

Abstract

A. S. Nikolaienko,
*Sumy State University, Medical
Institute, Department of Surgery,
Traumatology, Orthopedics, and
Phthiisology*

**PROTEOLYTIC SYSTEM CONDITION AS IN THE CASE OF
NEUTROPHIL ELASTASE IN PATIENTS WITH TROPHIC
ULCERS ON THE BACKGROUND OF DIABETES MELLITUS**

Relevance. According to the WHO and the International Diabetes Federation (IDF), the number of patients with diabetes in the world reached 463 million people in 2019. A typical manifestation of complications of diabetes mellitus is the development of trophic ulcers of the lower extremities, which leads to poor quality of life, disability, and high financial costs for treatment

Objective. To study the effect of autogenic platelet-rich plasma (APRP) on fluctuations of neutrophil elastase level in patients with trophic ulcers on the background of diabetes mellitus, which may help influence the pathogenesis of ulcer formation in DFD.

Materials and methods. Ninety-eight patients with neuropathic ulcers of diabetic origin, divided into two groups, were treated at the Burn Department and the Vascular Surgery Department of Sumy Regional Clinical Hospital. The comparison group (Group II) included 47 patients who received treatment according to the standard scheme. The main (Group I) included 51 patients who had the standard scheme supplemented with platelet-rich autoplasm. The control group included 20 practically healthy people. To obtain the objective, we investigated the changes in elastase levels from the day of hospitalization up to the 20th day of treatment. The study took 25–30 minutes.

Conclusions. The study found that the average level of neutrophil elastase in patients of the main group was 272.4 ± 4.2 nmol/(min • mL). The average neutrophil elastase level in the subjects of the comparison group was 274.2 ± 3.2 nmol/(min • mL). The levels in both groups were 3.8 times higher than that in the control group ($p < 0.05$).

On day 20 of treatment, the level of neutrophil elastase in the main group decreased to 93.24 ± 1.78 nmol/ min • mL. In patients of the comparison group, the elastase level decreased to 164.17 ± 2.00 nmol/ min • mL. The decrease in neutrophil elastase levels in the main group occurred significantly faster than that in the comparison group, which indicated a positive effect of autogenic platelet-rich plasma on the corresponding link in the pathogenesis of ulcerative defect.

Keywords: neutrophil elastase, autogenic plasma, trophic ulcer, diabetes mellitus.

Corresponding author: nikolaenko_as@ukr.net

Резюме

А. С. Ніколаєнко,
Сумський державний університет, медичний інститут, кафедра хірургії, травматології, ортопедії та фізотерапії

СТАН ПРОТЕОЛІТИЧНОЇ СИСТЕМИ НА ПРИКЛАДІ НЕЙТРОФІЛЬНОЇ ЕЛАСТАЗИ У ХВОРИХ З ТРОФІЧНИМИ ВИРАЗКАМИ НА ТЛІ ЦУКРОВОГО ДІАБЕТУ

Актуальність проблеми. За даними ВООЗ та Міжнародної Діабетичної Федерації (IDF) численність хворих на ЦД у світі на 2019 рік становила 463 мільйонів осіб. Типовим проявом ускладнень цукрового діабету є розвиток трофічних виразок нижніх кінцівок, що веде до зниження якості життя, втрати працездатності та значних фінансових витрат на лікування

Мета роботи. Вивчити вплив аутогенної плазми збагаченої тромбоцитами (АПЗТ) на коливання показників нейтрофільної еластази у хворих з трофічними виразками на тлі цукрового діабету, що можливо дасть змогу впливати на патогенез виразкоутворення при СДС

Матеріали і методи. На базі опікового відділення та відділення судинної хірургії Сумської обласної клінічної лікарні проліковано 98 хворих з нейропатичними виразками діабетичного генезу, які були розподілені на дві групи. У групу порівняння (II група) увійшло 47 хворих, які отримували лікування за стандартною схемою У основну (I групу) входило 51 хворих, у яких стандартний комплекс лікування був доповнений використанням аутоплазми збагаченої тромбоцитами. У контрольну групу увійшло 20 практично здорових осіб. У процесі вирішення поставленої мети ми дослідили у динаміці рівень еластази в день госпіталізації на 20 добу лікування. На дослідження використовується 25–30 хвилин

Висновки. У процесі дослідження встановлено, що середній рівень нейтрофільної еластази у хворих основної групи становив $272,4 \pm 4,2$ нмоль/(хв • мл). Середній показник нейтрофільної еластази у досліджених групи порівняння становив $274,2 \pm 3,2$ нмоль/(хв • мл). Показники обох груп були більшими, ніж у групі контролю у 3,8 рази ($p < 0,05$).

На 20 добу лікування рівень нейтрофільно еластази у основній групі зменшувався до $93,24 \pm 1,78$ нмоль/ хв • мл. У хворих групи порівняння зменшення еластази відбулося до $164,17 \pm 2,00$ нмоль/ хв • мл. Зменшення рівня нейтрофільно еластази у основній групі відбувалося значно швидше ніж у групі порівняння, що свідчить про позитивний вплив аутогенної плазми збагаченої тромбоцитами на відповідну ланку патогенезу виразкового дефекту.

Ключові слова: нейтрофільна еластаза, аутогенна плазма, трофічна виразка, цукровий діабет.

Автор, відповідальний за листування: nikolaenko_as@ukr.net

Introduction

Diabetes mellitus is one of the most important medical and social problems of nowadays that affects most economically developed countries in the world. According to WHO and the International Diabetes Federation (IDF), the number of patients with diabetes in the world reached 463 million people in 2019, and up to 2045, this number may increase to 700 million people. One-third of them

are senior people – over 65 years of age, which is evidence of limited use of surgical methods of treatment in them. In 2019, 760 billion US dollars were spent on the treatment of diabetes mellitus and its complications worldwide. [1]. One of the most severe local complications of DM is diabetic foot syndrome [2]. It typically manifests with the development of trophic ulcers of the lower

extremities, which leads to poor quality of life, disability, and high financial costs for treatment [3].

Proteolysis processes occupy a significant place in the processes of ulcer formation, namely in the reactions of inflammation and tissue destruction [4]. Neutrophil elastase (NE) is known to be the main representative of this system. Recently, a number of scientific papers have been devoted to the study of this enzyme and have proven an increase in the level of neutrophil elastase (NE) activity in various pathological conditions [6]. A significant part of these works was aimed at studying cystic fibrosis, acute respiratory distress syndrome, arterial hypertension, bronchiectasis, chronic obstructive pulmonary diseases, bullous emphysema, spontaneous pneumothorax, pulmonary and pleural tuberculosis, and acute appendicitis. [5, 6, 7, 8]. The proteolytic system state in patients with trophic ulcers of diabetic origin and the NE changes remain poorly studied.

It was found that the predominant amount of elastase is formed in neutrophils, which release elastase after its activation. After release, elastase participates in the lysis of matrix proteins, mainly elastin, collagen, fibronectin, proteoglycans, accompanied by the destruction of the stroma of the affected organ up to the formation of destructive processes [9].

Thus, neutrophil elastase plays a leading role in inflammatory processes and tissue destruction, regardless of their etiology. Taking this into account, the study of the peculiarities of proteolysis in ulcerative processes of the lower extremities as in the case of neutrophil elastase and fluctuations in its level during treatment determines the relevance of the problem, which is of theoretical and practical interest.

Objective: to study the effect of autogenic platelet-rich plasma (APRP) on fluctuations in neutrophil elastase level in patients with trophic ulcers on the background of diabetes mellitus, which may help influence the pathogenesis of ulcer formation in DFD.

Materials and methods

The study was conducted at the Burn Department and the Vascular Surgery Department of Sumy Regional Clinical Hospital. Ninety-eight patients with neuropathic ulcers of diabetic origin were examined and treated. Patients were divided into two groups. The comparison group (Group II) included 47 patients who received standard treatment according to the recommendations of the International Working Group on the Diabetic Foot (IWGDF 2015), which included: wound management (treatment of ulcers with antiseptic solutions, necrectomy), correction of carbohydrate metabolism (hypoglycemic drugs), antibiotic therapy, foot pressure offloading with orthopedic insoles. The main (Group I) included 51 patients who had the standard treatment supplemented with APRP according to our proposed method, i. e. by administration along the periphery of the trophic ulcer and local APRP applications. The control group included 20 practically healthy people. To obtain the objective, we investigated the changes in elastase levels during the treatment. Fifty-one people were observed in the main group, and 47 people were observed in the comparison group.

On the day of hospitalization and on day 20, 5 ml blood samples were drawn from the ulnar vein. Further research was performed according to the well-known scheme, based on centrifugation at 1500 rpm for 20 minutes. The research took 25 – 30 minutes [10, 11].

Results and discussion

During the study of neutrophil elastase in patients with neuropathic ulcers of diabetic origin, it was found that the level of neutrophil elastase in patients of both groups was increased on the day of hospitalization. Thus, in patients of the main group, the average level was 272.4 ± 4.2 nmol/min • mL, and in patients of the comparison group, it was 274.2 ± 3.2 nmol/min • mL, which was 3.8 times higher than in the control group (71.2 ± 3.7). The results are presented in Table 1.

Table 1 – Neutrophil elastase level

Group	Number of patients	NE level, nmol/min • mL, at hospitalization	NE level, nmol/min • mL, on the 20th day of treatment
	(n)	(M±m)	(M±m)
Group I	51	272.4 ± 4.2	93.24 ± 1.78
Group II	47	274.2 ± 3.2	164.17 ± 2.00
Control group	20	71.2 ± 3.7	

On the background of significant clinical-macroscopic improvement, accompanied by a decrease in edema and hyperemia, ulcer clearing, and improvement of the general condition, the level of NON-venous blood in the main group decreased to 93.24 ± 1.78 nmol/min • mL, and remained 1.3 times higher than that in the control group. In patients of the comparison group, the elastase level

decreased to 164.17 ± 2.00 nmol/min • mL and remained 2.3 times higher than the level in the control group. That is, the decrease in NE level in the main group occurred much faster than in the comparison group, which indicated the effect of APRP on the corresponding link in the pathogenesis of ulcerative defect.

Conclusions

In trophic ulcers of the lower extremities on the background of diabetes mellitus, there is an imbalance of the proteolytic system, namely, an increase in the level of neutrophil elastase in the blood serum. During the study, it was found that the level of neutrophil elastase in patients of the main group was in the range of 268–276 nmol/(min • mL), and the average level of the enzyme was 272.4 ± 4.2 nmol/(min • mL). The neutrophil elastase level in patients of the comparison group was in the range of 269–279 nmol/(min • mL), and the average level of the

enzyme was 274.2 ± 3.2 nmol/(min • mL). The levels in both groups were 3.8 times higher than that in the control group ($p < 0.05$).

During 20 days after treatment, the level of elastase remained 1.3 times higher in the patients of the main group as compared to the control levels, while in the comparison group, the decrease in elastase levels was significantly lower, remaining 2.3 times higher as compared to the control level. Thus, the positive effect of APRP on the proteolytic link (neutrophil elastase) in the pathogenesis of ulcerative defect has been proved.

References

1. International Diabetes Federation (IDF). Diabetes Atlas 9th edition IDF. 2020. Retrieved from: <https://www.idf.org/aboutdiabetes/complications.html>
2. Guariguata L, Whiting D, Weil C. The International Diabetes Federation Diabetes Atlas methodology for estimating global and national prevalence of diabetes in adults. *Diabetes Res Clin Pract.* 2011;94:322–32. doi: <http://dx.doi.org/10.1016/j.diabres.2011.10.040>
3. Oksuz E, Malhan S, Sonmez B, Numanoglu R. Cost of illness among patients with diabetic foot ulcer in Turkey. *World J Diabetes.* 2016;7(18): 462–469. doi: 10.4239/wjd.v7.i18.462
4. Liu Z, Shapiro S. A critical role for neutrophil elastase in experimental bullous pemphigoid. *J.Clin.Invest.*2000;5(1):113-123.
5. Holubnychi SO, Holubnycha VM, Piddubna HP, Hupalo M.V. [Features of spontaneous pneumothorax of tuberculous and nonspecific genesis]. *Tuberkuloz v suchasnomu sviti – chastota, symptomy, likuvannia.* [Proceedings of scientific conference: strategy and practice in a fight against infectious diseases]. Lyublin, 2013,pp.129– 140.(In Poland).
6. El-Eshrawy MM, El-Adawy EH, Mousa AA, Zeidan AE, El-Baiomy AA, Abdel-Samie ER et al. Elevated serum neutrophil elastase is related to prehypertension and airflow limitation in obese women. *BMC Womens Health.* 2011;11:1. doi.org/10.1186/1472-6874-11-1
7. Duzhyi ID, Oleshchenko GP, Hnatenko IA. [The state of the proteolytic system in patients with pulmonary tuberculosis]. *Tuberkuloh, lehenevi khvoroby, VIL infektsii.* 2019; 3. DOI: <https://doi.org/10.30978/TB2019-3-20>
8. Duzhyi ID, Oleshchenko GP, Hnatenko IA. [The state of the proteolytic system in patients with tuberculous pleurisy]. *Tuberkuloh, lehenevi khvoroby, VIL infektsii.* 2019; 2. DOI: <https://doi.org/10.30978/TB2018-2-41>
9. Khokha R, Murthy A, Weiss A. Metalloproteinases and their natural inhibitors in inflammation and immunity. *Nat Rev Immunol.* 2013;13(9):649-65. doi: 10.1038/nri3499.
10. Makinskij AI, Spirina AY, Docenko V L, Neshkova EA, inventors. *Sposob opredeleniya aktivnosti tuberkul'oznyh izmenenij v legkih* [Method for determining

the activity of tuberculous changes in the lungs]. Russian patent, no. 99121292, 2000.

11. Duzhyi ID, Nikolaienko AS, Popadynets VM, Medvedieva IM. [The state of the proteolytic system on the example of neutrophilic elastase in patients with trophic

ulcers of arterial and venous origin]. *Sertse i sudyny*. 2017; 4:108—108.

(received 19.11.2020, published online 29.12.2020)

(одержано 19.11.2020, опубліковано 29.12.2020)

Conflict of interest

The authors declare no conflict of interest.

Відомості про авторів/Information about the authors

НИКОЛАЄНКО Андрій Сергійович, аспірант кафедри хірургії, травматології, ортопедії та фізйотерапії, Медичний інститут Сумського державного університету.

40022, м. Суми, вул. Троїцька, 48

Сумська обласна клінічна лікарня

(хірургічний корпус, II поверх)

Телефон: +38 (099) 73-49-741

email: nikolaenko_as@ukr.net