CORRELATIONS OF ADENOSINE DEAMINASE ACTIVITY IN THE PLASMA OF DIABETIC PATIENTS WITH ARTERIAL HYPERTENSION

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Abstract

This work presents the preliminary data of one center study of the total activity of adenosine deaminase (tADA) and its isoforms (ADA1 and ADA2) in the blood plasma of patients with type 2 diabetes mellitus (T2DM), including cases with complication with arterial hypertension. As a nonspecific indicator of cellular immunity, altered serum tADA activity is used to evaluate diseases related to cell-mediated immune responses, it is considered a useful tool in the monitoring of clinical status of various diseases.

The results demonstrated significant differences between ADA activity levels in the blood plasma of type 2 diabetes mellitus patients and non-diabetic relatively healthy controls. This difference was mainly due to an increase in the activity of the ADA2 isoenzyme. In the peripheral blood plasma of patients with type 2 diabetes mellitus, the tADA (20.5 ± 0.39 *U/L*) and the activity of the ADA2 isoform (16.0 ± 0.75 *U/L*) were significantly higher than in non-diabetic controls (15.96 ± 0.75 *U/L* and 11.23 ± 0.38 *U/L*), tADA and ADA2, respectively, $p < 0.0001$.

The positive correlation between tADA, ADA1 and ADA2 isoform activity was observed with fasting blood glucose, only among type 2 diabetes mellitus patients.

The difference was observed between age groups in nondiabetic patients: there is an increase in ADA2 activity in patients older than 65 years (12.49±1.05 *U/L* vs. 9.86±0.33, $p < 0.0106$).

In diabetic group the tADA, ADA1 and ADA2 isoforms activity in insulin-dependent patients was significantly higher than in non-insulin-dependent patients, especially in men group the tADA activity (21.56 ± 1.85 *U/L* vs. 14.52 ± 1.54 *U/L*) was significantly higher, $p<0.0083$.

In nondiabetic patients with hypertension the significant elevation of ADA2 isoform activity was also observed (11.23 ± 0.38 *U/L vs*. 8.00 ± 0.3 *U/L*), $p < 0.0001$.

We determined the normative range of peripheral blood plasma ADA activity in relatively healthy controls and proved the alteration in activity of ADA enzyme implication in type 2 diabetes mellitus and arterial hypertension pathogenesis.

In conclusion, we note that the measurement of plasma ADA activity is important for understanding the clinical aspects of T2DM and may be useful in predicting the glycemic and immunological status of patients with type 2 diabetes mellitus and hypertension.

Keywords and phrases: blood plasma, adenosine deaminase 1, adenosine deaminase 2, type 2 diabetes mellitus, arterial hypertension.

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Համառոտագիր

Աշխատանքում ներկայացված է ընդհանուր ադենոզինդեամինազի (tADA) և դրա իզոձևերի (ADA1 և ADA2) ակտիվության մակարդակների ուսումնասիրության նախնական տվյալները 2-րդ տիպի շաքարային դիաբետով (ՇԴ) հիվանդների, ներառյալ զարկերակային հիպերտոնիայով հիվանդների արյան պլազայում:

Որպես բջջային իմունիտետի ոչ սպեցիֆիկ ցուցիչ՝ արյան պլազմայում tADA-ի ակտիվությունն օգտագործվում է բջջային միջնորդավորված իմունային պատասխանների հետ կապված հիվանդությունները գնահատելու համար, այն համարվում է օգտակար գործիք տարբեր հիվանդությունների կլինիկական կարգավիճակի մոնիթորինգի համար:

Արդյունքները ցույց տվեցին զգալի տարբերություններ tADA-ի և դրա իզոձևերի (ADA1 և ADA2) ակտիվության մակարդակների միջև 2-րդ տիպի ՇԴ հիվանդների և համեմատաբար առողջ, ոչ դիաբետիկ մարդկանց (որպես ստուգիչ խումբ) արյան պլազմայում: Այդ փոփոխությունը հիմնականում պայմանավորված էր ADA2 իզոֆերմենտի ակտիվության բարձրացմամբ:

2-րդ տիպի ՇԴ հիվանդների ծայրամասային արյան պլազմայում tADA-ի ակտիվությունը (20,5 ± 0.39 *U/L*) և ADA2 իզոֆերմենտի ակտիվությունը (16,0 ± 0,75 *U/L*) զգալիորեն ավելի բարձր են եղել, քան ոչ ՇԴ հիվանդների մոտ (tADA և ADA2, համապատասխանաբար՝ 15.96 ± 0.75U/L և 11.23 ± 0.38 U/L, p <0.0001):

Դիտվել է նաև դրական tADA, ADA1 և ADA2 ակտիվության և քաղցած վիճակում որոշված արյան գլյուկոզի մակարդակի միջև միայն ՇԴ հիվանդների մոտ:

tADA ֆերմենտի ակտիվության էական տարբերություններ են նկատվել նաև ստուգիչ տարիքային խմբերի միջև՝ 65 տարեկանից բարձր խմբի մոտ եղել է ADA2 իզոֆերմենտի ակտիվության աճ (12,49 ± 1,05 *U/L*), ի տարբերություն 65 տարեկանից ցածր խմբի (9.86 ± 0,33 *U/L*), p <0,0106):

Հատկանշական է, որ ՇԴ խմբում ADA իզոֆերմենտների ակտիվությունն ինսուլինկախյալ հիվանդների մոտ զգալիորեն ավելի բարձր է եղել, քան ոչ ինսուլինկախյալ հիվանդների, հատկապես՝ տղամարդկանց մոտ. tADA ակտիվությունն եղել է 21.56 ± 1.85 U/L vs. 14.52 ± 1.54 U/L, p<0.0083:

Հիպերտոնիայով ոչ դիաբետիկ հիվանդների մոտ ևս նկատվել է ADA2 իզոֆերմենտի ակտիվության զգալի բարձրացում՝ 11.23 ± 0.38 *U/L*, ի տարբերություն համեմատաբար առողջ մարդկանց՝ 8.00 ± 0.3 *U/L*, *p* <0,0001:

Աշխատանքի արդյունքում որոշվել է համեմատաբար առողջ մարդկանց ծայրամասային արյան պլազմայում ADA ֆերմենտի ակտիվության նախնական նորմատիվ տիրույթը և ապացուցվել 2-րդ տիպի ՇԴ-ի և զարկերակային հիպերտոնիայի պաթոգենեզում ADA ֆերմենտի ակտիվության փոփոխությունները: Եզրափակելով կարելի է նշել, որ արյան պլազմայի ADA-ի ակտիվության որոշումը կարևոր է 2-րդ տիպի ՇԴ-ի կլինիկական հայեցակետերը հասկանալու համար և կարող է օգտակար լինել 2-րդ տիպի ՇԴ-ով և հիպերտոնիայով հիվանդների գլիկեմիկ և իմունոլոգիական կարգավիճակը որոշելու համար:

Բանալի բառեր և բառակապակցություններ՝ արյան պլազմա, ադենոզինդեամինազ 1, ադենոզինդեամինազ 2, շաքարային դիաբետ 2-րդ տիպ, զարկերակային հիպերտոնիա:

ВЗАИМОСВЯЗЬ УРОВНЯ АКТИВНОСТИ АДЕНОЗИНДЕЗАМИНАЗЫ В ПЛАЗМЕ КРОВИ БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ С АРТЕРИАЛЬНОЙ ГИПЕРТОНИЕЙ

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Аннотация

В работе представлены предварительные данные исследования суммарной аденозиндезаминазы (tADA) и ее изоформ (ADA1 и ADA2) активности в плазме крови больных сахарным диабетом 2-го типа, в том числе с осложнением артериальной гипертонией. В качестве неспецифического маркера клеточного иммунитета изменение tADA в сыворотке крови используется для оценки тяжести заболеваний, связанных с клеточно-опосредованными иммунными реакциями, и считается полезным инструментом для мониторинга клинического состояния. Наши результаты выявили существенные различия в активности tADA в плазме крови больных сахарным диабетом 2-го типа и относительно здоровых лиц без диабета (контроль). Это возрастание активности фермента происходит, в основном, за счет увеличения активности изоформы ADA2.

В плазме периферической крови больных сахарным диабетом 2-го типа активности tАДА (20,5 ± 0.39 U/L) и изоформы ADA2 (16,0 ± 0,75 U/L) были достоверно выше, чем в контрольной группе (15.96 ± 0.75U/L и 11.23 ± 0.38 U/L) для суммарной ADA и ADA2, соответственно, $p<0,0001$.

Выявлена положительная корреляция между активностью tADA (АДА1, АДА2) и уровнем утренней глюкозы в крови, в группе больных диабетом в контроле корреляции не наблюдалось.

Существенные различия в активности ADA наблюдалась также между возрастными группами в контроле: отмечается повышение активности изоформы ADA2 у пациентов старше 65 лет (12.49 ± 1.05 U/L) против (9.86 ± 0.33 U/L) у пациентов до 65 лет, $p<0,0106$.

В группе пациентов с сахарным диабетом активность изоформ аденозиндезаминазы у инсулинозависимых пациентов была достоверно выше, чем у инсулинонезависимых пациентов, в особенности у мужчин активность tADA составляла 21.56 ± 1.85 U/L к 14.52 ± 1.54 U/L, $p<0.0083$.

У пациентов с артериальной гипертонией без диабета также наблюдалось повышение активности изоформы ADA2 (11.23 ± 0.38 U/L к 8.00 ± 0.3 U/L), $p < 0.0001$.

Нами определен нормативный диапазон активности ADA в плазме крови относительно здоровых лиц, и доказана роль изменения активности фермента в патогенезе сахарного диабета 2-го типа с артериальной гипертонии.

В заключение отметим, что измерение активности ADA в плазме крови важно для понимания клинических аспектов сахарного диабета 2 типа и может быть полезным для прогнозирования гликемического и иммунологического статуса пациентов с сахарным диабетом 2-го типа и артериальной гипертонией.

Ключевые слова и словосочетания: плазма крови, аденозиндезаминаза 1, аденозиндезаминаза 2, сахарный диабет 2-го типа, артериальная гипертония

Introduction

The prevalence of diabetes mellitus (DM) is increasing worldwide and it is now one of the leading causes of death [1]. Insulin resistance and impaired insulin secretion are the main physiological abnormalities associated with type 2 diabetes mellitus. Immunological disturbances involving the cell mediated immune system and improper T-lymphocyte function, also contribute to the pathophysiology of DM [2].

Hypertension is an important comorbidity, which presents in more than 50% of people with diabetes about 50% of them demonstrate insulin resistance [3, 4]. The co-existence of diabetes and hypertension significantly increases the risk of cardiovascular disease [5, 6].

In order to effectively manage diabetes and hypertension, an understanding of their underlying pathophysiology is important. As these two conditions commonly co-exist, it is postulated that they share similar pathogenetic mechanisms [7].

An increasing amount of evidence highlights the critical role for the adenosine system in regulation of glucose homeostasis, the pathophysiology of diabetes mellitus, including both, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), as well as the associated complications [8]. This system has considered as a central player in some pathophysiological conditions, particularly those linked to inflammatory responses such as diabetes and hypertension [9]. Although several studies have revealed that DM and hypertension are major risk factors for complications and death from COVID-19, it is still unclear whose are the mechanisms related to this condition [10-11]. Evidently, much more research on these specific topics is required.

The purinergic signaling pathway constitutes a ubiquitous system of cell–cell communication and expressed in almost every cell type [12]. The potential role of this system is explored in the regulation of many other biological functions such as inflammatory responses [13, 14].

While ATP acts as an excitatory neurotransmitter, adenosine (Ado), the main metabolite of ATP degradation, presents antagonist neuromodulatory effect acting primarily in neuroprotection. Extracellular ATP and its breakdown product Ado are also well-known mediators of inflammatory responses. ATP generally functions as a pro-inflammatory molecule while Ado is recognized as an anti-inflammatory agent [16, 17].

The participation of Ado in the modulation of glucose metabolism in T1DM and T2DM has been proposed. Ado functions mostly as an anti-inflammatory mediator through binding to its cell-surface receptors, also largely expressed on immune cells [12]. Changes in β cell metabolism commonly cause toxicity and result in cell death by apoptosis or necrosis [18]. In the early stage of DM, there is an increase in pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6 [19]. A chronic inflammatory state is established as the disease evolves, with the persistence of pro-inflammatory cytokines above normal levels, a process that may be associated with atherosclerosis in diabetic individuals [20]. Furthermore, immune cell infiltration triggers a releasing of pro-inflammatory cytokines, macrophages, and T cells, all of which contribute to insulin resistance [21].

The final key component of the adenosine pathway is adenosine deaminase (ADA) that is present in humans in two genetically and catalytically different isoforms – ADA1 and ADA2 and is catalyzing the irreversible deamination of (2'-deoxy) adenosine into (2'-deoxy) inosine. The result of ADA activity is depletion of adenosine in the local microenvironment, limiting its immunomodulatory effects. Considering the relevance in the purinergic cascade, ADA1 has a more prominent role and is widely expressed in

intestine, thymus, spleen and other lymphoid and non-lymphoid tissues and it is also involved with neurotransmission [22]. ADA1 can also be expressed as an ectoenzyme on the surfaces of lymphocytes [23] and dendritic cells [24].

ADA2, with a low substrate affinity (Km for adenosine ~2 mM) is a major component of the tADA activity in serum or plasma and is also found in liver, and monocytes/macrophages in negligible amounts [25]. This isoform has a key role in the regulation of immune responses, can induce proliferation of T helper cells and macrophages and prompt the differentiation of monocytes into macrophages and dendritic cells [26]. ADA2 can be active at sites of inflammation during hypoxia and in areas of tumor growth, etc.

Ubiquitous ADA isoenzymes are considered as suitable markers of cell-mediate immunity and have been used for monitoring severe diseases associated with immune system disorders [9, 27].

A number of reports are devoted to the studies of ADA in T2DM. An increase in the ADA activity level of, which is mainly due to ADA2 isoform, was observed in the blood plasma of diabetic patients compared to healthy controls. In diabetes, ADA activity correlated with the level of the glycated hemoglobin and other clinical parameters [28, 29].

Our study showed differences in the activity of two ADA isoenzymes at T2DM. The normative range of ADA2 activity in peripheral blood of healthy Armenian population was established. We demonstrated specific sex- and age- changes in the activity of the ADA2 isoform in the plasma of relatively healthy subjects (controls) and patients with hypertension and T2DM with and without hypertension. The changes were mainly associated with the ADA2 isoform. Our findings demonstrated the importance of alterations in the ADA activity in T2DM and in patients with hypertension. Therefore, testing of the ADA activity may be useful in assessing the severity of the disease and the effectiveness of the therapy, helping in developing new approaches to the prevention and treatment of diabetes.

Materials and methods

Chemicals Adenosine and erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other reagents were of high purity degree.

Patients The patients with type 2 diabetes mellitus (T2DM) and non-diabetic (NDM) (case/control study) were patients of Maralik Medical Center (Shirak, Armenia). Informed consent was obtained from all patients included in the investigation in accordance with Good Clinical Practice (GCP) standards and the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. Patients with T2DM were diagnosed according to guidelines of American Diabetes Association [30].

The preliminary study comprises of 359 patients (249 women and 110 men), in the age from 18 to 89 years, 165 (120 women and 45 men) with T2DM of different duration and 194 (129 women and 65 men) NDM patients, including those with hypertension, as controls. Individuals with any kind of infection or inflammatory diseases, which lead to increase in ADA activity, were excluded. The exclusion criteria were acute or chronic liver, kidney or cardiac diseases, malignancy, surgical operations and recovering from SARS-CoV-2 disease and vaccination.

Procedures were performed at the Maralik Medical Center. All the participants were examined early in the morning, fasted, having avoided caffeinated beverages, cigarettes and strenuous exercise since the previous evening.

The glycemic control and ***counting*** blood cells in the Goryaev chamber were performed in clinical laboratory.

Isolation of peripheral blood plasma A blood sample was taken from the antecubital vein in Biochemical Laboratory in Maralik Medical Center. Freshly obtained venous blood was drawn into vacuum blood collection tubes (Lind-Vac) with 3.2% sodium citrate anticoagulant. After centrifugation at 6000 *rpm* for 10 *min*, the supernatant, plasma, free of platelets, was obtained and immediately used in the assay. Stability of the ADA enzyme in the serum lasts 24 *hours* at 25°C, 7 days at 4°C and 3 months at −20°C.

ADA assay The total ADA activity in plasma was assessed by measuring of ammonia, produced in the catalyzed reaction of adenosine deamination at incubation at 37°C for 40 *min* of assay mixture, containing in 0.5 *ml*: 0.04 *M* K-phosphate buffer, pH 7.0; 6 *mM* adenosine and an aliquot of a sample. The enzymatic reaction was stopped by addition per 1 *ml* of phenol-nitroprusside and hypochlorite reagents [30]. The intensity of the developed color was measured at 630 *nm* against blank with all the reagents, excluding substrate adenosine. The ammonia content was evaluated using ammonium sulfate as a standard. The ADA activity was expressed as *μmole* of produced ammonia per 1 *L* of plasma for 1 *minute* (*U/L*). ADA2 activity was obtained in identical experiment conducted in the presence of 0.04 *mM* EHNA, a selective inhibitor of ADA1. ADA1 activity was calculated by subtracting ADA2 activity from the total ADA activity. All samples were duplicated/triplicated.

Statistical analysis The data were analyzed using GraphPad Prism 3 (GraphPad Prism Software Inc. San Diego, CA, USA) [31]. The results were expressed as the mean (*M*) ± standard error of the mean (*SEM*). The nonparametric Mann–Whitney test was used for statistical analysis of different biochemical parameters. Spearman's correlation coefficient (*r*) was assessed. The differences among groups were considered as statistically significant when the two-tailed *p values* were <0.05.

Results and discussion

The preliminary study comprises 359 of T2DM cases (165) and NDM controls (194) which were age and sex matched (case/control). The study was carried out in Maralik Medical Center (Shirak, Armenia) over a period of 12 months.

The following groups of the patients with T2DM included in this study: 1) T2DM: new-onset, who was diagnosed with the first manifestation of diabetes and long term patients; insulin-dependent diabetes mellitus (IDDM) and insulin-independent (IIDM); with hypertension (T2DM**H**) and without hypertension (T2DMN**H** - type 2 diabetes mellitus non-hypertension). Patients distributed into two groups by sex: adult women and men; and subgroups by age: patients from 18-65 years old (<65); adults over 65 (>65) years old; 2) NDM: non-diabetic patients with arterial hypertension (NDMH) and without (NDMNH).

T2DM patients studied were 73% - women, and 27% - men; IDDM - 67% women, 33% - men. The total activity of ADA and the activities of its isoenzymes ADA1 and ADA2 in the blood plasma regarding the sex and age for the T2DM and NDM patients presented in **Tables 1** and **2**, respectively.

Table 1 — ADA activity in type 2 diabetes mellitus patients and nondiabetic controls by sex and age

Variables	Age (years), median (range)	Total ADA U/L) p value	ADA1 (U/L) p value	ADA2 (U/L) p value
T2DM patients (165)				
Women (120)	63.1 [28-85]	19.23±0.93 (<0.7230)	4.42±0.4 (<0.4646)	16.32±0.9 (<0.5505)
Men (45)	60.6 [39-79]	19.93±1.43 (<0.7230)	4.84±0.66 (<0.4646)	15.23±1.33 (<0.5505)
NT2DM controls (194)				
Women (129)	50.7 [18-87]	14.36±0.58 (<0.2656)	4.21±0.28 (<0.3059)	11.27±0.38 (<0.068)
Men (65)	48.9 [18-84]	12.87±0.64 (<0.2656)	3.49±0.31 (<0.3059)	10.1±0.58 (<0.068)
Patients <65 years (150)	47.4 [18-65]	13.3±0.38 (<0.1057)	4.00±0.21 (<0.4696)	**9.86±0.33 (<0.0106)**
Patients >65 years (44)	71.6 [66-87]	16.03±1.29 (<0.1057)	4.33±0.91 (<0.4696)	**12.49±1.05 (<0.0106)**

Data are presented as mean ± SEM

The data in **Table 1** show, that there was not differences of the ADA activity level between sex groups of T2DM and NDM patients. The difference observed between age groups in NDM patients; there is an increase in ADA2 activity in patients older than 65 years (12.49 ± 1.05*U/L* vs. 9.86 ± 0.33*U/L*, p<0.0106), which confirmed by the literature data [32]. In T2DM group the age difference was not observed (data not shown).

The comparison of ADA activity in T2DM and NDM showed that both, tADA and ADA2 activities in T2DM patients (20.5 ± 0.39 *U/L* and 16.0 ± 0.75 *U/L*, respectively) were significantly higher than in NDM controls (15.96 ± 0.75 *U/L* and 11.23 ± 0.38 *U/L*, tADA and ADA2, respectively), *p* <0.0001, **Table 2, Fig. 1.** There was no difference between T2DM and NDM patients in ADA1 activity.

Table 2 — ADA activity in plasma of T2DM and NT2DM patients with and without hypertension

Patients	**total ADA (U/L) p value**	**ADA1 (U/L) p value**	**ADA2 (U/L) p value**
T2DM (165)	**20.5±0.39 (<0.0001)**	4.69±0.40 (<0.3946)	**16.0±0.75 (<0.0001)**
IDDM women (53)	**21.8±1.44 (<0.0306)**	5.11±0.64 (<0.1008)	**18.5±1.46 (<0.0830)**
IIDM women (67)	**17.61±1.25 (<0.0306)**	3.82±0.51 (<0.1008)	**14.65±1.17 (<0.0830)**
IDDM men (18)	**21.56±1.85 (<0.0083)**	5.41±1.1 (<0.1738)	16.41±1.8 (<0.0641)
IIDM men (27)	**14.52±1.54 (<0.0083)**	3.08±0.52 (<0.1738)	11.81±1.41 (<0.0641)
T2DMH (77)	19.44±1.1	4.80±0.5	15.56±1.17

	(<0.8401)	(<0.0772)	(<0.9007)
T2DMNH (88)	19.03±1.04 (<0.8401)	3.96±0.45 (<0.0772)	16.22±1.2 (<0.9007)
NDM (194)	**15.96±0.75** **(<0.0001)**	3.88±0.27 (<0.3946)	**11.23±0.38** **(<0.0001)**
NDMNH (91)	**11.18±0.35** **(<0.0001)**	3.51±0.20 (<0.2310)	**8.00±0.3** **(<0.0001)**
NDMH (103)	**16.02±0.61** **(<0.0001)**	4.48±0.38 (<0.2310)	**11.94±0.53** **(<0.0001)**

Data are presented as mean ± SEM

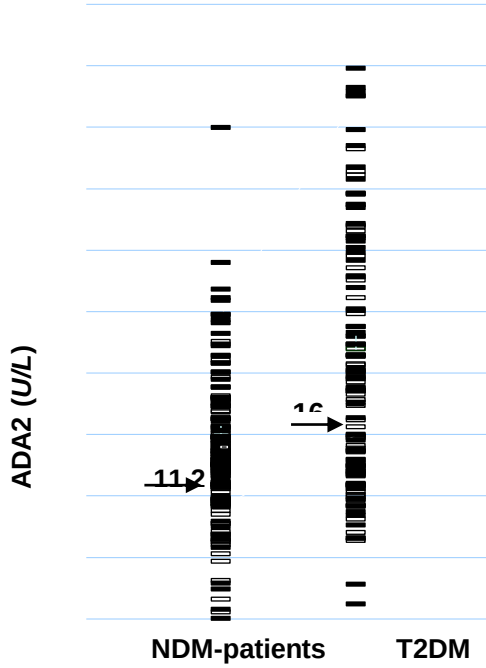

Fig. 1 – ADA2 activity in blood plasma of patients with T2DM and NDM controls

Arrows show the mean values of ADA2 activity

Table 2 presented data concerning the total activity of ADA and the activities of its isoenzymes ADA1 and ADA2 in the blood plasma of T2DM and NDM patients, and those with and without hypertension. There is an increase in the activity of tADA and ADA2 in NDMH patients, which is higher in relation to NDMNH patients, but lower, than ADA activity level in all T2DM subjects, (p value is 0.0001 for ADA2 and tADA). The differences were observed in tADA activity level in T2DM patients between IDDM and IIDM groups, but in men group the tADA activity in IDDM patients was significantly higher, 21.56±1.85 *U/L vs.* 14.52±1.54 *U/L*, p<0.0083.

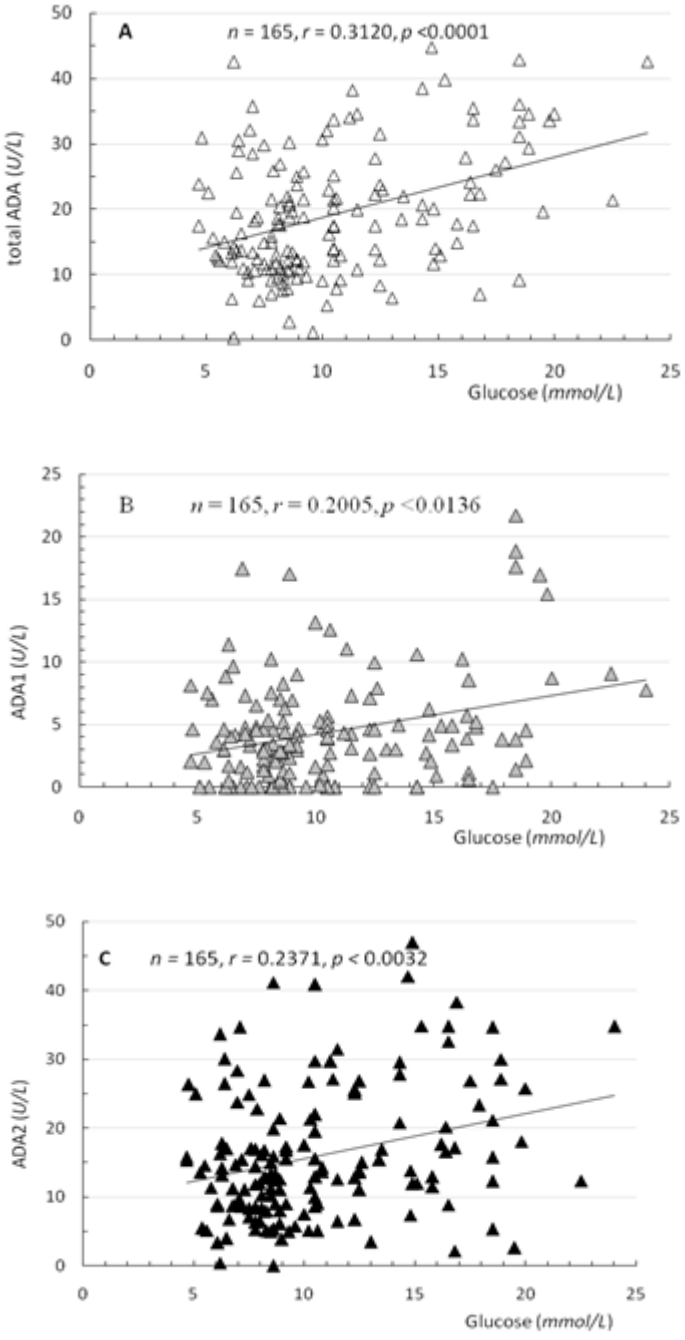

Fig. 2 – The correlation between fasting blood glucose and ADA (tADA, ADA1 and ADA2) activity
in plasma of T2DM patients

As presented in **Fig. 2** plasma tADA (A) and ADA1 (B) and ADA2 (C) level showed a positive correlation with fasting plasma glucose (r = 0.3120; p < 0.0001 – tADA; r = 0.2005; p < 0.0136 – ADA1 and r = 0.2371;

p <0.0032 – ADA2) level only among T2DM subjects, but no significant correlation was observed in NDM controls (data not shown).

The ADA2 activity of plasma in T2DMH patients is comparable to ADA2, observed in patients with T2DM. The hypertension as a complication of T2DM does not significantly add to ADA2 activity, **Fig. 3.**

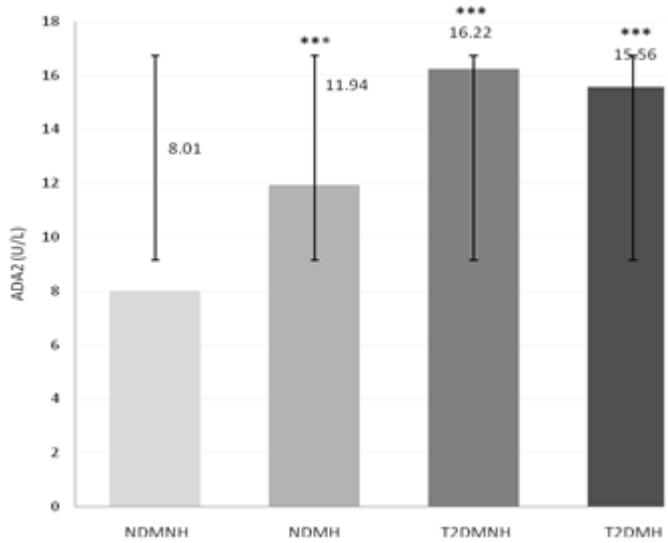

Fig. 3 – ADA2 activity in plasma of patients with T2DM, complicated with hypertension
***p<0.0001 – between NDM and patients with T2DM with and without hypertension

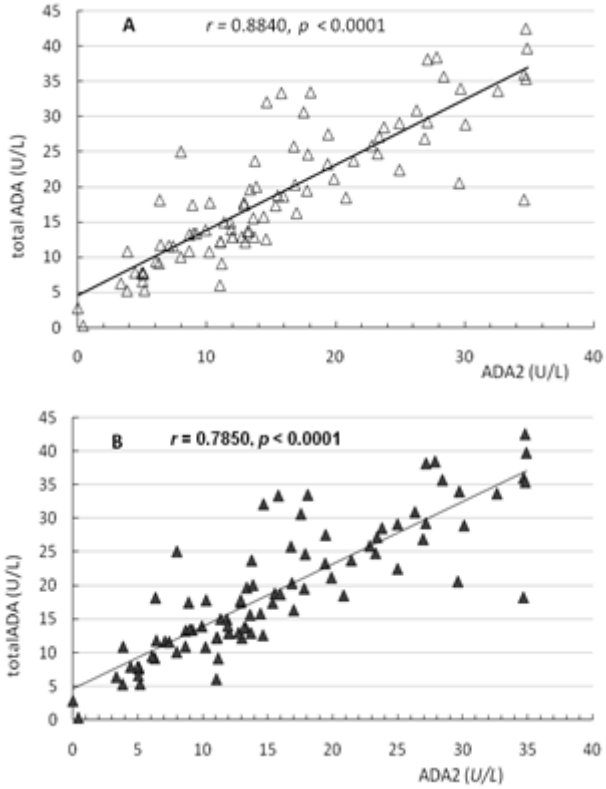

Fig. 4 – Correlation between plasma tADA and ADA2 activity
A – in 88 T2DMNH patients, B – in 77 T2DMH patients
r – Spearman's correlation coefficient

The comparison of correlations between tADA and ADA2 activities in plasma of T2DMNH (A) and T2DMH (B) patients shows, that the difference is not significant (p <0.0001, is the same), but Spearman coefficient - a little lower for patients with diabetes, complicated with arterial hypertension, **Fig. 4**, confirming the additional changes in ADA2 activity in plasma of diabetic patients with hypertension (**Fig. 3)**.

T2DM is a multifactorial disease and characterized by deranged protein and fat and carbohydrate metabolism, secondary to insulin resistance [2]. The role of ADA1 in the cellular immunity was first identified in patients with severe combined immune deficiency [33]. The pathogenesis of ADA2 deficiency includes lymphoproliferation, cytopenia, and variable degrees of immunodeficiency, and still is poorly understood [34]. During inflammation, increased ADA2 activity has been found in macrophage-rich tissues and was considered to be a reflection of immunological disturbance observed in these severe diseases, making ADA2 activity a convenient marker to improve the diagnosis and follow-up treatment of these disorders. In clinical laboratory, ADA activity detection has been used for diagnosing tuberculous pleural effusion and tubercular meningitis. In contrast to ADA1, ADA2 activity for adenosine requires high levels of adenosine and low optimum pH of 6.5, and it shows a weak affinity for substrate. This suggests that ADA2 expresses its activity only at conditions that are associated with hypoxia or inflammation [32, 34–36].

As a nonspecific indicator of cellular immunity, altered serum ADA activity is used to evaluate diseases related to cell-mediated immune responses, and is considered a useful tool in the monitoring of clinical status.

Conclusions

The results demonstrated significant differences between ADA activity levels in the blood plasma of T2DM patients and NDM relatively healthy controls. This increase was mainly due to an elevation in activity of the ADA2 isoenzyme.

The positive correlation between tADA, ADA1 and ADA2 isoforms activity was observed with fasting blood glucose only among T2DM patients.

We determined the normative range of peripheral blood plasma ADA2 activity in NDMNH relatively healthy patients and proved the alteration in activity of ADA enzyme implication in T2DM and arterial hypertension pathogenesis.

Our study revealed also the elevation of ADA2 activity with aging in NDM group of relatively healthy individuals, but not in T2DM patient group.

The measurement of plasma ADA activity is important for understanding the clinical aspects of T2DM and may be useful in predicting the glycemic and immunological status of patients with type 2 diabetes mellitus and hypertension.

Competing Interests

None declared.

Acknowledgments

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