

THE METABOLIC CONNECTION BETWEEN DIABETES MELLITUS AND AUTOIMMUNE HYPOTHYROIDISM

**Kuchkarova Lubov, Kayumov Khasan, Berdiyeva Sevara,
Rashid Achilov, Murtazaeva Nurshat, Jurakulov Shokhlakhon**
*National University of Uzbekistan, Tashkent named after M.
Ulugbek, Uzbekistan, Lyubakuchkarova@yahoo.com*

Abstract. *It is supposed that there is a connection between diabetes mellitus (DM) and autoimmune hypothyroidism (AIH). The aim of this study was to compare some biochemical blood parameters in experimental DM and AIH. The experiments were carried out on outbred white rats. DM was induced by feeding animals with a high-fat diet and a single injection of streptozotocin (35 mg/kg). AIH was induced by three subcutaneous injections of thyroglobulin (100 μ /rat) and Freund's adjuvant. For the first injection complete adjuvant, for the second and third treatments, incomplete adjuvant was used on the sixth and seventh days after the first injection. Results show that level of insulin does not change but the level of thyroxine decreases slightly in diabetic rats. In rats with AIH the insulin level was sharply increase, but thyroxine level sharply decreased. Almost all blood parameters in rats with DM and AIH (some organic metabolites, activity of enzymes) were changed unidirectional. Analogical blood parameters shift in both diseases shows the metabolic ways of both autoimmune diseases intersect. This phenomenon should be taken in attention to understand the etiology of DM and AIH.*

Key words. *Rats, diabetes mellitus, autoimmune hypothyroidism, thyroxine, insulin, serum biochemical parameters.*

Introduction

Autoimmune diseases are specific diseases that include various painful syndromes associated with disorders of the immune system and metabolism. In autoimmune diseases a protective reaction of the body to a trigger of unknown nature develops. In result the formation of autoantibodies and T-cell reactions to the own tissues occurs. Reactivity of B- and T-cells to normal components of the body deviates from the norm. The most obvious immunological manifestation is the production of autoantibodies [13].

Almost 4% of the world's population suffers from more than 140 different types of AIDs, which include tissue-specific diseases (lupus, autoimmune hepatitis, rheumatoid arthritis, psoriasis, Crohn's disease, multiple sclerosis, autoimmune thyrotoxicosis, insulin-independent and insulin-dependent diabetes, etc. The causes of autoimmune diseases are unknown. These diseases are difficult to diagnose [2, 13]. Autoimmune thyroid disease and type 2 diabetes are the most widespread chronic autoimmune pathologies worldwide. Autoimmune thyroid disease and DM and often cluster in individuals and families, seen in the formation of autoimmune polyendocrinopathy [15].

Autoimmune hypothyroidism is the most common organ-specific pathology of the thyroid gland, which leads to the destruction of the thyroid glands tissue and the deficiency of the thyroid hormones [10, 11]. DM is an autoimmune disease, the main diagnostic sign of which is chronic hyperglycemia – elevated blood glucose levels. DM results in group of metabolic diseases involving carbohydrate, lipid, and protein metabolism [8]. Recently it was found that autoimmunity is an important element in the relationship between AIH and DM [10, 11].

In addition, connection between these two diseases is expressed in the thyroid gland and the pancreas beta-cell dysfunction effect on glucose homeostasis [3]. The data from the examination of insulin-dependent diabetic patients for the presence of AID showed that 26.6% of them were diagnosed with AIH [12]. A higher frequency of thyroid destruction is discovered in patients with DM compared to the general population [5].

These facts indicate the need for research aimed at a more in-depth biochemical analysis of the pathogenesis of DM and associated changes in AIH and vice versa for to identify common shifts in the blood and select adequate treatment for DM and AIH.

The aim of the research is to identify common and specific parameter of type 2 DM and AIH in experimental rats.

Materials and methods

The outbred white male rats weighing 200 ± 20 g were used in experiments. The rats were housed under standard vivarium conditions with an unlimited access to water and standard food and natural light-dark regime. All animal procedures were performed according to the Guideline of the Ethical Committee on the use and care of laboratory animals at the Faculty of Biology of the National University of Uzbekistan.

Experimental type 2 diabetes was induced by feeding a high-fat diet for 28 days and a single injection of streptozotocin (35 mg/kg in 0.1 M citrate, pH 4.5) [16]. In further observations, only animals with high glucose levels were used.

For inducing AIH rats were injected with three subcutaneously injections of bovine thyroglobulin (100µg per rats) and Freund's adjuvant, For the first injection complete adjuvant was used for the second and third injections on the sixth and seventh days after the first one, respectively) incomplete Freund adjuvant were used. The control rats were subcutaneously injected with an equal volume of 0.9% saline at the same time. For determination of AIH the level of anti-TPO was analyzed on 21st days after first immunization in the blood serum samples. For that after cutting gum with a sharp blade the releasing blood was collected into heparin-treated microtubes. Then the blood cells were sedimented in a centrifuge (DLAB D2012, China) at a speed of 3000 rpm for 10 minutes. The serum was carefully aspirated with an automatic pipette. Serum anti-TPO titer were determined using ELISA assay kits (Dublin, Ireland) for rat anti-TPO on the enzyme immunoassay analyzer RT-2100C Microplate Reader Rayto (China). Only rats with the anti-TPO titer more than 0,5 were used in the experimental groups

Rats were divided into control group and two control group with experimental DM and AIH respectively, with 6 animals each. Blood was collected in heparinized centrifuge tubes after decapitation on the 60th day after the first immunization of rats. Blood was sedimented in a DLAB D2012 centrifuge (China) at a speed of 3000 rpm for 10 minutes. The serum was carefully aspirated and used for biochemical analyses.

The level of thyroxine and insulin in the serum was determined using a special reagent set ELISA Kits (Dublin, Ireland) on the immunoassay analyzer RT-2100C Microplate Reader Rayto (China). For analysis of some serum organic metabolites, enzyme activity and inorganic cations a biochemical analyzer RT 1904C (China) and a set of reagents Human Diagnostic firm (Germany) were applied.

Calculation of the mean and standard error was carried out by pairwise comparison using Student's t-test. The arithmetic mean (M), the standard error of the mean (m), and the significant coefficient (P) were defined. If the P value was less than 0.05, the difference between the control and experimental rat groups was considered statistically significant.

Results and discussing

As shown in table 1 the insulin level was not changed, but thyroxine level was decreased by 9,8% in the blood serum in rats with DM compared to control. However, in rats with AIH the level of insulin was increased by 82,3%, The level of thyroxine was almost 2 times lower compared to the control group.

Table 1. Thyroxine and insulin level in the serum of rats with type 2 DM and AIH (n=6)

Parameters	AIH							
	Animal groups							
	Control		DM			AIH		
	(M±SE)	%	(M±SE)	%	P*	(M±SE)	%	P
Insulin	0.50 ±0.03	100	0.47±0.03	94,0	>0,5	0.91 ±0.04	182,3	<0,001
Thyroxine	1.34 ±0.08	100	1.21±0.08	90.2	<0,05	0.68 ±0.05	50,7	<0,001

Note: P – statistically significant difference between control and experimental groups

As shown in Table 2 the level of organic metabolites in the rat blood serum with DM and AIH was also changed. The level of glucose, albumin, cholesterol and uric acid was sharply increased in both diseases. But increasing of total protein level was statistically significant only in rats with AIH.

Table 2. Serum metabolites level in the serum of rats with type 2 DM and AIH (n=6)

Parameters	Animal groups							
	Control		DM			AIH		
	(M±SE)	%	(M±SE)	%	P*	(M±SE)	%	P
Glucose (mmol/l)	4,5±0.2	100	12,2±1,1	271,1	<0.001	6,8.0±0.1 <0.001	151,1	>0,2
Total protein (g/l)	101,4±2.8	100	103,2±6,9	101,7	>0,5	124.1±3.2	119,3	<0.001
Albumin (g/l)P	40.2±0.3	100	44,3±0,0	110,2	<0,01	55.1±0.8	137,2	<0.001
Cholesterol (mmol/l)	2.0±0.3	100	4,2±0,5	210,0	<0.001	2.8±0.2	140,0	<0.01
Uric acid (μmol/l)	151.1±23.	100	300,4±11,2	198,8	<0.001	577.2±51.4	381,9	<0.001

Note: P – statistically significant difference between control and experimental groups.

As table 3 shows the activity of alanine aminotransferase and aspartate aminotransferase and α -amylase, in the rat with DM and AIH

was notably increased in both autoimmune decreases. compared with control values.

Table 3. Enzyme's activity in the serum of rats with varying severity of AIH (M±m; n=6)

Enzyme	Animal groups						
	Control		DM			AIH	
	(M±SE)	%	(M±SE)	%	P*	(M±SE)	% P
ALT	61.2±4.1	100,0	101,2±8.2	165,9	<0,001	222.0±14.4	363,9 <0,001
ACT	50.4±4.2-	100,0	68,2±0,5	135,3	<0,001	84.7±3.1	168,0 <0,001
α-Amylase	321.7±22.1-	100,0	677,9±81.2	210,7	<0,001	5623.1±324.1	174,8 <0,001

Note: P – statistically significant difference between control and experimental groups; ALT – alanine aminotransferase; AST – aspartate aminotransferase.

The main goal of this study was to identify common changes in blood parameters in experimental DM and AIH. Obtained data show that practically of all blood parameters: hormones (thyroxine, insulin), organic metabolites (glucose, total protein, albumin, cholesterol, uric acid) and enzyme activity (alanine aminotransferase, aspartate aminotransferase, α-amylase) change analogically in both diseases.

Its fact, in some studies the association of AIH with a wide range of organ-specific and non-organ-specific autoimmune diseases has been proven [7]. The current experiment findings also confirm that AIH causes a wide range of metabolic changes associated with type 2 DM and vice versa.

Thus, the results show that in rats with diabetes, the insulin level does not change, but the thyroxine level slightly decreases. In rats with AIH, the insulin level changes inversely proportional to the thyroxine level, which is manifested in a parallel increase in hypothyroidism and hyperinsulinemia. The manifestation of insulin at the same level as in control animals and in diabetic rats with marked hyperglycemia indicates the development of type 2 diabetes [16]. We suggest that the increase in the insulin level in rats with AIH against the background of relatively low glucose levels also indicates type 2 diabetes. Some researchers suggest that insulin resistance triggers the production of antibodies to the thyroid gland and the development of AIH, and if so, this may even lead to the progression of hypothyroidism [1]. Therefore, the progression of insulin resistance may also cause an increase in hypothyroidism. This assumption is consistent with the opinion of the authors who believe that

AIH contributes to the development of insulin resistance and related disorders [11].

However, it is known that functional insufficiency of the thyroid gland leads to hypoglycemia [3]. Increased glucose levels in rats with AIH suggest the development of insulin-independent diabetes mellitus. The opposite effects of hypothyroidism and diabetes on glucose concentration, in this case, may be responsible for the relatively small increase in glucose concentration in rats with AIH.

Almost all changes in blood parameters that were recorded in rats with experimental AIH were noted by other researchers in diabetes mellitus. Protein metabolism disorders in our studies were manifested in an increase in the concentration of protein, albumin and uric acid associated with the severity of AIH, observed by other researchers both in AIH and in diabetes mellitus [9].

Diabetes and thyroid insufficiency are both caused by hormonal changes. Although more and more studies have shown a link between diabetes and thyroid diseases, there is no definitive data on what causes them to occur together. The association between these conditions is largely explained by a common genetic background. HLA antigens DQ2 (DQA1*0501-DQB1*0201) and DQ8 (DQA1*0301-DQB1*0302) are common predisposing factors for both diseases [4].

Clinical observations also confirm the relationship between autoimmune diseases of the thyroid and pancreas. Autoimmune thyroid diseases occur in 17–30% of patients with diabetes mellitus [6]. The results of examination of patients with non-insulin-dependent diabetes mellitus for autoimmune thyroid diseases showed that the prevalence of AIH among them was 26.6%. Of these, 42.0% were euthyroid, and 58.0% had hypothyroidism [12]. We suggest that metabolic disturbances in one of these diseases can serve as a trigger for the other through changes in immune homeostasis. Understanding the mechanism of this interaction will be crucial for the development of new mechanism-based therapies for AIH and diabetes

Conclusion

DM and AIH cause similar shifts in the parameters of organic metabolites and enzyme activities in the blood serum, which indicates the mutual causality of these diseases. This fact will contribute to understanding the development of autoimmune diseases of the pancreas and thyroid gland and the creation of new approaches to their therapy.

Bibliography

1. Blaslov K., Gajski D., Vucelić V., Gačina P., Mirošević G., Marinković J., Vrkljan M., Rotim K. The association of subclinical insulin resistance with thyroid autoimmunity in euthyroid individuals. *Acta. Clin. Croat.* 2020. 59(4), 696-702.
2. Elkoshi Z. Cancer and Autoimmune Diseases: A Tale of Two Immunological Opposites? *Front. Immunol.* 2022. 13:821598. doi: 10.3389/fimmu.2022.821598
3. Eom Y., Wilson J., Bernet V. Links between thyroid disorders and glucose homeostasis. *Diab. Metab. J.* 2022. 46(2), 239-256.
4. Frommer L, Kahaly GJ. Type 1 Diabetes and Autoimmune Thyroid Disease-The Genetic Link. *Front Endocrinol (Lausanne).* 2021. Mar 10; 12:618213. doi: 10.3389/fendo.618213.
5. Gu Y., Li H., Bao X., Zhang Q., Liu L. and Meng G. The relationship between thyroid function and the prevalence of type 2 diabetes mellitus in euthyroid subjects. *J. of Clin. Endoc. Metab.* 2017. 102(2), 434-442.
6. Hollowell J.G., Staehling N.W., Dana Flanders W., Harry Hannon W., Gunter E.W., Spencer C.A., Braverman L.E. Serum TSH, T4, and Thyroid Antibodies in the United States Population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J. Clin. Endocrinol. Metab.* 2002. 87, 489-499.
7. Jenkins R., Weetman A. Disease associations with autoimmune thyroid disease. *Thyroid.* 2002. 12(11), 977-988. <https://doi.org/10.1089/105072502320908312>.
8. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E. Milestones in the history of diabetes mellitus: The main contributors. *World J Diabetes.* 2016. 7(1): 1-7.
9. Kunutsor S.K., Khan H., Laukkanen J.A. Serum albumin concentration and incident type 2 diabetes risk: new findings from a population-based cohort study. *Diabetolog.* 2015. 58, 961-967.
10. Li L., Liu S. and Yu J. Autoimmune thyroid disease and type 1 diabetes mellitus: same pathogenesis; new perspective? *Ther. Adv. Endoc. Metab.* 2020. 14(11), 1-19. <https://doi.org/10.1177/2042018820958329>.
11. Li Y., Zhou G., Ozaki T. Distinct histopathological features of Hashimoto's thyroiditis with respect to IgG4-related disease. *Mod. Pathol.* 2012. 25, 1086-1097. <https://doi.org/10.1038/modpathol.2012.68>.
12. McCanlies E., O'Leary L., Foley T., Kramer M., Burke J., Libman A., Swan J., Steenkiste A., Mccarthy B., Trucco M., Dorman J. Hashimoto's Thyroiditis and Insulin-Dependent Diabetes Mellitus: Differences among Individuals with and without Abnormal Thyroid Function. *J. of Clin. Endoc. and Metab.* 1998. 83(5), 1548-1551.
13. Pisetsky D.S. Pathogenesis of autoimmune disease. *Nat Rev Nephrol.* 2023. 19, 509-524 <https://doi.org/10.1038/s41581-023-00720-1>.

14. *Sharp PM, Hahn BH.* Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011. Sep; 1(1): a006841. doi: 10.1101/cshperspect.a006841.

15. *Steck AK, Rewers M.* Genetics of type 1 diabetes. Clin Chem. 2011. 57(2):176-85.

16. *Кайдаш О.А., Иванов В.В., Венгеровский А.И., Буйко Е.Е., Щепеткин И.А.* Экспериментальная модель сахарного диабета 2-го типа у крыс, вызванная диетой с высоким содержанием жиров и стрептозотоцином в низкой дозе. Бюллетень сибирской медицины. 2020. 19(2):41-47.