Acta Biochimica Iranica 2(4): 221-226, 2024

Research Article

Comparison of the serum level of glycine N-methyl transferase (GNMT) enzyme in prostate cancer, benign prostatic hyperplasi and healthy subjects

Elham Esmaili[1], Fateme Khalaj[1], Hamid Shafee[2], Mohammad Ranaee[3], Sohrab Halalkhor[1*], Sadra Samavarchi Tehrani[4*]

[1] *Department of Biochemistry, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran*
[2] *Department of Urology, School of Medicine, Clinical Research Development Center, Shahid Beheshti Hospital, Babol University of Medical Sciences, Babol, Iran*
[3] *Department of Pathology, Rouhani Hospital, Babol University of Medical Science, Babol, Iran*
[4] *Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran*

Article info:
Received: 10 September 2024
Revised: 10 October 2024
Accepted: 15 October 2024

[*] **Corresponding Authors:**

Sohhrab Halalkhor; Department of Biochemistry, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
Email: Sohrabhalalkhor@yahoo.com

Sadra Samavarchi Tehrani; Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran
Email: Tehranisadra94@gmail.com

ABSTRACT

Objectives: Prostate cancer (PCa) is the most common malignancy in men and is associated with elevated levels of prostate-specific antigen (PSA). Recently, Glycine N-methyltransferase (GNMT) has been recognized for its critical role in sarcosine production. Evidence suggests that serum GNMT levels fluctuate in various malignancies, including hepatocellular carcinoma, colorectal cancer, and gastric cancer. The current study evaluates serum GNMT levels in PCa, benign prostatic hyperplasia (BPH), and healthy subjects.

Methods: Serum samples were obtained from 85 adult males (29 PCa patients, 28 BPH patients, and 28 healthy participants) referred to Shahid Beheshti Hospital, Babol, and Shahid Hasheminejad Hospital, Tehran.

Results: Our findings revealed that PSA levels were significantly higher in the PCa group compared to BPH patients and healthy individuals. Additionally, PCa patients exhibited higher GNMT levels compared to BPH patients and healthy controls, though this difference was not statistically significant. Serum GNMT levels were positively correlated with age in the PCa group. In the BPH group, GNMT levels were significantly correlated with PSA concentrations.

Conclusion: Serum GNMT levels appear to be elevated in PCa patients; however, further research with a larger sample size is necessary to validate these findings.

Keywords: Prostate Cancer, Glycine N-Methyltransferase, Benign Prostatic Hyperplasia

Use your device to scan and read the article online

Citation: Esmaili E, Khalaj F, Shafee H, Ranaee M, Halalkhor S, Samavarchi Tehrani S. Comparison of the serum level of glycine N-methyl transferase (GNMT) enzyme in prostate cancer, benign prostatic hyperplasi and healthy subjects. Acta Biochimica Iranica. 2024;2(4):221-226.

https://doi.org/10.18502/abi.v2i4.19110

Introduction

Prostate cancer (PCa) is the most common malignancy in men and the sixth leading cause of cancer-related deaths (1). Although PCa typically exhibits slow or mild progression in diagnosed patients, at the metastatic stage, it is considered the second deadliest cancer among men (2). Several risk factors contribute to PCa, including age, race, family history, high-fat diet, inflammatory disorders, and androgens (3). Various diagnostic strategies are employed to detect PCa, such as prostate biopsy, PSA (prostate-specific antigen) testing, digital rectal examination (DRE), magnetic resonance imaging (MRI), and health screening. However, these methods are not entirely satisfactory due to shortcomings, including the poor sensitivity and specificity of PSA testing (4). Early detection of PCa significantly improves treatment outcomes, which underscores the importance of identifying sensitive biomarkers in the early stages of the disease. Although extensive research has been conducted on gene and protein expression (5, 6), the metabolite changes associated with PCa progression remain unclear. Metabolites, as end products of molecular processes, are influenced by the transcriptome, proteome, and genome. One of the metabolites identified in PCa is sarcosine, which has been found to be significantly elevated in urine during PCa progression (7). Sarcosine is derived from the methylation of glycine, a reaction catalyzed by glycine N-methyltransferase (GNMT)—an enzyme highly expressed in the liver, exocrine pancreas, and prostate of mammals (8).

GNMT is a multifunctional enzyme that influences genetic stability by modulating the ratio of S-adenosyl methionine (SAM) to S-adenosyl homocysteine (SAH), binding to folate, and interacting with environmental carcinogens such as benzo(a)pyrene (9). Given the role of GNMT in sarcosine production, evaluating this enzyme is essential for controlling PCa invasion (10). In this context, Sreekumar et al. demonstrated that PCa invasion was reduced when GNMT expression was knocked down, highlighting a connection between GNMT activity and PCa progression (7). Furthermore, the critical role of GNMT in other cancers has been widely studied (11, 12). For instance, research has shown that GNMT levels are decreased in hepatocellular carcinoma (HCC) (13). Additionally, this enzyme protects liver cells from environmental toxins by binding to carcinogens such as aflatoxin (9). Therefore, in the current study, we aim to compare serum GNMT levels in PCa patients, benign prostatic hyperplasia (BPH) patients, and healthy individuals to further explore its potential role in PCa progression.

Methods

Study participants

Serum samples were collected from 29 men over 40 years old with previously diagnosed PCa, 28 BPH (benign prostatic hyperplasia) patients, and 28 healthy participants referred to Shahid Beheshti Hospital (Babol) and Shahid Hasheminejad Hospital (Tehran). Healthy individuals were those who attended their annual checkup, had normal serum PSA levels, and exhibited no abnormal clinical symptoms upon expert physician examination. Additionally, they showed no abnormalities in digital rectal examinations (DRE). BPH patients were referred to a physician due to associated symptoms, and their diagnosis was confirmed via serum PSA levels, DRE, transrectal ultrasonography (TRUS), and prostate tissue biopsy tests. All cancer patients, who had no metastatic disease, were diagnosed based on PSA levels (>4 ng/mL) and prostate tissue biopsy confirmation. These patients had not received any therapy, such as surgery, preoperative radiotherapy, or chemotherapy. There were no underlying medical conditions, such as thyroid diseases or diabetes, among participants in the three groups. Prior to blood sample collection, each participant provided written informed consent. This study was approved by the Ethics Committee of Babol University of Medical Sciences (IR.MUBABOL.HRI.REC.1398.251).

Serum collection

From each individual, a 5ml blood sample was taken, then the serum of the blood samples was immediately separated and stored at -20˚C until further analysis. Moreover, the demographic information on the participated subjects, including age, weight, height, smoking, history of the disease and clinic pathological features such as serum PSA level, TNM stage, and Gleason score received. The BMI of subjects calculated by following equations; BMI = Weight (kg)/ Height^2 (m^2).

Assessment of serum level of GNMT enzyme

ELISA technique (Zell Bio Company Lot number: ZB-15159C-H9648) was used to measure the level of GNMT enzyme in the serum of the subjects. Prior to the assay, all samples were gradually defrosted and mixed using a Vortex. Afterward, standards were diluted in accordance with the kit's protocol (with concentrations: 0 / 0.312 / 0.625 / 1.25 / 2.5 / 5 and 10 ng/ml). After preparing standard solutions, 50 µL of each standard were added to distinct wells. Then 40µL of serum sample, 10 µL of GNMT antibody and 50 µL of HRP (horseradish peroxidase) were added and incubated at 37 °C for 60 min. After incubation, the wells were washed 4 times to remove unbound enzymes. Then 100 µl of TMB substrate or chromogen was added to each well, and again the plates were incubated at 37 °C for 10

minutes. Next, the color of the solution turned to blue, and after adding the stop solution, it changed to yellow. Finally, the amount of the target enzyme (GNMT) was determined by reading the solution's optical density (OD) at 450 nm.

Statistical analysis

SPSS version 20.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA) were used for statistical analysis. All data are presented as mean ± SEM. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test.

Results

The purpose of this study was to compare serum levels of GNMT enzyme and PSA among PCa patients, BPH patients, and healthy individuals. To achieve this, GNMT enzyme and PSA levels were evaluated using an ELISA assay. The demographic information of the study participants is provided in Table 1. There were no significant differences in age or BMI among the three groups.

Comparison of serum levels of GNMT and PSA among PCa, BPH patients and healthy individuals

The results of the ELISA assay demonstrated that PSA levels were significantly higher in the PCa group compared to BPH patients and healthy individuals. In contrast, serum PSA levels did not show a significant difference between the BPH group and healthy subjects; however, BPH patients exhibited higher PSA levels compared to healthy individuals. Additionally, as shown in Figure 2, patients with PCa had higher GNMT levels than BPH patients and healthy controls, but this difference was not statistically significant.

Correlation between serum levels of GNMT enzyme with other parametrs in PCa, BPH and healthy subject

As presented in Table 2, serum GNMT levels were positively correlated with age in the PCa group. Furthermore, our findings demonstrated that, in the BPH group, GNMT levels were significantly correlated with PSA concentrations.

Discussion

Prostate cancer (PCa) is a highly prevalent malignancy with a significant mortality rate among men (14). Therefore, research in this field is essential for early diagnosis and improved treatment. Several studies have identified PSA as a key diagnostic biomarker for PCa (15, 16). Consistent with these findings, our study also demonstrated a significant elevation in PSA levels in PCa patients compared to control groups. However, the use of PSA alone as a diagnostic marker for PCa screening remains challenging (14), primarily due to its limitations, such as low sensitivity (17). Additionally, studies indicate that PSA levels do not provide accurate information regarding the aggressiveness of PCa (18). As a result of these shortcomings, further research into PCa diagnostics has been actively pursued (19, 20). Apart from PSA, the GNMT enzyme, which is highly expressed in the prostate, liver, and exocrine pancreas, plays an important role in PCa invasion (8, 10, 21).

GNMT is an enzyme that forms sarcosine and S-adenosyl-L-homocysteine by removing a methyl group from S-adenosyl-L-methionine and transferring it to glycine. As a result, it can be said that this enzyme clears methionine in mammals (22). A study showed that sarcosine, a byproduct of GNMT, induces the malignancy of bladder urothelial cancer (BUC). Additionally, the muscle-invasive bladder carcinoma (MIBC) and distant metastases of this disease may also be predicted by its urine concentration (23). Furthermore, a number of chronic liver disease manifestations, such as Non-alcoholic fatty liver disease (NAFLD), cholestasis, cirrhosis, and liver cancer, have been associated to the down regulation of GNMT (24, 25). Additionally, an investigation revealed that this enzyme promotes the beta-oxidation of fatty acids and that GNMT regulation contributes to the amelioration of fatty liver disease (26). Moreover, the importance of GNMT in PCa was

Table1. Comparison of demographic characteristics of the studied groups

Variables	PCa group n=29	BPH group n=28	Healthy group n=28	P-value
Age (Year)	67.3±8.5	67.6±9.7	66.3±8.1	0.23
BMI(kg/m^2)	25.65 ± 2.79	25.11 ± 3.13	25.32 ± 4.61	0.848

Table 2. Correlation between the serum level of GNMT enzyme and parameters in PCa, BPH and healthy subjects

Variables	Healthy group n=28	BPH group n=28	PCa group n=29
Age (Year)	0.71*	0.33	-0.09
BMI (Kg/m^2)	0.16	0.08	-0.41
PSA	0.09	0.67*	0.04

The data in the table are the Pearson correlation coefficient (r) between GNMT enzyme with age, BMI and PSA level. * P.value<0.05

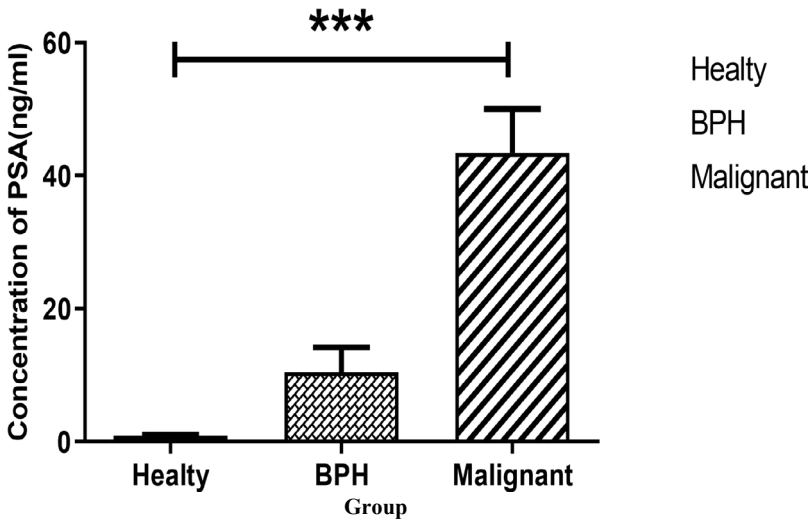

Figure 1. Comparison of PSA serum levels in the PCa, BPH and healthy subjects. *** P.value<0.001

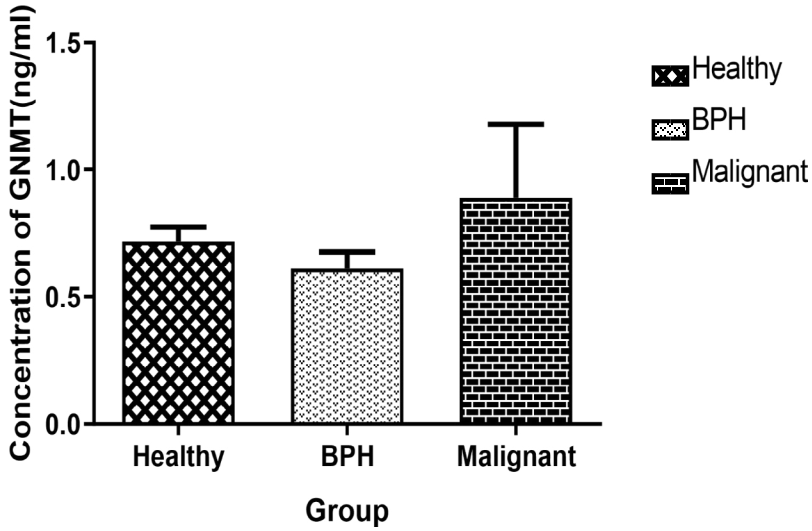

Figure 2. Comparison of serum concentration of GNMT in the group of PCa, BPH and healthy subjects

also investigated in various studies. In these studies, increased expression of GNMT was observed in PCa cells compared to normal prostate epithelial cells. It has also been reported that sarcosine, a product of GNMT, is a possible marker of aggressive PCa. And, this enzyme promotes a number of carcinogenic characteristics in PCa cells (7, 27).

The present study demonstrated that GNMT levels were higher in PCa patients compared to BPH patients and healthy individuals; however, this difference did not reach statistical significance. Consistent with a previous study showing increased GNMT expression in PCa patients, our findings also indicate elevated GNMT levels, although the increase was not statistically significant, potentially due to sample size limitations (28). Similarly, in vivo results support the positive role of GNMT enzyme in PCa invasion. While our study also reported elevated GNMT expression in PCa patients, the lack of statistical significance may be attributed to study limitations (29). Furthermore, in agreement with our findings, previous research indicated that sarcosine, a byproduct of GNMT, is not a reliable marker for PCa detection in urine sediments (30). However, the measurement of urine sarcosine and its associated metabolites has been shown to be useful in early PCa detection. In line with these findings, our study demonstrated GNMT elevation, Nonetheless, the lack of significance may be due to failure to assess enzyme activity (31, 32). Interestingly, plasma sarcosine levels, as measured by gas chromatography-mass spectrometry, were found to be higher in PCa patients than in BPH patients. Consistent with this result, our study observed higher GNMT enzyme levels—an enzyme responsible for sarcosine production—in PCa patients compared

to other groups, but this increase was not statistically significant (33).

Conclusion

In summary, our study demonstrated higher level of GNMT enzyme in PCa patients compared to healthy and BPH patients, however, it was not statistically significant. So, further studies are needed to clarify whether GNMT is significance in detection and better therapy of PCa.

Conflict of interests

The authors declare no conflict of interest.

Funding

There is no funding in this study.

References

1. Yousefi T, Mir SM, Asadi J, Tourani M, Karimian A, Maniati M, et al. In silico analysis of non-synonymous single nucleotide polymorphism in a human KLK-2 gene associated with prostate cancer. Meta Gene. 2019;21:100578. https://doi.org/10.1016/j.mgene.2019.100578
2. Chinigo G, Ruffinatti FA, Munaron L. The potential of TRP channels as new prognostic and therapeutic targets against prostate cancer progression. Biochim Biophys Acta Rev Cancer. 2024;1879(6):189226. https://doi.org/10.1016/j.bbcan.2024.189226
3. Huang YC, Lee CM, Chen M, Chung MY, Chang YH, Huang WJ, et al. Haplotypes, loss of heterozygosity, and expression levels of glycine N-methyltransferase in prostate cancer. Clin Cancer Res. 2007;13(5):1412-20. https://doi.org/10.1158/1078-0432.CCR-06-1551
4. Adhyam M, Gupta AK. A Review on the Clinical Utility of PSA in Cancer Prostate. Indian J Surg Oncol. 2012;3(2):120-9. https://doi.org/10.1007/s13193-012-0142-6
5. Junejo NN, AlKhateeb SS. BRCA2 gene mutation and prostate cancer risk. Comprehensive review and update. Saudi Med J. 2020;41(1):9-17. https://doi.org/10.15537/smj.2020.1.24759
6. Kumar S, Gurshaney S, Adagunodo Y, Gage E, Qadri S, Sharma M, et al. Hsp70 and gama-Semino protein as possible prognostic marker of prostate cancer. Front Biosci (Landmark Ed). 2018;23(11):1987-2000. https://doi.org/10.2741/4684
7. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009;457(7231):910-4. https://doi.org/10.1038/nature07762
8. Yeo EJ, Wagner C. Tissue distribution of glycine N-methyltransferase, a major folate-binding protein of liver. Proc Natl Acad Sci U S A. 1994;91(1):210-4. https://doi.org/10.1073/pnas.91.1.210
9. Yen CH, Hung JH, Ueng YF, Liu SP, Chen SY, Liu HH, et al. Glycine N-methyltransferase affects the metabolism of aflatoxin B1 and blocks its carcinogenic effect. Toxicol Appl Pharmacol. 2009;235(3):296-304. https://doi.org/10.1016/j.taap.2008.12.013
10. Ottaviani S, Brooke GN, O'Hanlon-Brown C, Waxman J, Ali S, Buluwela L. Characterisation of the androgen regulation of glycine N-methyltransferase in prostate cancer cells. J Mol Endocrinol. 2013;51(3):301-12. https://doi.org/10.1530/JME-13-0169
11. Heinzman Z, Schmidt C, Sliwinski MK, Goonesekere NCW. The Case for GNMT as a Biomarker and a Therapeutic Target in Pancreatic Cancer. Pharmaceuticals (Basel). 2021;14(3). https://doi.org/10.3390/ph14030209
12. Oh S, Jo S, Kim HS, Mai VH, Endaya B, Neuzil J, et al. Chemical Biopsy for GNMT as Noninvasive and Tumorigenesis-Relevant Diagnosis of Liver Cancer. Anal Chem. 2023;95(2):1184-92. https://doi.org/10.1021/acs.analchem.2c03944
13. Liu HH, Chen KH, Shih YP, Lui WY, Wong FH, Chen YM. Characterization of reduced expression of glycine N-methyltransferase in cancerous hepatic tissues using two newly developed monoclonal antibodies. J Biomed Sci. 2003;10(1):87-97. https://doi.org/10.1007/BF02256001
14. Senapati D, Sahoo SK, Nayak BS, Senapati S, Kundu GC, Bhattamisra SK. Targeting and engineering biomarkers for prostate cancer therapy. Mol Aspects Med. 2025;103:101359. https://doi.org/10.1016/j.mam.2025.101359
15. Van Poppel H, Albreht T, Basu P, Hogenhout R, Collen S, Roobol M. Serum PSA-based early detection of prostate cancer in Europe and globally: past, present and future. Nat Rev Urol. 2022;19(9):562-72. https://doi.org/10.1038/s41585-022-00638-6
16. Duffy MJ. Biomarkers for prostate cancer: prostate-specific antigen and beyond. Clin Chem Lab Med. 2020;58(3):326-39. https://doi.org/10.1515/cclm-2019-0693
17. Nevo A, Navaratnam A, Andrews P. Prostate cancer and the role of biomarkers. Abdom Radiol (NY). 2020;45(7):2120-32. https://doi.org/10.1007/s00261-019-02305-8
18. Tkac J, Gajdosova V, Hroncekova S, Bertok T, Hires M, Jane E, et al. Prostate-specific antigen glycoprofiling as diagnostic and prognostic biomarker of prostate cancer. Interface Focus. 2019;9(2):20180077. https://doi.org/10.1098/rsfs.2018.0077
19. Haldrup C, Pedersen AL, Ogaard N, Strand SH, Hoyer S, Borre M, et al. Biomarker potential of ST6GALNAC3 and ZNF660 promoter hypermethylation in prostate cancer tissue and liquid biopsies. Mol Oncol. 2018;12(4):545-60. https://doi.org/10.1002/1878-0261.12183
20. Ravi P, Wang V, Fichorova RN, McGregor B, Wei XX, Basaria S, et al. IGF-1 axis changes with ADT and docetaxel in metastatic prostate cancer. Endocr Relat Cancer. 2023;30(11). https://doi.org/10.1530/ERC-23-0241
21. Kerr SJ, Heady JE. Modulation of tRNA methyltransferase activity by competing enzyme systems. Adv Enzyme Regul. 1974;12:103-17. https://doi.org/10.1016/0065-2571(74)90009-0
22. Johnson AA, Cuellar TL. Glycine and aging: Evidence and mechanisms. Ageing Res Rev. 2023;87:101922. https://doi.org/10.1016/j.arr.2023.101922
23. Kishi S, Mori S, Fujiwara-Tani R, Ogata R, Sasaki R, Ikemoto A, et al. ERVK13-1/miR-873-5p/GNMT Axis Promotes Metastatic Potential in Human Bladder Cancer though Sarcosine Production. Int J Mol Sci. 2023;24(22). https://doi.org/10.3390/ijms242216367
24. Moylan CA, Pang H, Dellinger A, Suzuki A, Garrett ME, Guy CD, et al. Hepatic gene expression profiles differentiate presymptomatic patients with mild versus severe nonalcoholic fatty liver disease. Hepatology. 2014;59(2):471-82. https://doi.org/10.1002/hep.26661
25. Fernandez-Alvarez S, Gutierrez-de Juan V, Zubiete-Franco I, Barbier-Torres L, Lahoz A, Pares A, et al. TRAIL-producing NK cells contribute to liver injury and related fibrogenesis in the context of GNMT deficiency. Lab Invest. 2015;95(2):223-36. https://doi.org/10.1038/labinvest.2014.151
26. Fernandez-Tussy P, Fernandez-Ramos D, Lopitz-Otsoa F, Simon J, Barbier-Torres L, Gomez-Santos B, et al. miR-873-5p targets mitochondrial GNMT-Complex II interface contributing to non-alcoholic fatty liver disease. Mol Metab. 2019;29:40-54. https://doi.org/10.1016/j.molmet.2019.08.008
27. Song YH, Shiota M, Kuroiwa K, Naito S, Oda Y. The important

role of glycine N-methyltransferase in the carcinogenesis and progression of prostate cancer. Mod Pathol. 2011;24(9):1272-80. https://doi.org/10.1038/modpathol.2011.76
28. Miao L, Liu Y, Chen W, Gao C, Zhang Y, Wei J, et al. Brucine Suppresses Malignant Progression of Prostate Cancer by Decreasing Sarcosine Accumulation via Downregulation of GNMT in the Glycine/sarcosine Metabolic Pathway. Cell Biochem Biophys. 2024;82(3):2373-85. https://doi.org/10.1007/s12013-024-01348-z
29. Zabala-Letona A, Arruabarrena-Aristorena A, Fernandez-Ruiz S, Viera C, Carlevaris O, Ercilla A, et al. PI3K-regulated Glycine N-methyltransferase is required for the development of prostate cancer. Oncogenesis. 2022;11(1):10. https://doi.org/10.1038/s41389-022-00382-x
30. Bianchi F, Dugheri S, Musci M, Bonacchi A, Salvadori E, Arcangeli G, et al. Fully automated solid-phase microextraction-fast gas chromatography-mass spectrometry method using a new ionic liquid column for high-throughput analysis of sarcosine and N-ethylglycine in human urine and urinary sediments. Anal Chim Acta. 2011;707(1-2):197-203. https://doi.org/10.1016/j.aca.2011.09.015
31. Yamkamon V, Yee PP, Yainoi S, Eiamphungporn W, Suksrichavalit T. Simultaneous determination of sarcosine and its related metabolites by gas chromatography-tandem mass spectrometry for prostate cancer diagnosis. EXCLI J. 2018;17:965-79. https://doi.org/10.17179/excli2018-1352
32. Passornraprasit N, Hinestroza JP, Rodthongkum N, Potiyaraj P. Cellulose nanofibers/polyacrylic acid hydrogels integrated with a 3D printed strip: A platform for screening prostate cancer via sarcosine detection. Carbohydr Polym. 2025;352:123134. https://doi.org/10.1016/j.carbpol.2024.123134
33. Markin PA, Brito A, Moskaleva N, Fodor M, Lartsova EV, Shpot YV, et al. Plasma Sarcosine Measured by Gas Chromatography-Mass Spectrometry Distinguishes Prostatic Intraepithelial Neoplasia and Prostate Cancer from Benign Prostate Hyperplasia. Lab Med. 2020;51(6):566-73. https://doi.org/10.1093/labmed/lmaa008