DOI: 10.31793/1680-1466.2024.29-3.201

Bradykinin and interleukin-6 content in the blood of patients with COVID-19 and diabetes mellitus

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Abstract. To explain the causes of acute respiratory distress syndrome, which occurs as a result of SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2) infection, two main hypotheses are proposed: a cytokine storm theory and a bradykinin (BK) storm theory. From a cytokine storm perspective, elevated levels of cytokines, primarily interleukin-6 (IL-6), cause multisystem pathologic manifestations of COVID-19 (Coronavirus disease 2019), including acute lung injury and respiratory distress syndrome in critically ill patients. The BK storm theory emphasizes the importance of decreased angiotensin-converting enzymes in lung epithelial cells, resulting in an inability to break down BK and the BK analog des-Arg9-BK. **The aim** of the work was a comparative study of BK levels in the blood of patients with diabetes mellitus (DM) and patients with mild and severe forms of COVID-19. **Material and methods.** Blood was obtained by standard venipuncture and stored in EDTA tubes. Plasma was separated by centrifugation within 10 minutes after blood sampling. The amount of BK was determined using enzyme immunoassay kits ab136936 («Abcam», Great Britain), IL-6 – EH2IL6 («Invitrogen», Austria). **Results.** It was shown that the BK level increased significantly in patients with DM. Patients with DM and COVID-19 had a slight increase in the amount of BK. At the same time, the amount of IL-6 increased significantly in patients with DM and, especially, in patients with acute COVID-19. Conclusions. The BK level increase in the blood of patients with DM and COVID-19 is caused mainly by DM. In patients with acute COVID-19, the level of the pro-inflammatory cytokine IL-6 increased almost threefold compared to controls.

Keywords: coronavirus infection, bradykinin, diabetes mellitus, interleukin-6.

Introduction

There is still no clear understanding of the causes of acute respiratory distress syndrome, which occurs as a result of SARS–CoV-2 infection. There are two main hypotheses: a cytokine storm theory and a BK storm theory [1-3]. From a cytokine storm perspective, elevated cytokine levels cause multisystem pathological manifestations of COVID-19 (Coronavirus disease 2019), including acute lung injury and respiratory distress syndrome in critically ill patients. The more recent theory of the BK storm emphasizes the importance of a decrease in the number of angiotensin-converting enzymes (ACE and ACE2) in the lung epithelial cells, which leads to the inability to break down BK and the BK analog

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des-Arg9-BK [2, 3]. BK is a short-lived vasoactive compound that acts as a vasodilator and mediator of inflammation in various signaling cascades. It is part of the kallikrein-kinin system, which in turn is part of the renin-angiotensin-aldosterone system, which plays a key role in the pathogenesis of COVID-19. Acute respiratory distress syndrome caused by SARS-CoV-2 disrupts the kallikrein-kinin system and renin-angiotensin-aldosterone system, triggering a BK storm, a process that leads to increased BK expression and downstream effects mediated by its signaling [1-7]. There are also a number of urgent problems related to the most optimal methods for treatment of patients with DM – the most vulnerable and large-scale category of patients, both during the coronavirus pandemic and in the post-coronavirus period [8].

The aim of the work was to compare the BK levels in the blood of patients with DM, patients with DM who suffered from mild or severe COVID-19, and patients with acute COVID-19.

Material and methods

The research protocol was approved by the Ethics Committee of the SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine». All patients signed informed consent for further diagnostic and scientific research on their biomaterials.

Blood was obtained by standard venipuncture and stored in EDTA tubes. Plasma was separated by centrifugation within 10 minutes after blood sampling. Samples were stored at -80°C until use. The amount of BK (n=70) was determined using enzyme immunoassay kits ab136936 («Abcam», Great Britain), IL-6 (n=36) – EH2IL6 («Invitrogen», Austria). Measurements were performed at an optical wavelength of 450 nm on an immunoenzymatic plate analyzer Stat Fax 3200 («Awareness Technology», USA). The calibration curve (**Fig. 1**) indicates that there is no scatter in the data.

70 patients with DM and COVID-19 participated in the study. Controls were individuals without DM who did not have COVID-19. The groups were represented by patients with DM (group 2, n=10), DM patients after mild COVID-19 (group 3, n=19), DM patients after severe COVID-19 (group 4, n=20), acute COVID-19 patients (patients during COVID-19) without DM, or with DM lasting no more than 5 years (group 5, n=9), acute COVID-19 patients with DM lasting more than 10 years (group 6, n=12). Groups 2-4 included 19 men and 30 women, groups 5 and 6 (patients during COVID-19) had 10 men and 11 women.

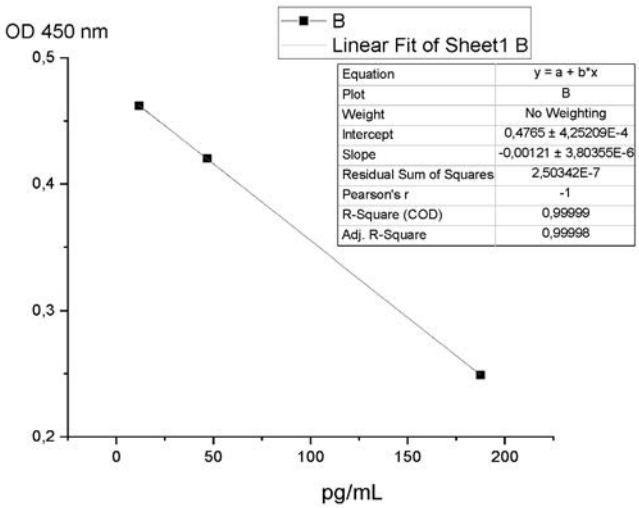

Fig. 1. Calibration curve for determination of BK in blood plasma.

The groups were representative of age (mean 51.50±0.93 years; groups 5 plus 6 – 64.90±2.01 years), body mass index (mean 28.89±0.39 kg; groups 5 plus 6 – 34.89±1.56 kg). Treatment of DM and associated pathology was in accordance with the ADA/EASD recommendations for patients with high cardiovascular risk and was similar in all groups.

The presence of COVID-19 in patients was determined by PCR, the average oxygen saturation in groups 5 and 6 was 88.14±0.73 %, the average maximum temperature was 38.13±0.19 °C. From the groups 5 plus 6, ten patients were treated with ACE inhibitors (iACE), and 15 received metformin. Determination of the degree of severity of COVID-19, as well as treatment of the coronavirus disease, was in accordance with the protocol «Providing medical assistance for the treatment of the coronavirus disease COVID-19», approved by the order of the Ministry of Health of Ukraine (April 2, 2020 No. 762). The group with severe COVID-19 included patients who used oxygen during treatment or got sick 2 or more times.

Statistical analysis and data presentation were performed using Origin 7.0 software. The results of the study are presented as M±SE. Student's *t*-test was used to compare data groups. Values of $p<0.05$ were considered significant.

Results and discussion

The level of BK increased significantly in patients with DM **(Fig. 2, column 2)**. A slight additional BK amount increase was observed in patients with DM who have suffered from COVID-19 **(Fig. 2, columns 3, 4)**. There was no difference between patients who contracted a mild and severe form of COVID-19 (Fig. 2, columns 3 and 4).

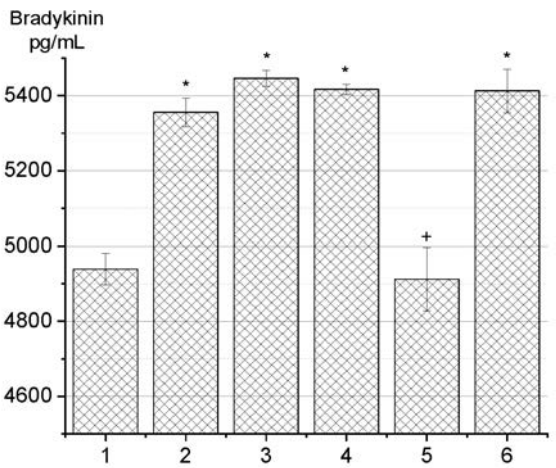

Fig. 2. BK levels in patients with DM and COVID-19.

Notes: 1 – control (n=8), 2 – patients with DM (n=10), 3 – patients with DM who suffered from mild COVID-19 (n=19), 4 – patients with DM who suffered from severe COVID-19 (n=20), 5 – patients with acute COVID-19 without DM, with newly detected DM or with DM lasting up to 5 years (n=9), 6 – patients with acute COVID-19 with DM lasting more than 10 years (n=12).

Therefore, the BK level increase under the conditions of our study is determined mainly by DM. This can be explained by the fact that current guidelines recommend the use of iACE/angiotensin II type 1 receptor blockers (iACE/ARB) in patients with concomitant cardiovascular pathologies, but there are assumptions that an increase in BK levels and the severity of the COVID-19 course when using some drugs are observed. In particular, therapy with peptidase inhibitors, such as iACE and neprilysin inhibitors, increases the concentration of BK [9]. Therefore, to maintain normal blood pressure, the body must balance the levels of ACE and ACE2. It is assumed that because the coronavirus attaches to the angiotensin receptor on the cell surface and increases the synthesis of ACE2, entering the cell with the help of this molecule, but decreases its activity – it causes a significant increase in the concentration of BK (BK storm) and critical complications, especially in patients with arterial hypertension who take iACE for pressure regulation. Currently available ARBs or sartans are able to block this pathological process. Sartans can contribute to renin-angiotensin-aldosterone system inhibition more effectively than iACE, while it does not inhibit ACE with the subsequent formation of excess BK and does not cause the development of a BK storm.

It is important to note that in patients with acute COVID-19 without DM, with newly diagnosed DM or with DM lasting no more than 5 years, the BK level decreased to the control level **(Fig. 2, column 5)**, in contrast to patients with DM duration of more than 10 years **(Fig. 2, column 6)**. At the same time, the number of blood monocytes increased in the second group compared to the first group **(Table 1)**, which indicates the development of inflammatory processes, probably due to viral infection and DM. The average level of glucose in patients at the time of admission to the clinic and at the time of discharge was also higher in the group with DM duration of more than 10 years. This fact further indicates that diabetes makes the main contribution to the increase in BK concentration in the body of patients with DM and COVID-19.

Table 1. The glucose level and number of monocytes in the blood of patients with DM and COVID-19 (groups 5 plus 6)

Indicators	DM duration in patients with COVID-19	
	≤5 years	>10 years
BK (pg/mL)	4912.40±84.15	5412.67±57.66+
Glucose (mmol/L)*	6.62±1.11	10.36±1.16+
Glucose (mmol/L)**	4.96±0.54	8.01±0.68+
Monocytes (%)	5.40±0.36	8.83±0.59+

*Notes. * – glucose level at the time of patient hospitalization; ** – glucose level at the time of patient discharge. + – differences between groups are significant, p<0.05.*

It is worth noting that the BK level in COVID-19 patients (groups 5 plus 6) treated with metformin is significantly lower than in untreated patients (5181.27±69.70 pg/mL vs 5332.89±54.18 pg/mL) while iACE treatment led to the opposite result (5395.04±40.98 pg/mL vs 5173.25±65.71 pg/mL). In addition, patients with a body mass index >30 kg/m² had higher BK levels compared to pa-

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tients with a lower index (5392.04±38.60 pg/mL vs 5077.69±34.92 pg/mL). Lower levels of BK in the group of patients treated with metformin can be explained by a decrease in renin-angiotensin-aldosterone system activity under treatment of drug. Therefore given the pathophysiology of insulin resistance and its implication for vascular function, many have hypothesized a potential beneficial effect of metformin on endothelium-dependent vasodilation, finding positive relational evidence in many animal studies and human trials [10].

Increased BK plasma concentrations may be due to reduced BK degradation because of iACE treatment or decreased levels of kinin degrading enzymes.

The cytokine storm theory, which is an uncontrolled inflammatory response, is attributed to IL-6, which is involved in immune responses and inflammatory processes, including the cytokine storm in COVID-19. IL-6, transcription factors nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3) are the main factors driving the cytokine storm. Synergistic interactions between NF-κB and STAT3 induce hyperactivation of NF-κB with subsequent production of inflammatory cytokines. Because IL-6 is a target of NF-κB, simultaneous activation of NF-κB and STAT3 in non-immune cells orchestrates a positive feedback loop of NF-κB activation by the IL-6/STAT3 axis. This positive feedback loop is termed the IL-6 amplifier (IL-6-Amp). IL-6-Amp is activated by a variety of local initiators, demonstrating that the IL-6/STAT3 axis is a critical target for disease therapy [1, 11].

According to our data, the level of IL-6 increased from 0.89±0.01 pg/mL in healthy individuals to 1.10±0.04 pg/mL in patients with DM who became ill with COVID-19 and almost three times – up to 2.56±0.67 pg/mL – in patients with acute COVID-19 (**Table 2**).

Table 2. The interleukin-6 level (pg/mL) in the blood of patients with DM and COVID-19

Groups	IL-6 level (n)
Control	0.888±0.097 (4)
DM	0.973±0.064 (6)
DM after suffering from COVID-19	1.097±0.038 (8)+
During COVID-19	2.561±0.673 (21)+*

*Notes. + – differences from control are significant, p<0.05; * – differences from groups 2 and 3 are significant, p<0.05.*

BK is a potent short-lived vasoactive peptide that is part of kallikrein-kinin system, associated with the inflammatory response and mediating various functions of vascular permeability, such as thrombosis and blood coagulation. BK induces vasodilatation in the peripheral circulatory system by reducing arterial smooth muscle tone and increasing blood flow [1, 2, 4-7]. In addition, it initiates extravasation of plasma, due to its effect on the endothelium of capillaries, and vasoconstriction through smooth muscle fibers of veins induction. During inflammatory conditions, such as asthma, it promotes the movement of cells from blood to tissues and activates mast cells, fibroblasts, macrophages, and smooth muscles of organs [12]. BK-mediated signaling has also been implicated in vasculopathy, obesity, neuropathy, diabetes, cancer, and chronic pain [8, 13, 14]. Research by Turnic et al. [3] strongly supports the BK storm hypothesis. Since elevated BK levels have been found in the majority of fatal COVID-19 cases, future therapeutic strategies for COVID-19 should focus on reducing serum BK concentrations. At the same time, our data are more in favor of the cytokine storm theory than the BK storm theory, possibly due to the specificity of the patient treatment using a complex of iACE/blockers of angiotensin II type 1 receptors, that can influence BK level. Therefore, in the future, it is worth studying the connection between taking ACE inhibitors and the BK level.

Conclusions

The BK level increase in the blood of patients with DM and COVID-19 is caused mainly by DM.

In patients with acute COVID-19, the level of the pro-inflammatory cytokine IL-6 increased almost threefold compared to controls.

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Abbreviations

ACE – angiotensin-converting enzymes
BK – bradykinin
COVID-19 – Coronavirus disease 2019
DM – diabetes mellitus
iACE – angiotensin-converting enzymes inhibitors
IL-6 – interleukin-6

Рівні брадикініну та інтерлейкіну-6 у крові хворих на COVID-19 та цукровий діабет

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Резюме. Для пояснення причин гострого респіраторного синдрому, який виникає внаслідок інфікування SARS–CoV-2 (severe acute respiratory syndrome-related coronavirus 2) висунуті дві головні гіпотези: теорія цитокінового шторму та теорія брадикінінового (БК) шторму. З погляду цитокінового шторму, підвищення рівня цитокінів, у першу чергу інтерлейкіну-6, викликає багатосистемні патологічні прояви COVID-19, включаючи гостре ураження легенів і респіраторний дистрес-синдром у тяжкохворих пацієнтів. Теорія БК шторму підкреслює важливість зниження кількості ангіотензинперетворюючих ферментів в епітеліальних клітинах легенів, що призводить до нездатності розщеплювати БК та його аналог des-Arg9-BK. **Метою** роботи було порівняльне дослідження рівнів БК у крові хворих на цукровий діабет (ЦД) та пацієнтів, що перехворіли легкою і важкою формами COVID-19. **Матеріал і методи.** Кров отримували за допомогою стандартної венепункції та зберігали в пробірках з ЕДТА. Плазму відокремлювали центрифугуванням протягом 10 хв після забору крові. Кількість БК визначали за допомогою наборів для імуноферментного аналізу ab136936 («Abcam», Велика Британія), інтерлейкіну-6 (n=36) – EH2IL6 («Invitrogen», Austria). **Результати.** Показано, що рівень БК вірогідно зростав у хворих на ЦД. У хворих на ЦД і COVID-19 спостерігалося незначне зростання кількості БК. Водночас кількість інтерлейкіну-6 вірогідно зростала у хворих на ЦД і, особливо, у хворих гострим COVID-19. **Висновки.** Зростання рівня брадикініну в крові хворих на ЦД із COVID-19 визначається головним чином захворюванням на ЦД. У хворих гострою COVID-19 рівень прозапального цитокіна IL-6 зростав майже в три рази порівняно з контролем.

Ключові слова: коронавірусна інфекція-2, брадикінін, цукровий діабет, інтерлейкін-6.

For citation: Pushkarev VV, Levchuk NI, Sokolova LK, Pushkarev VM, Kovzun OI, Tronko MD. Bradykinin and interleukin-6 content in the blood of patients with COVID-19 and diabetes mellitus. Endokrynologia. 2024;29(3):201-206. DOI: 10.31793/1680-1466.2024.29-3.201.

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Personal contribution: Pushkarev V.V., Levchuk N.I. – setting up experiments, writing, design and translation; Sokolova L.K., Pushkarev V.M., Kovzun O.I. – analysis of literature sources, writing, design, editing text and translation; Tronko M.D. – idea of work and consultation when editing an article.
Funding: The article was prepared within the framework of budgetary funding of the National Academy of Medical Sciences of Ukraine according to the plan of research work of the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine».

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Declaration of ethics: the authors have declared no conflicts of interests or financial obligations.
Article: received April 29, 2024; revised August 17, 2024; accepted October 18, 2024; published October 30, 2024.

Для цитування: Пушкарьов ВВ, Левчук НІ, Соколова ЛК, Пушкарьов ВМ, Ковзун ОІ, Тронько МД. Рівні брадикініну та інтерлейкіну-6 у крові хворих на COVID-19 та цукровий діабет. Ендокринологія. 2024;29(3):201-206. DOI: 10.31793/1680-1466.2024.29-3.201.
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Особистий внесок: Пушкарьов В.В., Левчук Н.І. – постановка експериментів, написання, дизайн та переклад; Соколова Л.К., Пушкарьов В.М., Ковзун О.І. – аналіз літературних джерел, написання, оформлення, редагування тексту та переклад; Тронько М.Д. – ідея роботи та консультації при редагуванні статті.
Фінансування: стаття підготовлена в рамках бюджетного фінансування НАМН України за планом науково-дослідних робіт ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України».
Декларація з етики: автори заявили про відсутність конфлікту інтересів або фінансових зобов'язань.
Стаття: надійшла до редакції 29.04.2024 р.; перероблена 17.08.2024 р.; прийнята до друку 18.10.2024 р.; надрукована 30.10.2024 р.