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V.N. Komarevtsev, K.V. Balabanova[1], I.V. Kalinin[2], I.O. Komarevtseva, I.A. Vyshnitska[3], K.V. Shipilov, N.M. Seryogina
The State Establishment "Lugansk State Medical University", Rivne
[1]Center for nursing development of the MOH of Ukraine, Kyiv
[2]National University of Life and Environmental Sciences of Ukraine, Kyiv
[3]Saint James School of Medicine, The Valley, Anguilla

A NEW LOOK AT THE BIOMARKER ROLE OF PROSTANOIDS – COMMON LINKS IN THE PATHOGENESIS OF UROLOGICAL CANCERS AND COVID-19

e-mail: kialdmu@ukr.net

The purpose of the study was a comparative analysis of the level of eicosanoid excretion in urine in patients with COVID-19 and tumors of the kidneys, bladder and prostate gland, which, in our opinion, will make it possible to establish some common links in the pathogenesis of tumor lesions and COVID-19. Urinary excretion of PGI_2, PGE_2, TxB_2, $PGF_{2\alpha}$ in patients with COVID-19 , as in patients with bladder cancer and prostate cancer is significantly increased. Whereas in patients with kidney cancer without and with metastases, the entire spectrum of prostanoids was significantly reduced. The leading prostanoids in COVID-19 patients were prostacyclin PGI_2 and PGE_2; in patients with bladder cancer and prostate cancer – PGE_2, and in patients with kidney cancer without and with metastases – thromboxane B_2.

Key words: prostaglandins and thromboxane, COVID-19, tumors of the kidneys, bladder and prostate gland.

В.М. Комаревцев, К.В. Балабанова, І.В. Калінін, І.О. Комаревцева, І.А. Вишницька, К.В. Шипілов, Н.М. Серьогіна

НОВИЙ ПОГЛЯД НА БІОМАРКЕРНУ РОЛЬ ПРОСТАНОЇДІВ – ЗАГАЛЬНІ ЛАНКИ ПАТОГЕНЕЗУ УРОЛОГІЧНИХ РАКІВ І COVID-19

Метою дослідження був порівняльний аналіз рівня екскреції ейкозаноїдів із сечею у хворих на COVID-19 та пухлини нирок, сечового міхура та передміхурової залози, що, на нашу думку, дасть змогу встановити спільні зв'язки в патогенез пухлинних уражень та COVID-19. Екскреція з сечею PGI_2, PGE_2, TxB_2, $PGF_{2\alpha}$ у пацієнтів з COVID-19, як і у пацієнтів з раком сечового міхура та передміхурової залози, значно збільшується. Тоді як у хворих на рак нирки без метастазів і з метастазами весь спектр простаноїдів був суттєво знижений. Провідними простаноїдами у пацієнтів з COVID-19 були простациклін PGI_2 і PGE_2; у хворих на рак сечового міхура та передміхурової залози – $ПГЕ_2$, а у хворих на рак нирок без і з метастазами – тромбоксан B_2.

Ключові слова: простагландини та тромбоксан, COVID-19, пухлини нирок, сечового міхура та передміхурової залози.

The study is a fragment of the research project "Development of new highly economical methods of biomarker diagnostics and prediction of the course and complications of COVID-19 and community-acquired pneumonia in military personnel and civilians", state registration No. 0123U101246; "Development and implementation of innovative technologies for the diagnosis of oncogynecological and oncourological diseases based on liquid biopsy data of extracellular DNA and stem cells", state registration No. 0123U101248.

Prostaglandins (PG) and thromboxane A_2 (TXA_2) are pro-inflammatory lipid mediators produced through cyclooxygenase (COX) pathway. Eicosanoids are critical mediators of physiological processes, in particular inflammation, pain, fever, arthritis, asthma, cardiovascular disease and cancer [15]. Lipids play a central role in viral infection, as viruses interfere with the lipid synthesis and signalling in the host cells to control their entry and replication. Plasmatic levels of lipids in coronavirus disease 2019 (COVID-19) patients have been found to correlate with disease severity, and several lipids have been identified as potential markers of COVID-19 severity [2].

Prostaglandins, especially PGE_2, have been found to have proinflammatory effects in the pathophysiology of COVID-19 [9]. Smeitink J. et al. suggested that PGE_2 has a significant role in COVID-19 pathophysiology hyperinflammatory and immune responses; therefore, PGE_2 can be measured in patients with COVID-19 [14]. Meng H. et al. [10] findings indicate the presence of an eicosanoid storm in patients admitted to the intensive care unit (ICU) with sepsis caused by COVID-19. Notably, urinary eicosanoid appears to be disproportionately elevated in COVID-19 patients compared with other sepsis patients, despite their greater disease severity.

Coronavirus disease 2019, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is associated with various complications. Among them, renal complications, especially acute kidney injury (AKI), are associated with critical conditions [9]. These renal complications are known as COVID-19-associated kidney injuries. A high incidence of AKI has been reported, especially among critically ill patients, and patients with COVID-19-associated kidney injuries reportedly have a higher risk

of in-hospital death [5]. Moreover, recent studies have suggested prolonged kidney dysfunction in some patients with COVID-19 [11].

Considering the biological properties of eicosanoids, understanding their modulation in urine in urological cancers and COVID-19 will help us understand the mechanisms causing kidney injury associated with COVID-19 [9], as well as common acute kidney injury, and may prompt researchers to develop laboratory tests to identify the prediction of maximum severity and/or new reagents to suppress renal complications of COVID-19.

The purpose of the study was to perform a comparative analysis of the level of eicosanoid excretion in urine in patients with COVID-19 and tumors of the kidneys, bladder and prostate gland, which, in our opinion, will make it possible to establish some common links in the pathogenesis of tumor lesions and COVID-19.

Materials and methods. The studies were carried out on the basis of city and regional hospitals of the Luhansk region between 2015 to 2018 (patients with tumors of the kidneys, bladder and prostate gland), 2020 to 2022 (patients with COVID-19). In accordance with the provisions of the Declaration of Helsinki by the World Medical Association of the last revision (1964-2013) and informed consent for the use of biological material was obtained in all patients prior to inclusion in the study. Research permission was obtained from the Bioethics Committee of the Lugansk State Medical University (Rubizhne, Ukraine, number 25/2015 and 01.09.2023). The patients' epidemiological data, laboratory examination, complications, clinical outcomes, CT imaging data, and treatment plan were extracted from medical records. The date of onset of the disease was the date of the first symptom. All cases of SARS-CoV-2 infection confirmed by a positive result on real-time reverse transcriptase polymerase chain reaction tests of a nasal sample and/or diagnosed by a computed tomography chest scan were included and analyzed. The primary endpoint of this study was the advent of severe acute respiratory syndrome within 30 days of hospitalization.

To test our hypothesis, this case-control study consisted of 35 healthy donors (control group); 28 patients with a positive diagnosis of COVID-19 according to PCR analysis. During our work, we also examined 103 patients with kidney tumors, 113 patients with bladder cancer and 114 patients with prostate cancer. According to the TNM classification, patients with malignant kidney tumors had stages of the tumor process: $T_1N_0M_0$ – 20 (19 %); $T_2N_0M_0$ – 58 (56 %); $T_2N_1M_1$ – 3 (3 %); $T_3N_0M_0$ – 17 (17 %); $T_3N_1M_0$ – 1 (1 %); $T_3N_2M_0$ – 2 (2 %); $T_4N_2M_0$ – 2 (2 %). Patients with bladder cancer had stages of tumor growth: $T_1N_0M_0$ – 31 (27 %); $T_2N_0M_0$ – 40 (35 %); $T_2N_0M_1$ – 2 (2 %); $T_3N_0M_0$ – 27 (24 %); $T_4N_0M_0$ – 8 (7 %); $T_4N_0M_1$ – 1 (1 %); $T_4N_1M_0$ – 1 (1.8 %); $T_{4A}N_xM_0$ – 2 (1.2 %); $T_{4B}N_0M_0$ – 8 (4.7 %); $T_{4B}N_1M_0$ – 1 (0.6 %); $T_{4B}N_2M_0$ – 1 (1 %); $T_4N_2M_1$ – 3 (3 %). Patients with prostate cancer had stages of the tumor process: $T_1N_0M_0$ – 3 (3 %); $T_2N_0M_0$ – 29 (25 %); $T_2N_1M_0$ – 4 (4 %); $T_2N_1M_1$ – 5 (4 %); $T_3N_0M_0$ – 41 (36 %); $T_3N_1M_0$ – 11 (19 %); $T_3N_2M_1$ – 6 (5 %); $T_4N_1M_1$ – 15 (13 %).

Patients of all clinical groups were examined in specialized hospitals, and healthy volunteers were examined on an outpatient basis and partially in a hospital. During the examination, in-depth biochemical and instrumental diagnostics were carried out. To synchronize the scientific analysis of existing pathological processes, the collection of daily urine from both patients and healthy donors was always carried out at 9 a.m. before the start of therapeutic or surgical procedures in plastic tubes.

Determination of eicosanoids in urine. When collecting urine, the prostanoid synthesis blocker indomethacin (20 mg/ml) was added. In each visit, 24 h urine samples were collected and stored at −40°C until analysis. The concentration of urinary PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 was indirectly quantified by measuring the PGE_2, $PGF_{2\alpha}$, 6-keto prostaglandin $F_{1\alpha}$ metabolite and 11-dehydro thromboxane B_2, respectively. All 4 prostanoid subtypes were determined in urine using two competitive enzyme-linked immunosorbent assay (ELISA) kits acquired from Cayman Chem. Co. (Ann Arbor, MI, USA, Item № 514010, 516011, 515211, 501020), following the manufacturer's instructions. The urine samples were diluted 1:10. Concentrations are expressed as ng/24h.

Data Processing. Statistical and graphical analyses were done using STATISTICA 7.0 (StatSoft Inc. USA, version 7.0) and and MedCalc Version 20.218 64 bit (MedCalc Software, Ostend, Belgium). Parametric data were summarized as mean (standard error) (Mean±SEM). Kolmogorov–Smirnov test was applied to examine the normality of data distribution. To examine group-wise differences, unpaired Student's t-test was used. A p-value below 0.05 was considered statistically significant.

Results of the study and their discussion. Determination of the percentage of individual prostanoids in daily urine from their total amount, taken as 100 %, showed that the main product of the oxidation of polyunsaturated fatty acids in the human kidney is prostacyclin ($PGI_2 > TxB_2 > PGF_{2\alpha} > PGE_2$) (Table 1).

Patients with COVID-19 had higher the concentration of urinary PGE_2, $PGF_{2\alpha}$, PGI_2 and TXB_2 values as compared to those in control objects (controls PGE_2: 267.6±9.6 ng/24h; patient with COVID-19: 1017.2±20.8 ng/24h; $p< 0.00001$; controls $PGF_{2\alpha}$: 307.2±12.0 ng/24h; patient with COVID-19: 588.7±16.5 ng/24h; $p<0.00001$; controls PGI_2: 887.8±16.7 ng/24h; patient with COVID-19: 1109.9±19.0 ng/24h; $p<0.00001$;

controls TXB_2: 724.6±14.9 ng/24h; patient with COVID-19: 919.9±16.4ng/24h; p< 0.00001). The maximum level in COVID-19 patients was PGI_2 and PGE_2. The difference between PGI_2 and PGE_2 levels was not significant.The ratio of prostanoids was different from that in healthy people: $PGI_2 > PGE_2 > TxB_2 > PGF_{2\alpha}$.

Table 1

Indices of urinary excretion of prostanoids in patients with COVID-19 and urological oncology (Mean±SEM)

Groups	PGE_2, ng/24h	$PGF_{2\alpha}$, ng/24h	PGI_2, ng/24h	TxB_2 ng/24h
Healthy donors (control group) (n=35)	267.6±9.6	307.2±12.0	887.8±16.7	724.6±14.9
Patients with COVID-19 (n=28)	1017.2±20.8*	588.7±16.5*	1109.9±19.0*	919.9±16.4*
Patients with renal cell cancer without/metastases (n=95)	188.8±5.3*	523.0±9.2*	538.6±2.3*	1126.0±10.9*
Patients with renal cell cancer with/metastases (n=8)	97.3±8.8*	492.3±21.5*	171.8±6.5*	963.7±37.3*
Patients with bladder cancer (n=113)	988.4±9.3*	581.5±6.4*	345.3±4.5*	830.7±6.3*
Patients with prostate cancer (n=114)	1247.7±5.4*	545.9±5.3*	392.4±4.0*	948.3±7.1*

Notes: Data are Means ± SEM for Gaussian variables; Intergroup by the T-test Students * – p – significant differences between control (healthy donors) and test groups

Daily urinary excretion of thromboxane in patients with kidney tumors with and without metastasis did not differ from the control group. Excretion of $PGF_{2\alpha}$ increased only in kidney tumors without metastasis and did not differ from the initial values of healthy donors in patients with kidney tumors with metastasis. But the levels of PGE_2 and prostacyclin sharply decreased in patients without/metastases kidney tumors and especially with metastasis, which naturally affected the ratio of PGE_2 / $PGF_{2\alpha}$ and PGI_2 / TxB_2, namely, led to their decrease. The ratio of urinary excretion of all classes of prostanoids in patients with kidney tumors without metastases was mirrored compared to the control group: $TxB_2 > PGI_2 > PGF_{2\alpha} > PGE_2$. Thromboxane B_2 also took first place in the excretion of prostanoids in patients with metastases, and prostacyclin gave second place to the synergist thromboxane B_2 according to the mechanism of action – $PGF_{2\alpha}$: $TxB_2 > PGF_{2\alpha} > PGI_2 > PGE_2$.

In patients with prostate cancer, the quantitative predominance of the synthesis of all classes of prostanoids compared to the kidneys and bladder remains. The qualitative ratio of the main classes of prostanoids in urine and tissue in patients with bladder and prostate cancer is similar: $PGE_2 > TxB_2 > PGF_{2\alpha} > PGI_2$. Urinary excretion of PGE_2, TxB_2, $PGF_{2\alpha}$ in patients with bladder cancer and prostate cancer is significantly increased. Especially, the content of PGE_2 in the urine of patients with bladder cancer is 5 times higher than in controls, and in patients with prostate cancer – 6 times higher. But the excretion of PGI_2 in patients with bladder and prostate cancer decreased compared to healthy people.

Prostaglandin E_2 and leukotriene B_4 (LTB_4) are eicosanoids involved in modulation of the antiviral immune response. Recent studies have identified increased levels of several eicosa [6]. In most COVID-19 studies, the over-expression of pro-inflammatory lipid mediators such as prostaglandins and thromboxanes has been observed in infected subjects compared to controls [2].

Elevated levels of eicosanoids have been reported in plasma [12], tracheal aspirate [1], urine [13] and in stimulated peripheral blood monocytes [4] of patients with severe COVID-19.

Peripheral blood mononuclear cell (PBMC) PGE_2 was significantly elevated in COVID-19 patients, consistent with our results above in urine and with other studies. In addition, PBMC release of $PGF_{2\alpha}$ were also modestly but significantly increased in the COVID-19 group compared with the healthy controls [12]. PGE_2 and $PGF_{2\alpha}$ production are elevated in PBMCs from patients with COVID-19. $PGF_{2\alpha}$ release correlates with broad populations of immune cells in PBMCs from patients with COVID-19.

In Biagini D. et al. (2023) [2] study, they propose a comprehensive profiling of oxylipins and PUFAs in plasma samples of COVID-19 patients infected with Wild-type, Alpha (B.1.1.7), Delta (B.1.617.2), and Omicron (B.1.1.529) variants. Hypotheses are formulated regarding inflammation regulation depending on the wave of infection. The results showed that oxidative stress and inflammation resulting from COVID-19 were highly dependent on the SARS-CoV-2 variant, and that the Wild-type elicited the strongest inflammatory storm. The Alpha and Delta variants induced a comparable lipid profile alteration upon infection, which differed significantly from Omicron. The latter variant increased the levels of pro-inflammatory mediators in infected patients [2].

We conducted the study during the Wild-type and Alpha (B.1.1.7) of SARS-CoV-2 strains epidemic. Thus, our study showed that these SARS-CoV-2 strains are characterized by a storm of eicosanoids PGI_2 and PGE_2.

Studying the spectrum of excretion of renal prostanoids can serve as a prognostic criterion for the development of end-stage kidney diseases and the effectiveness of treatment [5, 9]. As our studies have shown, the production of PGE_2 and PGI_2 by the kidneys is reduced (and quite significantly) in patients with kidney tumors with and without metastasis against the background of unchanged urinary excretion of TxB_2 and increased $PGF_{2\alpha}$. Such a decrease in the content of PGE_2 and PGI_2 in daily urine may indeed be

associated with activation of metabolism, which is consistent with experimental data on the increased activity of NAD-dependent I5-OH dehydrogenase in the cytosolic fraction of the renal cortex in a model of ureteral obstruction, and also with activation of 9-keto reductase, which catabolizes PGE_2 into PGF2α, which is consistent with the data obtained.

In this case, tumor damage to the kidneys can also lead to a decrease in the endogenous biosynthesis of renal prostanoids, firstly, due to a decrease in the substrate due to cell death by growing tumor cells and reactive sclerosis around them; secondly, due to a violation of the glomerular-canalicular outflow of the resulting prostanoids, as evidenced by their significant content in the tumor contents.

Normally, the biosynthesis of prostanoids in the prostate gland is maximum, exceeding that in the kidneys [7, 8], as our study showed.

Thus, endogenous prostanoids play the role of a tissue factor in the extracellular environment of tumor cells, which in association with the cytokine link creates a signaling pathway for maladaptation of the processes of proliferation and apoptosis [3] in tumor cells, promoting their growth and progression. At the same time, the role of prostanoids is individual for the tumor process of the kidneys, bladder and prostate. In kidney cancer, TxB_2 plays a leading role in oncogenesis, and PGE_2 plays a leading role in the pathogenesis of bladder and prostate tumors.

Conclusions

1. PGs can have both pro-inflammatory and anti-inflammatory effects depending on the inflammation scenario. In this study, we showed the role that PGs may play during SARS-CoV-2 infection and in the development and progression of kidney, bladder and prostate cancer.

2. Urinary excretion of PGI_2, PGE_2, TxB_2, $PGF_{2\alpha}$ in patients with COVID-19, as in patients with bladder cancer and prostate cancer is significantly increased. Whereas in patients with kidney cancer without and with metastases, the entire spectrum of prostanoids was significantly reduced.

3. The leading prostanoids in COVID-19 patients were prostacyclin PGI_2 and PGE_2; in patients with bladder cancer and prostate cancer – PGE_2, and in patients with kidney cancer without and with metastases – thromboxane B_2. Which indicates an imbalance of eicosanoids depending on inflammation and localization of oncogenesis.

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