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Evaluation of the activity of prostate cancer biomarkers in post-massage urine

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Prostate cancer (PC) – refers to important and serious problems in urology. In Ukraine, PC ranks fourth in the structure of malignant neoplasms and third in the structure of mortality of men over 60 years. An important issue in the systemic approach to improving the quality of health care to ensure life expectancy while maintaining activity in men is not only the search for new highly sensitive, specific and non-invasive diagnostic methods that will detect PC in the early stages of the disease, but also the need to review and justify new threshold values of already known biomarkers, and the ability to find their optimal combinations. Many studies have shown that the detection of sarcosine by high performance liquid chromatography in combination with electrochemical analysis of the element has made it possible to prove the limit of detection of sarcosine in the urine of cancer patients. Imperfect study and limited use in clinical practice of prognostic features of acid phosphatase (PCP), citrate, zinc, sarcosine, spermine and myoinositol as biomarkers in post-massage urine prompted this kind of study.
The objective: **to evaluate the activity of acid phosphatase, citrate, zinc, sarcosin, spermine and monoinositol in post-massage urine as biomarkers of prostate cancer.**
Materials and methods**. During the period 2016–2020, 246 men, aged 45 to 84, were examined on the basis of the Institute of Urology of the National Academy of Medical Sciences of Ukraine with complaints of urination disorders who were hospitalized or underwent a comprehensive examination. All patients received written consent to participate in the study, which was approved by the Ethics ComMission in the Institute of Urology of the National Academy of Medical Sciences of Ukraine.**
All PC patients underwent 12-point prostate biopsy under TRUS control using a G18 biopsy needle. During pathomorphological examination PC was classified according to Gleason's score. Patients were divided as follows: with verified PC (1st group), patients with benign prostatic hyperplasia (BPH) (2nd group) and relatively healthy (3rd group).
The content of PC biomarkers in post-massage urine was determined by known methods. Thus, acid phosphatase (EC 3.1.3.2) was analyzed by reaction with 1 mM para-nitrophenyl phosphate in 0.1 M flow buffer, pH 6.2; determination of citrate – ultraviolet; sarcosine – enzyme-linked colorimetric study and spermine – measurement of the amount of quinone-imine dye based on the formation of hydrogen peroxide; spermidine – by accumulation of l-pyrroline. All these methods were performed on a spectrophotometer «Specoll-211» (Germany). Zinc was determined by atomic absorption spectroscopy on an Analytik Jena ContrAA 300 spectrophotometer (Germany). Myoinositol was evaluated by the enzymatic cyclic method and measured by increasing the optical density of thio-NADH at 405 nm with an incubation of 37 °C.
Results**. Among the 246 patients studied after the examination, 107 (43.5%) had verified PC, 62 (58.0%) of which had a non-aggressive and 45 (42.0%) had aggressive form; 71 (28.9%) were diagnosed with BPH and 68 (27.6%) were found to be relatively healthy and included in the reference group. PAP data indicate that in almost healthy values of the indicator was the highest compared to those found in patients with BPH and PC, the lowest values were found in cases of PC, when its median was 1.5 IU/l. There is no evidence of a difference between the levels of PAP activity in patients with PC in the clinical stage T1-T2 and stages T3b. The use of PAP for the differential diagnosis of indolent and aggressive malignancies has not shown clinically significant results. In men without signs of prostate pathology, the concentration of citrate in post-massage urine, in contrast to patients with BPH, significant increased, and in patients with PC showed the opposite trend. Quantitative content of citrate in prostatic urine in the case of non-aggressive indolent tumors (Gleason index 5–6) significantly exceeded the value of the indicator in aggressive (Gleason 7–9 points) The concentration of zinc in prostatic urine in normal prostate and BPH almost did not differ. With increasing proliferation, especially under conditions of accelerated uncontrolled cell growth, apoptogenic function of zinc is first inhibited and then disappears at all, as a result of the comparison of zinc content in post-massage urine in patients with PC and in cases of pathology, its values, however, could not be established between patients with indolent and aggressive tumors. In terms of sarcosine content, patients without signs of prostate pathology and BPH did not differ statistically, while patients with PC in all parameters significantly exceeded the previous two groups. The median concentration of myoinositol in the functionally normal prostate was 35 mM, in patients with BPH there was a slight increase to 38 mM, and in PC the median value was 3 and 3.5 times smaller. In the absence of signs of prostate pathology, the concentration of spermine was 87 mM, in BPH the median increased to 114 mM, managed to identify significant differences. However, it was possible to trace the value of spermine content in determining the stage of the tumor process, namely between T1c – T2 and T3a.**
Conclusion**. Enzymatic activity of acid phosphatase allows to detect PC but makes it impossible to make a differential diagnosis of the nature of the cancer process. The level of zinc in post-massage urine did not show significant prognostic and differential diagnostic potential. Changes in the content of citrate, sarcosine and spermine in prostatic urine allow us to clearly predict not only the presence of PC, but also to make a differential diagnosis of cancer. Monoinositol showed only differential diagnostic potential in PC.**
Keywords: *prostate cancer, benign prostatic hyperplasia, biomarkers, urine, acid phosphatase, zinc, citrate, sarcosine, spermine, monoinositol.*

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Оцінка активності біомаркерів раку передміхурової залози в сечі після масажу

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Рак передміхурової залози (РПЗ) – важлива і серйозна проблема в урології. В Україні він посідає четверте місце у структурі злоякісних новоутворень і третє місце у структурі смертності чоловіків віком понад 60 років. Підвищення якості медичної допомоги, збільшення тривалості життя, збереження його якості у чоловіків із РПЗ потребують не лише пошуку нових високочутливих, специфічних та неінвазивних методів діагностики, які дозволять виявляти захворювання на ранніх стадіях, але і перегляду та обґрунтування нових порогових значень вже відомих біомаркерів, а також можливість знаходити їхні оптимальні комбінації. Результати багатьох досліджень продемонстрували, що виявлення зміни концентрації саркозину в сечі асоціюються з ризиком розвитку РПЗ. Недоліки вивчення та обмежене використання в клінічній практиці таких біомаркерів, як кисла фосфатаза (КФ), цитрат, цинк, саркозин, спермін та міоінозитол спонукають до проведення такого роду досліджень.

Мета дослідження: оцінювання активності кислої фосфатази, цитрату, цинку, саркозину, сперміну та моноінозитолу в сечі після масажу як біомаркерів РПЗ.

Матеріали та методи. За період 2016–2020 рр. на базі Інституту урології НАМН України обстежено 246 чоловіків віком від 45 до 84 років зі скаргами на порушення сечовипускання, які госпіталізовані або пройшли комплексне обстеження. Усі пацієнти надали письмову згоду на участь у дослідженні, яку схвалила Комісія з етики Інституту урології НАМН України. Усім пацієнтам з РПЗ була проведена 12-точкова біопсія передміхурової залози під контролем ТРУЗД за допомогою біопсійної голки G18. За результатами патоморфологічного дослідження РПЗ класифікували за шкалою Глісона. Пацієнти були розподілені таким чином: з верифікованим РПЗ (1-а група), пацієнти з доброякісною гіперплазією передміхурової залози (ДГПЗ) (2-а група) та відносно здорові (3-я група).

Вміст біомаркерів РПЗ у сечі після масажу простати визначали відомими методами. Кислу фосфатазу (ЕС 3.1.3.2) визначали за допомогою реакції з 1 мМ паранітрофенілфосфату в 0,1 М проточному буфері, pH 6,2; цитрат – ультрафіолет; саркозин – ферментно-колориметричним методом, спермін – вимірюванням кількості хініонімінового барвника на основі утворення перекису водню; спермідин – шляхом накопичення l-піроліну. Усі ці методи застосовували на спектрофотометрі «Specoll-211» (Німеччина). Цинк визначали методом атомно-абсорбційної спектроскопії на спектрофотометрі Analytik Jena ContrAA 300 (Німеччина). Міоінозитол оцінювали ферментативним циклічним методом і вимірювали шляхом збільшення оптичної щільності тіо-NADH при 405 нм при інкубації 37 °С.

Результати. З 246 обстежених пацієнтів у 107 (43,5%) верифікований РПЗ, у 62 (58,0%) з них – неагресивна, у 45 (42,0%) – агресивна форма. У 71 (28,9%) була діагностована ДГПЗ, а 68 (27,6%) пацієнтів визнані відносно здоровими та включені до контрольної групи. Вміст КФ у майже здорових був найвищим порівняно з хворими на ДГПЗ та РПЗ. Найнижчі значення були виявлені у випадках РПЗ, коли його медіана становила 1,5 МО/л. Групи пацієнтів з ПР у клінічній стадії Т1-Т2 та Т3b за концентрацією КФ достовірно не відрізнялися. Визначення КФ у підгрупах з неагресивним та агресивним РПЗ не виявило клінічно значущих розбіжностей. У чоловіків без ознак патології передміхурової залози концентрація цитрату в сечі після масажу, на відміну від пацієнтів з ДГПЗ, значно підвищилася, а у пацієнтів з РПЗ спостерігалась протилежна тенденція. Вміст цитрату в сечі після масажу передміхурової залози при неагресивному РПЗ (індекс Глісона 5–6) значно перевищував такий при агресивному (7–9 балів за Глісоном). Концентрація цинку в простатичній сечі при нормальній простаті та ДГПЗ майже не відрізнявся. Також за цим показником не відрізнялися підгрупи з неагресивною та агресивною формою РПЗ. За вмістом саркозину пацієнти без ознак патології передміхурової залози та ДГПЗ статистично не відрізнялися, тоді як пацієнти з РПЗ за цим показником достовірно перевищували дві попередні групи. Середня концентрація міоінозитолу у чоловіків з функціонально нормальною простатою становила 35 мМ, у пацієнтів з ДГПЗ спостерігалося незначне збільшення до 38 мМ, а при РПЗ медіана була у 3 та 3,5 разу меншою. За відсутності ознак патології передміхурової залози концентрація сперміну становила 87 мМ, при ДГПЗ медіана зросла до 114 мМ ($p<0,05$). Проте вдалося простежити значення вмісту сперміну при визначенні стадії пухлинного процесу, а саме між Т1с – Т2 та Т3а.

Заключення. Ферментативна активність кислої фосфатази дозволяє виявити РПЗ, але унеможливлює диференційну діагностику природи ракового процесу. Рівень цинку в сечі після масажу не виявив значного прогностичного та диференційно-діагностичного потенціалу. Зміни вмісту цитрату, саркозину та сперміну в простатичній сечі дозволяють чітко прогнозувати не тільки наявність РПЗ, але й проводити диференційну діагностику раку. Моноінозитол показав лише диференційно-діагностичний потенціал при РПЗ.

Ключові слова: *рак передміхурової залози, доброякісна гіперплазія передміхурової залози, біомаркери, сеча, кисла фосфатаза, цинк, цитрат, саркозин, спермін, спермідин, моноінозитол.*

Оценка активности биомаркеров рака предстательной железы в моче после массажа

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Рак предстательной железы (РПЖ) – важная и серьезная проблема в урологии. В Украине он занимает четвертое место в структуре злокачественных новообразований и третье место в структуре смертности мужчин старше 60 лет. Повышение качества медицинской помощи, увеличение продолжительности жизни, сохранение ее качества у мужчин с РПЖ предполагают не только поиск новых высокочувствительных, специфических и неинвазивных методов диагностики, которые позволят выявлять заболевания на ранних стадиях, но и пересмотр и обоснование новых пороговых значений уже известных биомаркеров, а также подбор их оптимальных комбинаций. Многие исследования показали, что обнаружение изменения концентрации саркозина в моче ассоциируется с риском развития РПЖ. Недостатки изучения и ограниченное использование в клинической практике таких биомаркеров, как кислая фосфатаза (КФ), цитрат, цинк, саркозин, спермин и миоинозитол в качестве биомаркеров побуждают к проведению такого рода исследований.

Цель исследования: оценить активность кислой фосфатазы, цитрата, цинка, саркозина, спермина и моноинозита в моче после массажа в качестве биомаркеров РПЖ.

Материалы и методы. За период 2016–2020 гг. на базе Института урологии НАМН Украины обследовано 246 мужчин в возрасте от 45 до 84 лет с жалобами на нарушение мочеиспускания, которые госпитализированы или прошли комплексное обследование. Все пациенты дали письменное согласие на участие в исследовании. Протокол исследования был одобрен Комиссией по этике Института урологии НАМН Украины.
Всем пациентам с РПЖ была проведена 12-точечная биопсия предстательной железы под контролем ТРУЗИ с помощью биопсийной иглы G18. По результатам гистологического исследования РПЖ классифицировали по шкале Глиссона. Пациенты были распределены следующим образом: с верифицированным РПЖ (1-я группа), пациенты с доброкачественной гиперплазией предстательной железы (ДГПЖ) (2-я группа) и относительно здоровые (3-я группа). Содержание биомаркеров РПЖ в моче после массажа простаты определяли известными методами. Кислую фосфатазу (ЕС 3.1.3.2) определяли посредством реакции с 1 мМ паранитрофенилфосфата в 0,1 М проточном буфере, pH 6,2; цитрат – ультрафиолет; саркозин – ферментно-колориметрическим методом, спермин – измерением количества хинониминового красителя на основе образования перекиси водорода; спермидин – путем накопления l-пирролина. Все эти методы проводились на спектрофотометре Specoll-211 (Германия). Цинк определяли методом атомно-абсорбционной спектроскопии на спектрофотометре Analytik Jena ContrAA 300 (Германия). Миоинозит оценивали ферментативным циклическим методом и измеряли путем увеличения оптической плотности тио-NADH при 405 нм при инкубации 37 °С.
Результаты. Из 246 обследованных пациентов у 107 (43,5%) верифицирован РПЖ, из них у 62 (58,0%) – неагрессивная, а у 45 (42,0%) – агрессивная форма. У 71 (28,9%) была диагностирована ДГПЖ, а 68 (27,6%) пациентов были признаны относительно здоровыми и включены в контрольную группу. Содержание КФ у почти здоровых было самым высоким по сравнению с больными ДГПЖ и РПЖ. Самые низкие значения были обнаружены в случаях РПЖ, когда его медиана составляла 1,5 МЕ/л. Группы пациентов с РПЖ в клинической стадии T1-T2 и T3b по концентрации КФ достоверно не отличались. Определение КФ в подгруппах с неагрессивным и агрессивным РПЖ не показало клинически значимых различий. У мужчин без признаков патологии предстательной железы концентрация цитрата в моче после массажа, в отличие от пациентов с ДГПЖ, значительно повысилась, а у пациентов с РПЖ наблюдалась противоположная тенденция. Содержание цитрата в моче после массажа предстательной железы при неагрессивном РПЖ (индекс Глисона 5–6) значительно превышал таковой при агрессивном (7–9 баллов по Глисону). Концентрация цинка в простатической моче при нормальной простате и ДГПЗ почти не отличалась. Также по этому показателю не отличались подгруппы с неагрессивной и агрессивной формой РПЖ. По содержанию саркозина пациенты без признаков патологии предстательной железы и ДГПЖ статистически не отличались, тогда как пациенты с РПЖ по этому показателю достоверно превышали две предыдущие группы. Средняя концентрация миоинозита у мужчин с функционально нормальной простатой составляла 35 мМ, у пациентов с ДГПЖ наблюдалось незначительное увеличение до 38 мМ, а при РПЖ медиана была в 3 и 3,5 раза меньше. При отсутствии признаков патологии предстательной железы концентрация спермина составляла 87 мМ, при ДГПЖ медиана выросла до 114 мМ ($p<0,05$). Однако удалось проследить значение содержания спермина при определении стадии опухолевого процесса, а именно между T1c – T2 и T3a.
Заключение. Ферментативная активность кислой фосфатазы позволяет выявить РПЖ, но делает невозможным дифференциальную диагностику природы ракового процесса. Уровень цинка в моче после массажа не выявил значительного прогностического и дифференциально-диагностического потенциала. Изменения содержания цитрата, саркозина и спермина в простатической моче позволяют четко прогнозировать не только наличие РПЖ, но и проводить дифференциальную диагностику рака. Моноинозитол показал только дифференциально-диагностический потенциал при РПЖ.
Ключевые слова: *рак предстательной железы, доброкачественная гиперплазия предстательной железы, биомаркеры, моча, кислая фосфатаза, цинк, цитрат, саркозин, спермин, спермидин, моноинозитол.*

Prostate cancer (PCa) – belongs to the solid tumors and ranks fourth in Ukraine in the structure of malignant neoplasms and third – in the mortality of men over 60 years [1, 2, 3].

A key issue in a systematic approach to improve the quality of medical care is not only the search for new highly sensitive, specific and non-invasive diagnostic methods that can detect PCa in the early stages of the disease, but also the need to review and justify new thresholds for known biomarkers and searching for optimal combinations.

One of the first biomarkers of PCa, which was determined in blood plasma, was the enzyme acid phosphatase (PAP), which is represented by a glycoprotein dimer. It was used to identify malignant tumors with metastases [12]. However, PAP has a very low sensitivity in the identification of local adenocarcinoma without metastases throughout the body. Therefore, this test was later replaced by a more reliable analysis based on prostate-specific antigen (PSA) [13-14].

In 1969, Cooper and Infeld demonstrated in their work that citrate can be used to diagnose PCa, because its concentration in normal tissue and under conditions of BPH is quite high (at least 100 mM), while in malignant neotransformation the content of citrate is reduced by several times. In 1979, Costello and co-authors confirmed this assumption by biochemical determination of citrate in semen and prostatic fluid and proved that citrate can serve as a diagnostic marker of adenocarcinoma [10, 15]. Subsequent studies have found that cancer patients with PCa have a reduction of citrate in seminal plasma and post-massage urine by almost 2.7 times compared with healthy men [12].

Measuring the concentration of ions of calcium, magnesium, potassium, sodium, chlorine, zinc and some other anions and cations in normal secretion of the prostate and under the conditions of adenocarcinoma. Costello L.S. (2009) noticed that a significant decrease of the concentration of citrate is also accompanied by a marked decrease of the concentration of potassium, calcium, magnesium, zinc and, conversely, the increase of the chlorine content in the secretion [11]. Deeper analysis showed that the drop in zinc concentration occurs in the case of adenocarcinoma and is in the range of 2–100 mcg/ml, while in healthy men, with BPH and prostatitis, the average zinc concentration is 500 mcg/ml of secretion. In view of the above, zinc has

been proposed as an additional biomarker for the determination of oncotransformation in the prostate.

Giskeodegard et al. in the study of tumor fragments removed during radical prostatectomy, demonstrated by high-sensitivity magnetic resonance that the increase in tumor aggressiveness as the corresponding increase in the Gleason index is accompanied, compared with indolent tumors, with significant decrease not only in citrate but also spermine and increase of [total spermine + creatine + polyamines / citrate] (UPC/C) with p = 2,17.10-4 [12].

Subsequently, citrate and spermine, as well as myoinositol, were proposed as components of the biomarker panel to predict the risk of prostate cancer. The main advantage of this series of biomarkers was its independence from the age of patients, as well as high specificity (up to 60%), combined with a sensitivity of 90% [13].

In many studies, it has been shown that the concentration of choline increases with the aggressiveness of tumors. Instead, more informative from this point of view was sarcosine, which is derived from choline and is N-methylglycine. Normally, sarcosine is dimethylated to glycine by an oxidative process involving dehydrofolate and FADN2-dependent sarcosine dehydrogenase. In the work of Sreekumar A. et al. (2009) was showed that the concentration of sarcosine increases significantly in the prostate under conditions of tumor metastasis [14]. However, measurement of sarcosine in the blood did not reveal significant differences between healthy patients and cancer patients [15]. Subsequently, it was also not possible to use sarcosine in its detection in urine to diagnose adenocarcinoma and predict its aggressiveness. At the same time, another study showed that when using liquid chromatography in combination with tandem mass spectrometry of the urine, it is still possible to observe significant differences in the amount of sarcosine between healthy men and cancer patients. Further improvement of the method of determination of sarcosine in urine by high-performance liquid chromatography in combination with electrochemical analysis of eluent allowed to increase the limit of determination of sarcosine to 110 nM, which was sufficient for accurate analysis of the determination of sarcosine in the urine of cancer patients.

The imperfection of the study methods and the limited use in clinical practice of prognostic features of acid phosphatase (PCP), citrate, zinc, sarcosine, spermine and myoinositol as biomarkers in post-massage urine prompted to make a new study.

The objective: to evaluation of the activity of acid phosphatase, citrate, zinc, sarcosin, spermine and monoinositol in post-massage urine (PMU) as biomarkers of prostate cancer and evaluation of their differential diagnostic potential.

MATERIALS AND METHODS

During the period from 2016 to 2020, 246 men, aged 45 to 84, with complaints of urination disorders, who were hospitalized or underwent a comprehensive examination, were examined on the basis of the Institute of Urology of the National Academy of Medical Sciences of Ukraine. From all patients written consent to participate in the study was recieved, which was approved by the Ethics ComMittee at the Institute of Urology of the National Academy of Medical Sciences of Ukraine.

The survey was conducted according to the following criteria:

– general analysis of blood and urine;
– biochemical analysis of blood;
– blood test for PSA (total, free, ratio);
– digital rectal examination;
– Ultrasound of the urinary tract;
– MRI with intravenous prostate augmentation (if necessary);
– transrectal multifocal prostate biopsy under TRUS control (if necessary);
– MSCT with intravenous amplification (if necessary);

Prostate biopsy was performed under TRUS control with 12-dots technique, using a G18 biopsy needle. Pathomorphological examination was performed according to Gleason's classification. Classification of malignant tumors by clinical stages was performed according to the TNM system (2002).

Upon completion of the evaluation of the results of examinations, including pathomorphological analysis of biopsy material, patients were divided as follows: with verified PCa (prostate cancer) (1st group), patients with benign prostatic hyperplasia (BPH) (2nd group) and relatively healthy (3rd group).

The content of PCa biomarkers in post-massage urine was determined by known methods. Thus, acid phosphatase (EC 3.1.3.2) was analyzed by reaction with 1mM of para-nitrophenyl phosphate in 0.1 M flow buffer, pH 6.2; determination of citrate – with ultraviolet; sarcosine – with enzyme-linked colorimetric study and spermine – with measurement of the amount of quinone-imine dye based on the formation of hydrogen peroxide; spermidine – by accumulation of 1-pyrroline. All these methods were performed on a spectrophotometer «Specoll-211» (Germany). Zinc was determined by atomic absorption spectroscopy on the spectrophotometer Analytik Jena ContrAA 300 (Germany). Myoinositol was evaluated by the enzymatic cyclic method and measured by the increasing of the optical density of thio-NADH at 405 nm with an incubation of 37°C. Detailed technology for each of the above studies has been published [11, 12, 13].

RESULTS AND DISCUSSION

Among 246 patients in the study after the examination, 107 (43.5%) had verified PCa, 62 (58.0%) of which had non-aggressive and 45 (42.0%) aggressive form; 71 (28.9%) were diagnosed with BPH and 68 (27.6%) were found to be relatively healthy and were included to the reference group. The latest data were significant in comparative analysis during determining of prognostic values. The main purpose of this group was aimed to prove the effectiveness of the traditional diagnostic complex for the detection of PCa, preferably in the early stages, the probability, according to its volume, to predict the aggressiveness and prevalence of cancer. Subsequently, the data of their examination, according to the methodology defined by the program, were used to assess the prognostic properties of post-massage urine.

The content of markers in post-massage urine was determined in the following volume: CF – 196 examinations (89 – in patients with PCa, 61 – with

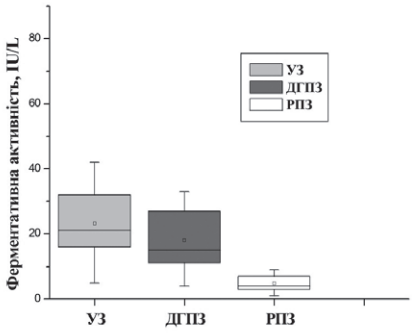

Fig. 1. The results of the study of acid phosphatase activity in the post-massage urine of patients with PCa, BPH and relatively healthy

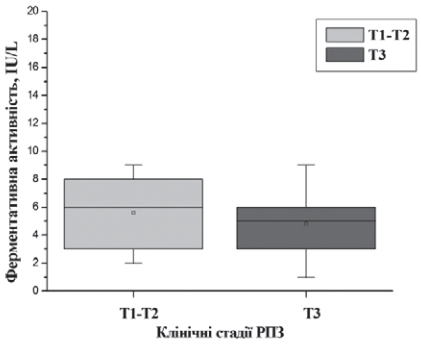

Fig. 2. Dependence of enzymatic activity of acid phosphatase in post-massage urine on the stage of carcinogen in patients with PCa

Note: */statistically significant differences between the two groups of patients by the selected criterion, $p<0.05$ are absent.

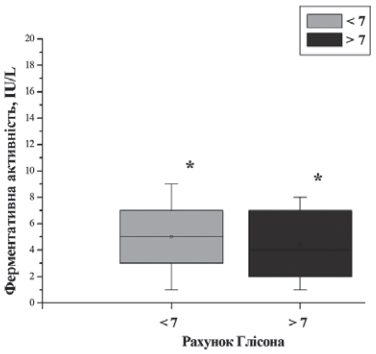

Fig. 3. Rates of acid phosphatase in post-massage urine in patients with indolent and aggressive malignancies

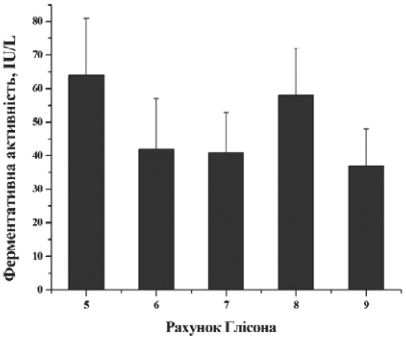

Fig. 4. Dependence of acid phosphatase activity in post-massage urine on the aggressiveness of tumors according to the Gleason index.

Note: */statistically significant differences with the PCa group (-), $p < 0.05$.

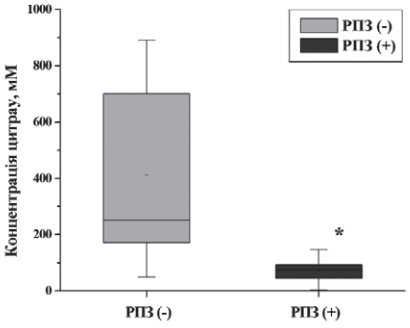

Fig. 5. Comparative data on the content of citrate in the post-massage urine of patients with PCa and in cases of its absence

Note: * / statistically significant differences between the two groups of patients by the selected criterion at $p < 0,05$.

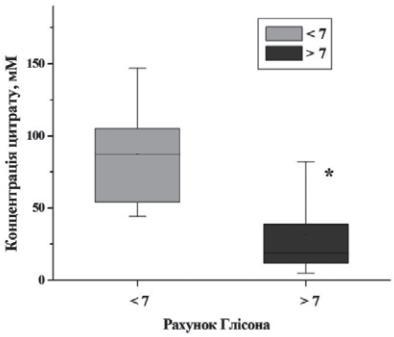

Fig. 6. Data on citrate content in post-massage urine of patients with indolent and aggressive malignant tumors

Note: * / statistically significant differences between groups of patients with PCA, $p<0.05$.

BPH, 46 – in relatively healthy patients); citrate – 204, including 87, 71, 46, respectively; zinc – 151, including 59, 53, 39, respectively; sarcosine – 159, including 65, 54, 40, respectively; spermine – 188, including 84, 61, 43, respectively; myoniositol – 184, including 75, 66, 43 respectively.

Prognostic signs of acid phosphatase (PAP) data are presented in Fig. 1 and show that in relatively healthy patients the value of the indicator was the highest compared to those found in patients with BPH and PCa. The normalized median value was 4 IU/l, ranging from 5 to 42 IU/l with an interquartile range of 16 to 32 IU/l. In patients with BPH, these parameters were: 2.1 IU/l, 4–33 IU/l and 11–7 IU/l, respectively. The lowest values were found in cases of PCa, when its median was 1.5 IU/l, the limit of the enzyme 3–9 in the interquartile range of 3–7 IU/l.

Dependence of marker value on clinical stages and aggression of the oncoprocess are presented in fig. 2. It was demonstrated the absence of differences between the levels of PAP activity in patients with PCa in the clinical stages T1-T2 and stages T3c. In cases of PCa of T1-T2 stages the median of this value was 6 IU/l, the interquartile range – 3-8 IU/l, whereas at T3 PCa – 5 IU/l, 3-6 IU/l, respectively; enzymatic activity of PAP varied in both cases almost to the same extent, namely 1-9 IU/l and 2-9 IU/l, respectively.

Application of PAP for differential diagnosis of indolent and aggressive malignancies did not show clinically significant, the results are shown in Fig. 3. These are results of the comparison of acid phosphatase activity in patients with Gleason index 6 and less and 7 and more points. In particular, for the Gleason index <7 median was 5 IU/l, interquartile range – 3-7 IU/l, a number of dynamic values for PAP activity was in the range of 1-9 IU/l. In patients with Gleason ≥7 the median was 4 IU/l, the interquartile range was 2 – 7 IU / l, and all values of PAP activity were in the range of 1-8 IU/l.

Analysis of the dependence of the enzymatic activity of acid phosphatase (AP) from Gleason scale of tumor showed that it was greatest at the levels 5 and 8 points, namely 64±17 IU/l and 58±14 IU/l, respectively, while the Gleason index of 6 and 7 points revealed its moderate

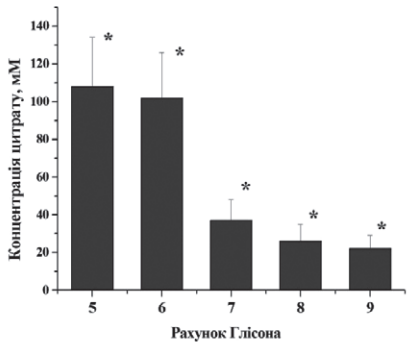

Fig. 7. Dependence of citrate content in post-massage urine on the aggressiveness of tumors according to the Gleason index

Note: * / statistically significant differences with the PCA group, p<0.05.

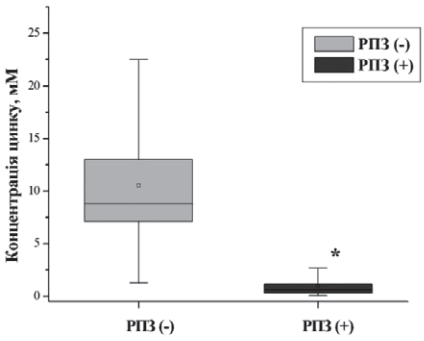

Fig. 8. Zinc content in post-massage urine of patients with PCa and patients without malignant neoplasms in prostate

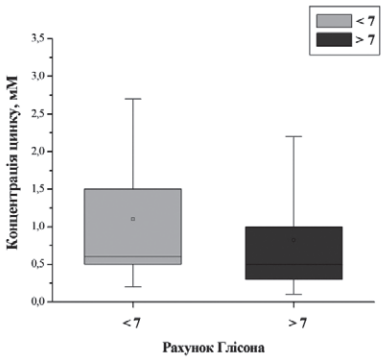

Fig. 9. Zinc content in post-massage urine of patients with indolent and aggressive malignant tumors

Note: * / groups have statistically significant differences at p<0.05.

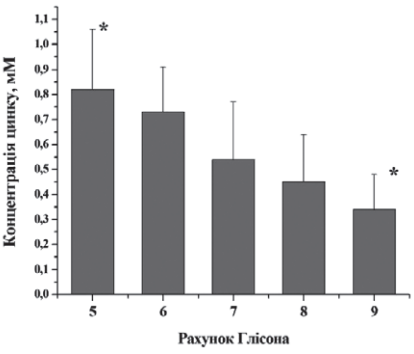

Fig. 10. Dynamics of zinc content in post-massage urine depending on the value of the Gleason index

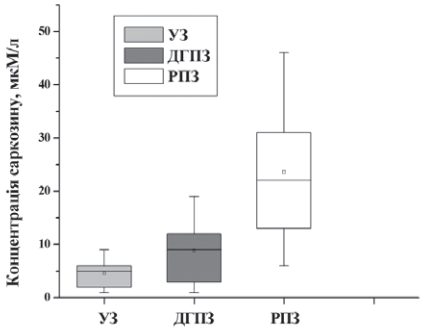

Fig. 11. The content of sarcosine in the post-massage urine of patients with PCa, BPH and relatively healthy men

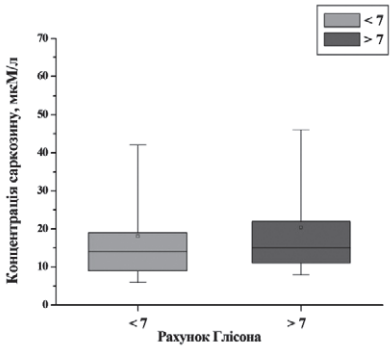

Fig. 12. The content of sarcosine in the post-massage urine of patients with indolent and aggressive malignant tumors

Note: * / statistically significant differences between groups, p<0.05.

activity – 41–42 IU/l (Fig. 4). When Gleason increased to 9, the activity of AP had the lowest value – 37±11 IU/l. The absence of any statistically significant differences between the five indicators makes it impossible to use this marker for the differential diagnosis of malignant tumors of the prostate by their aggressiveness.

In men without signs of prostate pathology, the concentration of citrate in post-massage urine was in the range of 5–370 mM with a median value of 283 mM and an interquartile range of 174–330 mM. In contrast, in patients with BPH there was a significant increase of the rate, which was manifested by an increase of the median to 720 mM, while practically the same interquartile range under conditions of shifting to higher concentrations: 650–810 mM; however, the lowest level was at least 170 mM, and the highest reached 890 mM. The opposite tendency was found in patients with PCa. The citrate content in prostatic urine decreased significantly: the median value was only 32 mM, the interquartile range was 18-48 mM, and the values did not exceed 72 mM and were not less than 5 mM (Fig. 5).

Quantitative content of citrate in prostatic urine in the case of non-aggressive indolent tumors (Gleason index 5–6) significantly exceeded the value of the indicator in aggressive (Gleason 7–9 points) (Fig. 6). It should be noted that the median value in aggressive tumors was 4,5 times less than the value in patients with non-aggressive, with the interquartile range in these cases did not exceed 27 mM and was between 12 mM and 39 mM. In contrast, the interquartile range of citrate levels in the latter was almost twice as large and was in the range of 54–105 mM. A detailed study of the dynamics of citrate content changes with increasing of Gleason index revealed a feature (Fig. 7), which manifested itself in a slight decrease of its value during the Gleason transition from 5 to 6 points (the difference between them was only 6 mM with the absolute values of 108 and 102 mM, respectively). Whereas in the cases with Gleason 7 points there was a different decrease in citrate content (2.8 times to 37 mM). The further decrease was less intense: up to 26 and 22 mM at 8 and 9 points of the Gleason index, by 30% and 15.4%, respectively, in their sequence. These data indicate the

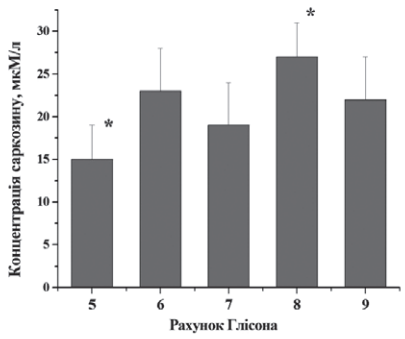

Fig.13. Dependence of sarcosine content in post-massage urine on the aggressiveness of tumors according to the Gleason index

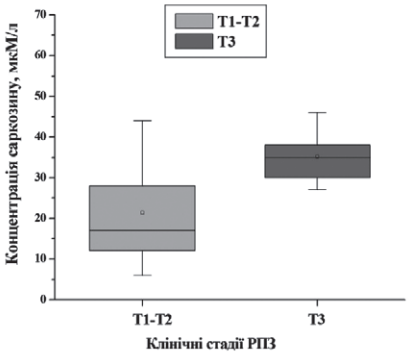

Fig. 14. Dependence of sarcosine content in post-massage urine on the cellular stage of the carcinogen in patients with PCa

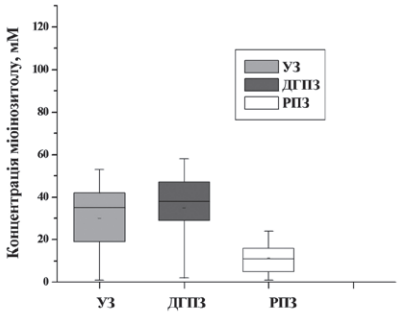

Fig. 15. The content of myoinositol in the post-massage urine of patients with PCa, BPH and men with normal glandular tissue

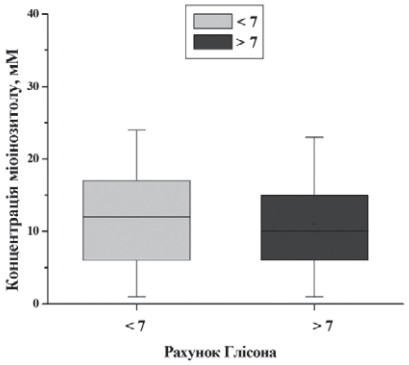

Fig. 16. The content of myoinositol in the post-massage urine of patients with indolent and aggressive malignant tumors

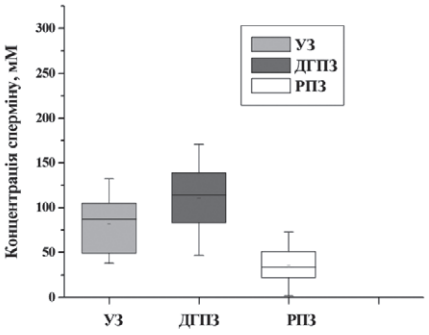

Fig. 17. The content of spermine in the post-massage urine of patients with PCa and in its absence, patients with BPH and relatively healthy men

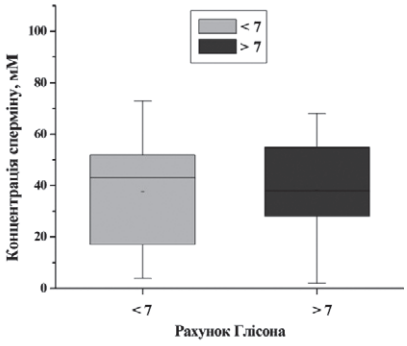

Fig. 18. Spermine content in post-massage urine in patients with indolent and aggressive malignancies

possibility of using citrate not only for the differential diagnosis of PCa, but also for determinating of the degree of aggression.

The concentration of zinc in prostatic urine with normal prostate and BPH almost did not differ. Thus, in the first case, the median was 8.7 mM, in the second – 9, 0 mM; the interquartile range was 6.2–13.6 mM and 7.4–12.5 mM, respectively, and the extreme values of the time series were in the range of 1.3–22.5 mM and 2.1 mM – 20.4 mM, respectively. While in patients with PCa the zinc content decreased sharply (10–20 times) to 0.6 mM by the median, the interquartile range decreased towards low concentration values and was equal to 0.3–1.2 mM, and their upper limit was almost at the level of the lowest in patients with BPH and only 2 times greater than this value in a normal gland. The differences can be explained by the ability of zinc in zinc-accumulating cells to perform apoptogenic function, which is associated with growth inhibition and high differentiation. Therefore, with increased proliferation, especially under conditions of accelerated uncontrolled cell growth, apoptogenic function of zinc at first is inhibited, and then disappears at all. Indeed, in the works of some authors it was shown that in cancer cells there are no protein transporters of zinc Zip 1 and Zip 2. In addition, a significant decrease of its concentration in the cell is observed at the stage of pre-malignancy, which precedes the oncotransformation.

As a result of the comparison of the indicators of the quantitative content of zinc in the post-massage urine in patients with PCa and without pathology, a significant difference between its values was revealed (Fig. 8). However, it could not be established between patients with indolent and aggressive tumors (Fig. 9).

Analysis of the dependence of zinc concentration on the value of the Gleason index showed that with an increase of the latter from 5 to 8 points there was a gradual decrease in zinc concentration, but there was no significant difference between the individual values; it took place only while comparing the extreme values: the Gleason index 5 and 9 (Fig. 10). Thus, the quantitative rate of zinc content in post-massage urine showed the ability to distinguish patients with PCa from patients with BPH and with normal prostate. However, its ability to differentiate indolent and aggressive tumors was imperfect, so it does not allow it's use to determine the likelihood of tumor aggressiveness.

The quantitative content of sarcosine in the post-massage urine of different groups of patients is presented in Fig. 11. In terms of sarcosine content, patients without signs of prostate pathology and with BPH did not differ

statistically, and in the latter it was slightly higher in median, as well as the lower and upper limits of the interquartile range. Whereas patients with PCa in all parameters significantly exceeded the previous two groups.

Thus, the ability of the value of the sarcosine content for its use as a laboratory diagnostic test in the differential diagnosis of PCa was confirmed. Instead, sarcosine was not sensitive enough biomarker to clearly distinguish patients with aggressive and indolent malignancies (Fig. 12). The analysis did not reveal a correlation between the concentration of sarcosine and the value of the Gleason index. However, significant differences were observed between cancer patients with PCa with an index of 5 and 6 and 72–8 points (Fig. 13). However, sarcosine has shown objectivity in determining the clinical stage of the tumor. With the help of this indicator it was possible to distinguish patients with PCa with cT1 – cT2 and cT3 stages (Fig. 14). This confirms the prospects for the use of sarcosine for the identification of extracapsular tumors of the prostate.

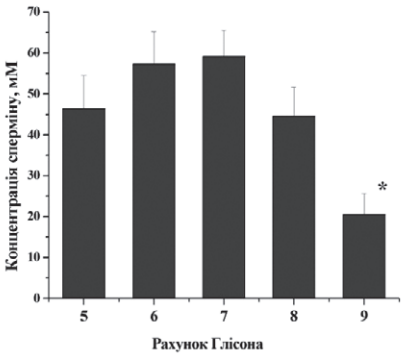

Fig.19. Dynamics of spermine content in post-massage urine of patients depending on the values of the Gleason index

Note: * / – statistically significant differences between groups at $p<0.05$.

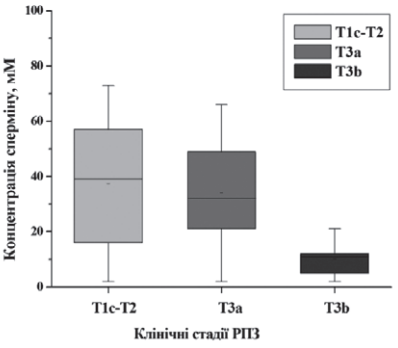

Fig.20. Dependence of spermine content in post-massage urine on the cellular stage of the carcinogen of patients with PCa

The concentration of myoinositol in the median in patients with functionally normal prostate was 35 mM with an interquartile range of 19–42 mM, with final values of its level 1 – 53 mM. In patients with BPH, there was a slight increase in the median concentration to 38 mM; the extreme limits were also larger (2–58 mM), and the interquartile range was larger – 29–47 mM. Significantly lower values of myoinositol were characteristic of patients with PCa. Thus, the median value was 3 and 3.5 times less than in men without signs of prostate pathology and in patients with BPH, respectively. In addition, the upper limit of changes in the indicator did not exceed 24 mM. There is a significant difference between the content of myoinositol in patients with PCa and BPH, as well as in men without prostatic pathology (Fig. 15). The results of the study of the dependence of myoinositol content in the post-massage urine in patients with PCa with tumors of silent (Gleason index <7) and aggressive type (Gleason index ≥ 7) are presented in Fig. 16. Statistically significant differences between indicators could not be established (with a median of 12 and 10, respectively).

In the absence of signs of prostate pathology, the concentration of spermine was 87 mM median, the interquartile range ranged from 49 mM to 105 mM, with the highest value reaching 132 mM, and the lowest – 38 mM, with BPH median increased to 114 mM, interquartile the range was wider 83 – 139 mM, and the final values of the indicator were in the range of 47–171 mM. In contrast, in patients with PCa the feature was manifested by a low average value of spermine concentration in the post-massage urine of patients, which was equal to 34 mM, a smaller intequartile range (22–51 mM) and much smaller extreme values of the time series: 2 – 73 mM (Fig. 17). No significant differences were found between indolent and aggressive tumors. This was confirmed by a comparative analysis between the values of spermine content in post-massage urine and the values of the Gleason index (Figs. 18 and 19). Thus, this biomarker can be used not only for laboratory and clinical diagnosis of malignant neoplastic transformation of the prostate, but also to determine the likelihood of delocalized processes that extend beyond the prostatic capsule (Fig. 20).

Thus, as a result of biochemical study of the content of postmassage secretion of patients with PCa of different aggressiveness, BPH and men with normally functioning prostate gland, it was found that the development of malignant neoplastic transformation causes synchronous decrease in enzymatic activity of acid phosphatase, zinc and citrate content. Instead, the concentration of sarcosine increases. The change in the concentration of these substances in the post-massage secretion was due, on the one hand, to the suppression of the secretory activity of the epithelial cells of the glandular epithelium of the prostate, as well as the appearance of cancer cells in the secretion. As it turned out, these substances, although they have biomarker properties, but their effectiveness for testing various signs of malignant neoplastic transformation of the prostate was different. Thus, with the help of some, such as citrate, it was possible to distinguish aggressive tumors from indolent, and with the help of others, such as sarcosine and spermine, to determine the presence of delocalized forms of prostate cancer. At the same time, biomarkers such as zinc and acid phosphatase were effective only to establish the very fact of malignant neoplastic transformation.

CONCLUSIONS

1. Enzymatic activity of acid phosphatase allows to detect PCaa but makes it impossible to make a differential diagnosis of the nature of the cancer process.

2. The level of zinc in post-massage urine did not show significant prognostic and differential diagnostic potential.

3. Changes in the content of citrate, sarcosine and spermine in prostatic urine allow us to clearly predict not only the presence of PCa, but also to make a differential diagnosis of cancer.

4. Monoinositol showed only differential diagnostic potential in PCa.

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REFERENCES

1. Bachurin GV, Bogun MU, Lomaka CC. Diagnostychna cinnist indeksu zdorovja proststy u rannjomu vyjavlenni raku peredmihurovoji zalozy. Urologia. 2019;4(91): 390-6.
2. Rak v Ukraini, buleten nacionalnogo cancer-reestru Ukrainy, 1989-2013. Kiev: Nacionalnyj Instytut raka MOZ Ukrainy.
3. Sajdakova NO, Starceva LM, Mejerytskij SM, Kravchenko OV, Kononova GE. Rak peredmihurovoji zalozy: medyko-demografichni osoblyvosti po administratyvnyh teritorijah Ukrainy. Zdorovje muzhchiny. 2014; 1: 21-3.
4. Golovachev SV, Nurgaliev NS, Kamarli ZP, Makimbetov EK. Sostojanie onkologicheskoj pomoshi I epidemiologija raka pedstatelnoj jelezy v Centralnoaziatskih respublikah. Onkourologia. 2016; 3(12):82-6.
5. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer. 2010; 127; 2893-2917.
6. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J. Clin. 2011; 61: 69-90.
7. Andriole GL, Crawford ED, Grubb RL, et al. Prostate cancer screening in the randomized prostate, lung, colorectal, and ovarian cancer screening trial: Mortality results after 13 years of follow-up. J. Natl. Cancer Inst. 2012; 104: 125-32.
8. Campbell G. General methodology I. Advanced in statistical methodology for the evaluation of diagnostic and laboratory tests. Statistics in Medicine. 1994; 13: 499-508.
9. Veliev EI, Tomilov AA, Goncharuk DA, Bogdanov AB, Golubcov EN. Diagnosticheskie vozmozhnosti PET/KT 68GA-PSMA dlja pacientiv s recidivom raka predstatelnoj zhelezy. Urologia. 2018;3: 105-10.
10. Golovko SV. Rol novitnih biomarkeriv tretynnogo pokaznyka Glisona ta comorbidnogo vyznachennja v pokrashenni vyznachennja gormonalno-nelikovanogo nemetastatychnogo raku peredmihurovoi zalozy. Zdorovje mujchiny. 2020;2(25):92-4.
11. Foti AG, Herschman H, Cooper JF. Comparison of human prostatic acid phosphatase by measurement of enzymatic activity and by radioimMunoassay. Clinical Cgemistry. 1977; 23(1): 95-9.
12. Kavanagh JP. Sodium, potassium, calcium, magnesium, zinc, citrate and chloride content of human prostatic and seminal fluid. J. Reprod. Fertil. 1985; 75: 35-41.
13. Şeker R, Fidancı V, Erol D, Yalbuzdag O, Saydam G, Senes M. A simple modified method for urine citrate determination. Turk J. Biochem. 2009; 34 (3): 173-7.
14. Ferlay J, Colombet M, Soerjomataram I, Mathers C. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8);1941-53.
15. Barron N, Keenan J, GamMel P, Martinez G Vanesa. Biochemical relapse following radical prostatectomy and miR-200a levels in prostate cancer. Prostate. 2012;72(11);1193-9.

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