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ADENOSINE DEAMINASE AND GLUTAMINASE IN DUPUYTREN'S CONTRACTURE COMBINED WITH DIABETES MELLITUS

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The etiology of Dupuytren's contracture (DC), a disease characterized by the thickening of the palmar aponeurosis, remains relatively understudied. Diabetes mellitus (DM) is identified as one of the risk factors for DC. The objective of the present research is to find out the relation of DM to the DC in the level of some essential enzymes. The object of the study was the hand's aponeurosis. The activities of adenosine deaminase (ADA) and glutaminase (GLS) enzymes were studied in the aponeurosis tissue homogenate. 15 individuals participated in the research and were divided into 3 groups per 5 persons: the group of individuals with only DC, the group consisted of individuals with DC and DM together (DC+DM), and the group of the relatively healthy subjects served as a control. The activity of GLS in the control group was equal to 0.92 ± 0.0180 *IU/μg* protein, and in the DC and DC+DM groups decreased down to 0.60 ± 0.03 *IU/μg* protein and 0.12 ± 0.012 *IU/μg* protein, relatively. On the contrary, the activity of ADA was in the control group of 0.057 ± 0.007 *IU/μg* protein and increased in the DC and DC+DM groups up to 0.47 ± 0.03 *IU/μg* protein and 0.94 ± 0.062 *IU/μg* protein, respectively. The results of the research indicated that these two enzymes are associated with the ethology and pathogenesis of DC development.

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***Keywords*:** Dupuytren's contracture, hand's aponeurosis, adenosine deaminase, glutaminase.

Introduction. Diabetes mellitus (DM) is an endocrine disease characterized by an absolute or relative deficiency of insulin, leading to consistently elevated blood glucose levels, known as hyperglycemia. Dupuytren's contracture (DC) is a condition impacting the palmar fascia, the fibrous tissue layer beneath the skin of the palm [1]. The fascia lies above the tendons, nerves, blood vessels, and bones in the palm and fingers. The DM and DC are related to each other, first being a risk factor for the second [2–4]. Alcoholic cirrhosis of the liver and DM are among the most significant risk factors for developing DC. The liver is considered as the crossroads where these diseases and the related enzymes intersect.

Alcohol use leads to increased intestinal permeability and the passage of bacterial products such as lipopolysaccharides (LPS) into circulation. The LPS upon

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joining Kupffer cells, activate the MyD88 pathological pathway through *Toll-like receptor 4*, resulting in the production of pro-inflammatory cytokines, among which TNF-α induces hepatocellular damage [5]. Decreased glutaminase activity is likely associated with hepatocellular damage. Acetaldehyde directly increases collagen expression [6] in the hepatic stellate cells (HSC). Probably, this might be one of the causes for the development of DC. People with alcohol hepatitis often suffer from DC. HSC can be activated by inflammatory mediators and oxygen free radicals at oxidative stress.

Adenosine deaminase (ADA, EC 3.5.4.4) is a key enzyme in purine metabolism, catalyzing the conversion of adenosine to inosine with the release of ammonia. ADA is present in all mammalian tissues, with higher concentrations found in lymphoid systems. This enzyme plays a vital role in the development and maintenance of the immune system [7]. At inflammations the ADA activity increases due to inflamed tissue and cell injury, resulting in the decrease of anti-inflammatory adenosine level and aggravating the inflammation.

Glutaminase (GLS, EC3.5.1.2) a key enzyme in regulating glutaminolysis, which produces glutamate from glutamine, is abundant in the liver and pancreatic β-cells [8]. It participates in various cellular processes, including energy production, *redox homeostasis* and biosynthesis of biomolecules necessary for cell function and growth. It has been reported that GLS inhibition removed senescent cells from various organs and tissues in aged mice, thereby improving age-related tissue dysfunction. However, GLS inhibition could lead to unintended consequences by disrupting essential cellular processes [9]. The later prompted the study of the GLS enzyme in DC.

In our previous researches of the DM and DC diseases we have registered significant changes in the levels of ADA and GLS enzymes at DC and DM diseases, respectively. The aim of the present study is to investigate these two enzymes in individuals with DC, and to compare their levels with those in healthy subjects as well as in DC patients who concurrently suffer from DM.

Materials and Methods. The research involved 15 participants. We divided them into 3 groups per 5 individuals in each group: with both DC and DM (DC+DM), individuals with only DC (DC), and practically healthy persons with mechanical trauma to the hand (Control group). The DC+DM and DC groups included only male participants consuming alcohol.

The research utilized tissue homogenate obtained from the hand's aponeurosis (fascia).

The used reagents Adenosine, BSA, Glutamine and Coomassie G-250 were purchased from "Sigma" (USA), the other reagents were of highest purity available.

Preparation of Homogenate: aponeuroses from healthy and diseased individuals were homogenised using Portr homogeniser in 10 *mM* phosphate buffer, pH 7.4 (100 *mg* in 10 *mL*) for 8–10 *min* under cold conditions. The resulting homogenate was centrifuged at 17 000 *rpm* for 20 *min* using an MPW-352 refrigerating table centrifuge. After centrifugation, the supernatant was subjected to further analysis.

By Bradford method was evaluated the protein quantity [10]. The standard curve was constructed using BSA in phosphate buffer, pH 7.4. The Bradford's reagent was added to the test tubes containing various aliquots of BSA at precisely defined time intervals. In 15 *min*, the absorbance of the samples was measured at wavelength of 596 *nm* at the same precisely defined time intervals. The graphical dependence of absorbance at 596 *nm* from the BSA concentration was used in evaluation of unknown protein concentrations in the assayed homogenates.

Assay of Enzymes: ADA activity is evaluated by the phenol-hypochlorite colorimetric method, measuring the amount of ammonia liberated in the enzymatically catalysed deamination of adenosine (Ado). The assay mixture, in a final volume of 0.5 *mL*, consisted of 100 μL of 0.2 *M* phosphate buffer, pH 7.4, and tissue homogenate under study (20–100 μg protein). The reaction was initiated by adding substrate (100 μL of 0.2 *M* Ado) to the test tubes. Following a 40-minute incubation at 37°C, the reaction in the test tubes was stopped by sequential adding per 1 *mL* of phenol-nitroprusside and hypochlorite reagents. After incubating for 15 *min* at 42°C, absorbance at 630 *nm* was recorded.

GLS activity is evaluated by the same phenol-hypochlorite colorimetric method, measuring the amount of ammonia liberated in the catalysed deamination of glutamine (Gln). The difference lies in the buffer (0.2 *M* phosphate buffer, pH 8.4,) and the substrate (glutamine) [11].

The activities of both enzymes were evaluated using the standard curve obtained with ammonium sulphate as a standard. They were expressed in IU, as the amount of liberated ammonia in $\mu mol/min$, per μg ($IU/\mu g$) of protein in the assay mixture.

All data are analysed using the statistical GraphPad Prism 8.0.1 Software. Specific differences are examined using Wilcoxon and Student's *t*-test. The data showing p values of <0.05 are considered as statistically significant. Results are expressed as means ± SEM.

Results. The study compared the levels of ADA and GLS enzymes in homogenates of fascia tissues among three group individuals: patients suffering by DC accompanied with DM (DM+DC group), patients suffering by DC without DM (DC group) and practically healthy individuals (control group).

The results of performed comparisons for GLS and ADA are presented in Figs. 1 and 2, respectively. From these Figure one can see that the patterns of changes in the levels of two enzymes in the studied groups are opposite.

Fig. 1 evidences that among the studied groups, the level of GLS activity is the lowest, 0.12 ± 0.01 $IU/\mu g$ protein in samples from patients with DC, who also have diabetes mellitus (DC+DM, $n = 5$). The level of GLS is higher, 0.6 ± 0.03 $IU/\mu g$ protein in samples from patients with DC, who do not have symptoms of diabetes mellitus (DC group, $n = 5$). Finally, the level of GLS is significantly higher, 0.92 ± 0.02 $IU/\mu g$ protein in individuals without diabetes mellitus and Dupuytren's disease (control, $n = 5$).

According to Fig. 2, the pattern of changes in ADA activity in the samples from the studied patient groups is opposite to that of GLS. The lowest ADA level, 0.057 ± 0.007 $IU/\mu g$ protein, was noted in samples from healthy individuals, both without diabetes and without DM (control, $n = 5$). The level of ADA activity, 0.47 ± 0.03 I $IU/\mu g$ protein, was noted in samples from individuals with only

DM, without diabetes mellitus (DM, n = 5). The highest level of ADA activity, 0.94 ± 0.06 *IU*/μg protein, was noted in samples from individuals with DM in combination with diabetes mellitus (DM + DM, n = 5).

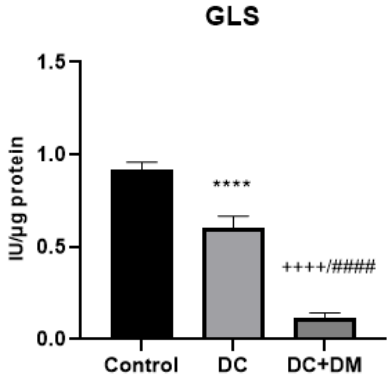


Fig. 1. Activity of GLS in three groups of participants, *IU*/μg protein:

**** p < 0.0001 DC to Control;
++++ p < 0.0001 DC + DM to Control;
p < 0.0001 DC+DM to DC.

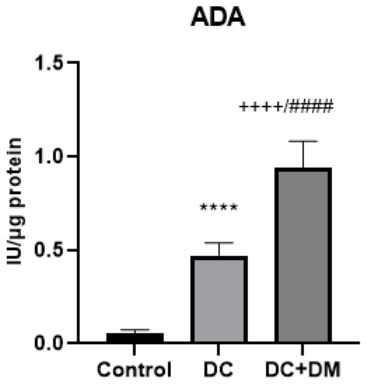


Fig. 2. Activity of ADA, in three groups of participants, *IU*/μg protein:

**** p < 0.0001 DC to Control;
++++ p < 0.0001 DC + DM to Control;
p < 0.0001 DC+DM to DC.

Discussion. The present studies have found significant changes in the enzymes studied, ADA and GLS, when patients with DC are compared with patients suffering from both DC and DM. The liver is considered as a crossroad where these diseases and enzymes intersect. If confirmed by an extension of an identical study with a larger number of participants, this observation may have important implications in the etiology of Dupuytren's disease and diabetes mellitus, especially when they are present together.

The results of the research evidence that ADA enzyme activity increases across the following groups: healthy individuals < patients with DC < patients who have both the DC and DM. The ADA enzyme level of DC patients increased approximately 8 times compared to healthy ones. People with both DC and DM exhibit ADA enzyme levels that are approximately 16 times higher than those found in healthy individuals. Finally, individuals with both DC and DM have ADA enzyme levels that are 2 times higher compared to individuals with DC alone.

On the contrary, according to this research, the GLS enzyme activity in the healthy individuals is highest compared with the other two groups. It decreases in the following sequence: healthy individuals > patients with DC > patients, who have DC+DM. In healthy people, enzyme activity is approximately 1.5 times higher than in DC patients, and approximately 7.9 times higher than in patients with both diseases. People with DC disease alone have enzyme levels that are approximately 5.4 times higher than those found in individuals who have both diseases.

The mentioned notable changes might be crucial in identifying the ethology of Dupuytren's disease and hold diagnostic.

No doubt that this suggestion needs to be continued in the study, involving more participants to enhance the statistical significance.

Conclusion. This work evidences, that adenosine deaminase and glutaminase enzymes having critical role in vital processes in mammals, are likely also important in the rare disease Dupuytren's contracture. The present study underlines this possibility, especially when DC is accompanied by diabetes mellitus, which is considered a risk factor for DC. Continuation of this preliminary study will hopefully be useful in determining the etiology of the rare disease Dupuytren's contracture and in finding pharmacological ways to treat it, avoiding the currently used surgical intervention.

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ԱԴԵՆՈԶԻՆԴԵԱՄԻՆԱԶԻ ԵՎ ԳԼՅՈՒՏԱՄԻՆԱԶԻ ԴԵՐԸ ԴՅՈՒՊՅՈՒԻՏՐԵՆԻ ԿՈՆՏՐԱԿՏՈՒՐԱՅԻ ԵՎ ՇԱՔԱՐԱՅԻՆ ԴԻԱԲԵՏԻ ՀԱՄԱԿՑՄԱՆ ԴԵՊՔՈՒՄ

Դյուպյուիտրենի կոնտրակտուրան կամ ափային ֆիբրոմատոզը քիչ հետազոտված հիվանդություն է, որը բնութագրվում է ափային ապոնևրոզի հաստացումով: Դյուպյուիտրենի հիվանդության զարգացման ռիսկի գործոններից է շաքարային դիաբետը: Տվյալ հետազոտության նպատակն է ֆերմենտատիվ մակարդակում պարզել շաքարային դիաբետի և դյուպյուիտրենի կոնտրակտուրայի միջև կապը: Որպես հետազոտության օբյեկտ հանդես է գալիս վիրահատական ճանապարհով վերցված, մարդկային հաստացած ափային ապոնևրոզը: Մարդկային ափային ապոնևրոզից պատրաստված հյուսվածքային հոմոգենատում որոշվել է Ադենոզինդեամինազ (ԱԴԱ) և գլյուտամինազ (ԳԼՍ) ֆերմենտների ակտիվությունը: Հետազոտությանը մասնակցել են 15 բուժառու, որոնց բաժանել ենք 3 խմբի, յուրաքանչյուրում՝ 5 բուժառու: Առաջին խմբում ընդգրկված բուժառուներն ունեին միայն Դյուպյուիտրենի կոնտրակտուրա (DC): Երկրորդ խմբի բուժառուներն ունեին շաքարային դիաբետ և դյուպյուիտրենի կոնտրակտուրա (DM+DC), իսկ գործնականում առողջները ներառված էին երրորդ խմբում և հանդես էին գալիս որպես ստուգիչ (C): Ստուգիչ խմբում ԳԼՍ ֆերմենտի ակտիվությունը հավասար է 0.92 ± 0.0180 *IU/μg* protein: Դյուպյուիտրենի կոնտրակտուրա (DC), ինչպես նաև, միաժամանակ՝ շաքարային դիաբետ և դյուպյուիտրենի կոնտրակտուրա (DC+DM) ունեցող բուժառուների խմբում ԳԼՍ ֆերմենտի ակտիվությունը նվազում է, համապատասխանաբար՝ 0.60 ± 0.03 *IU/μg* protein և 0.12 ± 0.012 *IU/μg* protein, ի հակադարձ ԳԼՍ ֆերմենտի, ԱԴԱ ֆերմենտի ակտիվությունը ստուգիչ խմբում հավասար է 0.057 ± 0.007 *IU/μg* protein և, համապատասխանաբար՝ ավելանում է դյուպյուիտրենի կոնտրակտուրա (DC) և շաքարային դիաբետ և դյուպյուիտրենի կոնտրակտուրա (DC+DM) ունեցող բուժառուների խմբում 0.47 ± 0.03 *IU/μg* protein և 0.94 ± 0.062 *IU/μg* protein: Վերը նշված հետազոտության արդյունքները հստակ կապ են ցույց տալիս ԱԴԱ ու ԳԼՍ ֆերմենտերի և Դյուպյուիտրենի կոնտրակտուրայի էթիոլոգիայի և պաթոգենեզի միջև:

Г. К. ЭКИМЯН

РОЛЬ АДЕНОЗИНДЕЗАМИНАЗЫ И ГЛУТАМИНАЗЫ В КОНТРАКТУРЕ ДЮПЮИТРЕНА ПРИ САХАРНОМ ДИАБЕТЕ

Контрактура Дюпюитрена или ладонный фиброматоз мало исследованная болезнь, которая характеризуется утолщением ладонного апоневроза. Одним из фактором риска болезни Дюпюитрена является сахарный диабет. Целью данного исследования является выяснение связи между контрактурой

Дюпюитрена и сахарным диабетом на ферментативном уровне. В качестве объекта исследования выступает человеческий утолщенный ладонный апоневроз, взятый хирургическим способом. В тканевом гомогенате, взятом хирургическим путем из человеческого ладонного апоневроза, определена активность ферментов аденозин деаминаз (АДА) и глютаминаз (ГЛС). В исследовании участвовали 15 человек, которые были разделены на три группы, в каждой по пять человек. В первую группу вошли пациенты с контрактурой Дюпюитрена. Во вторую группу вошли пациенты одновременно с контрактурой Дюпюитрена и сахарным диабетом. В третьей группе находились практические здоровые пациенты – контрольная группа. Активность ГЛС в контрольной группе равна 0.92 ± 0.0180 *IU/μg* protein. В группе пациентов с контрактурой Дюпюитрена и группе с двумя болезнями (контрактура Дюпюитрена и сахарный диабет) активность ГЛС снижается до 0.60 ± 0.03 и 0.12 ± 0.012 *IU/μg* protein соответственно. В контрольной группе активность фермента АДА равна 0.057 ± 0.007 *IU/μg* protein. В группе пациентов с контрактурой Дюпюитрена и группе с двумя болезнями (контрактура Дюпюитрена и сахарный диабет) активность АДА соответственно поднимается до 0.47 ± 0.03 и 0.94 ± 0.062 *IU/μg* protein. Вышеизложенное исследование показывает четкую связь между двумя ферментами и этиопатогенезом контрактуры Дюпюитрена.