparison of discrete values, nonparametric criteria were used, including the Mann-Whitney paired test for unrelated samples and the Wilcoxon test for related samples. $P < 0.05$ was considered to indicate statistical significance.

Table 1 presents the results of the analyses of LPO indicators in lymph. The table depicts that when simulating DM with streptozotocin, the concentration of LPO activation markers increased significantly. The biochemical analyses demonstrated that from the 5th day after starting DM simulation, the level of diene conjugates in the lymph exceeded the initial level by 66.6%, and the level of secondary LPO product of malondialdehyde exceeded it by > 2.6 times ($p < 0.001$).

As the period from the start of modeling DM in animals increased, the level of diene conjugates and malondialdehyde increased. A marked decrease was noted in the antioxidant potential that we evaluated using the level of reduced glutathione in the lymph. Thus, after 30 d, the level of glutathione in the lymph decreased to 73.8% of the initial value ($p < 0.05$). A steady increase in the level of LPO products in lymph was recorded during the 30 min of the study. The level of diene conjugates and malondialdehyde in the lymph exceeded the initial values by 3.2 and 2.2 times, respectively ($p < 0.001$); moreover, there was a slight tendency to decrease against reduced antioxidant potential of the lymph. By the end of the study, the level of reduced glutathione in the lymph decreased to 66.6% of the initial value ($p < 0.001$). Similar values were obtained in our earlier studies [10].

The results of the study on lymphatic coagulation are presented in Table 2; when modeling streptozotocin-induced DM, a significant increase in lymphatic coagulability occurs, characterized by a decrease in the lymphatic coagulability in terms of activated partial thromboplastin, prothrombin, and thrombin time.

On day 5 after the start of modeling, a marked increase in the level of von Willebrand factor was noted in the lymph that exceeded the initial level by 26.1% ($p < 0.05$). On day 30 from the start of the simulation, the activated partial thromboplastin time and thrombin time decreased by 42.2% and 32.9%, respectively, ($p < 0.01$) as compared to the initial values. In the lymph, there were markers of intravascular activation of lymphatic coagulation, including soluble fibrin-monomeric complexes and fibrinogen degradation products. A significant decrease was noted in the antithrombin-III activity.

With an increase in time since the start of modeling, intravascular lymphatic coagulation disorders aggravated. Thus, on day 60 from the start of modeling, markers of endothelial dysfunction (von Willebrand factor), soluble fibrin-monomeric complexes, and fibrinogen degradation products were detected in the lymph with the inhibition of fibrinolytic activity. Similar dynamics in the events in the lymphatic coagulation system persisted until the end of the study period.

This study showed that the rate of lymph drainage from the thoracic duct in presence of streptozotocin-induced DM underwent phase changes. Thus, the rate of lymph outflow from the thoracic duct ($p < 0.05$) increased slightly on day 5 from the start of the experiment and subsequently began to decrease gradually. On day 30, it was 81.8% of the initial value ($p < 0.01$). Such changes in time persisted throughout the experimental period.

Studies of the drainage function of the lymphatic system in experimental DM confirmed these assumptions. Our research results are presented in Table 3; in presence of experimental DM, the rabbit drainage function of the lymphatic system of the heart was markedly inhibited, as confirmed by an increased duration of stages I and II of removal of the lymphotropic stain from the heart. More-

Table 2. Coagulation lymphostasis against Streptozotocin-induced DM ($M \pm m$; $n = 19$)

Indices	Initial state	After administration of Streptozotocin, day			
		5	15	30	60
N	3	3	4	3	3
Von Willebrand factor, %	55.1±3.9	69.5±4.4*	85.3±4.7**	99.8±5.1***	90.9±4.8***
Activated partial thromboplastin time, s	53.4±2.1	47.3±3.1	30.9±1.3***	33.4±2.1***	32.3±1.8***
Prothrombin time, s	33.2±1.9	24.4±1.1**	27.1±1.2*	24.1±1.3**	27.2±0.9*
Thrombin time, s	27.4±1.3	20.9±0.7*	18.4±0.4***	20.4±7.9**	23.4±0.7
Concentration of fibrinogen, g/L	2.7±0.05	2.9±0.03	3.2±0.02*	3.0±0.01*	2.9±0.02*
Soluble fibrin-monomer complexes, +/–	–	–	+	+	+
Fibrinogen degradation products, +/–	–	–	+	+	+
Antithrombin-III, %	120.4±6.9	90.9±5.9	81.1±4.8**	75.9±6.4**	80.9±4.7**
Fibrinolytic activity, min	21.4±1.1	20.9±1.2	22.2±0.9	16.9±0.4*	12.4±1.1**
Rate of lymph efflux, ml/min/kg	0.22±0.02	0.25±0.01	0.20±0.02*	0.18±0.01**	0.15±0.01***

Note: statistically significant difference with the initial indicators is *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3. Duration of excretion of the lymphotropic stain from the heart in the presence of experimental DM in rabbits ($M \pm m$, $n = 19$)

Series of experiment	Stages of stain excretion	Initial state	After administration of Streptozotocin, day			
			5	15	30	60
N		4	3	3	3	3
Control	Stage I, s	182.4±6.8	176.7±8.4	170.9±9.3	155.4±8.7	171.4±6.8
	Stage II, s	355.7±9.3	346.7±12.4	349.7±11.2	366.9±9.7	359.5±12.3
Test group	Stage I, s	167.4±6.2	177.5±7.3	214.4±8.3**	257.6±9.3***	264.4±7.6***
	Stage II, s	373.4±11.3	390.7±9.7	428.9±12.3	456.4±10.7**	495.4±9.8***

Note: statistically significant difference with the initial indicators is *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

over, on day 30 of the study, stage I increased by 28.1% as compared to the initial level ($p < 0.05$). The dynamics of changes were unidirectional; by the end of the study period, the stage I duration exceeded the initial level by 57.9% ($p < 0.001$). A change in the duration of the lymphotropic stain removal from the heart at the stage II occurred, starting from month 2 of the experiment; it exceeded the initial level by 22.2% ($p < 0.05$); by day 90, it was 32.7 % ($p < 0.001$) more than the initial value.

Thus, the research results showed that when modeling DM, the drainage function of the lymphatic system of tissues was significantly impaired, and favorable conditions are created for the accumulation of toxic metabolites at the cellular and organ levels [10]. In our studies, in presence of experimental DM, LPO activation was accompanied by endothelial dysfunction, resulting in impaired coagulation of lymph and lymphatic drainage of the heart.

Based on a comparison with previous trials [7–10], we can conclude that with the use of streptozotocin-induced DM, intravascular activation of lymphatic coagulation and inhibition of lymphatic tissue drainage at the level of the thoracic duct has a negative effect on the drainage function of the heart lymphatic system, contributing to the accumulation of toxic products of impaired metabolism in the myocardium intercellular. This, in turn, exacerbates endotoxicosis at the cellular and organ levels, there-

by creating favorable conditions for the occurrence of cardiovascular complications. Thus, considering our results, we believe that it is appropriate to perform further research on the state of lymphatic coagulation and lymphatic drainage of the heart in case of cardiovascular complications in patients with DM.

CONCLUSION

Activation of lipid peroxidation and intravascular coagulation of the lymph, followed by inhibition of lymphatic drainage of tissues at the level of the thoracic lymphatic duct, in particular the heart creates a favorable condition for the accumulation of toxic products of impaired metabolism in the myocardium and contributes to the development of cardiovascular complications.

The authors declare no conflict of interest.

REFERENCES

1. Maslova O.V., Suntsov Yu.I. Epidemiology of diabetes mellitus and microvascular complications. *Diabetes mellitus.* 2011; 14 (3): 6–11. (In Russ.) DOI: 10.14341/2072-0351-6216.

2. Ametov A.S., Kurochkin I.O., Zubkov A.A. Diabetes mellitus and cardiovascular diseases. *RMZh*. 2014; (13): 954–959. (In Russ.)

3. Sizikov V.I., Nelayeva A.A., Hasanov Yu.V., Bykova I.Y. Dysfunction of endothelium and disorders of platelet-coagulation hemostasis in the development of diabetic nephropathy in type 2 diabetes mellitus. *Diabetes mellitus.* 2007; 10 (1): 46–48. (In Russ.) DOI: 10.14341/2072-0351-5915.

4. Krasnopevtseva I.P., Bondar I.A., Pikov I.V. Features of vascular platelet and coagulative hemostasis at patients with diabetes mellitus of 1 type. *Meditsina i obrazovanie v Sibiri*. 2013; (3): 11. (In Russ.)

5. Derevyanko I.A., Novakovskaya S.A. Microcirculatory myocardial channel at an early stage of diabetic cardiomyopathy development. *Novosti mediko-biologicheskikh nauk*. 2016; 13 (1): 23–28. (In Russ.)

6. Fang Z.Y., Schull-Meade R., Leano R. et al. Screening for heart disease in diabetic subjects. *Am. Heart J.* 2005; 149: 349–354. DOI: 10.1016/j.ahj.2004.06.021.

7. Levin Yu.M. *Prakticheskaya limfologiya*. (Practical lymphology.) Baku: Maarif. 1982; 302 p. (In Russ.)

8. Mammadov Y.D. *Infarkt miokarda. Limfaticheskaya sistema serdca. Patofiziologiya i patogeneticheskie osnovy lecheniya*. (Myocardial infarction. Heart lymphatic system. Pathophysiology and pathogenetic bases of treatment.) M.: Meditsina. 1989; 224 p. (In Russ.)

9. Minnebayev M.M. Physiology and pathophysiology of the lymphatic system. Research results and further prospects. *Kazanskiy meditsinskiy zhurnal*. 2015; 96 (1): 118–123. (In Russ.) DOI: 10.17750/KMJ2015-118.

10. Gusejnova Sh.M., Aliev S.D., Aliev M.H. Sostojanie svertyvaemosti limfy i limfaticheskogo drenazha serdca pri eksperimental'nom diabete. *Estestvennye i tehnicheskie nauki*. 2012; (3): 446–450. (In Russ.)

11. Gavrilova V.B., Gavrilova A.R., Hmara N.F. Change of dien conjugates in blood plasma by UV absorption of hapten and isopropane extracts. *Laboratornoe delo*. 1988; (2): 60–64. (In Russ.)

12. Andreeva L.A., Kozhemyakin L.A., Kishkun A.A. Modification of the method of determination of lipid peroxides in the test with thiobarbituric acid. *Laboratornoe delo*. 1988; (11): 41–43. (In Russ.)

13. Ellman G. Tissue sulfhydryl groups. *Arc. Biochem. Biophys*. 1959; 82: 70–77. DOI: 10.1016/0003-9861(59)-90090-6.