® Postepy Hig Med Dosw (online), 2015; 69: 436-439
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2014.05.06
Accepted: 2015.02.17
Published: 2015.04.08

Serum β-glucuronidase as a potential colon cancer marker: a preliminary study

β-glukuronidaza surowicy krwi jako potencjalny marker raka jelita grubego – badanie wstępne

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Aim: Colorectal cancer is characterized by high morbidity and mortality in developed countries. The lack of low-cost, easy-to-use screening diagnostic methods is one of the causes of late diagnosis of colorectal cancer. Beta-glucuronidase (GLU) is a lysosomal exoglycosidase involved in degradation of glycosaminoglycans of the cell membranes and extracellular matrix of normal and cancerous colon tissues. The aim of our research was to evaluate the activity of GLU in the serum of colorectal cancer and estimate its potential value in the diagnosis of colorectal cancer.

Material/Methods: Blood samples were collected from 21 patients with colorectal adenocarcinoma and 17 healthy subjects. GLU activity was determined by the colorimetric method of Marciniak et al. by measuring the amount of p-nitrophenol released from 4-nitrophenyl-beta-D-glucuronide, at λ = 405 nm.

Results: We found significantly greater activity of GLU ($p<0.0001$) in the serum of patients with colorectal cancer, as compared to the healthy subjects. The serum GLU activity significantly differentiates patients with colorectal cancer from healthy individuals.

Conclusions: Serum GLU activity has diagnostic value and may be used in the diagnosis of colon adenocarcinoma.

Keywords: beta-glucuronidase • colon adenocarcinoma • serum

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1148704

Word count: 1139
Tables: 2
Figures: 4
References: 17

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Introduction

Colorectal cancer is a common neoplasm in highly developed countries, with the survival rate over 60% in the USA and under 40% in less developed countries [14].

In Poland, colorectal cancer occupies the fourth position among men and fifth among women in regard to the morbidity rate and the third position among all malignant neoplasms occurring in both men and women in regard to the mortality rate [15]. Colorectal cancer requires popularization of early diagnosis and wide-ranging preventive care [4].

Evaluation of the activity of lysosomal hydrolases in serum and urine may be helpful in the diagnosis of colorectal adenocarcinoma [3,8,9,11,13]. Beta-glucuronidase (GLU) is a lysosomal hydrolase. It catalyzes the hydrolysis of natural (mostly proteoglycans and glycosaminoglycans of the cell surface and extracellular matrix) and synthetic β-D-glucuronides into glucuronic acid and aglycone. GLU may also catalyze the transferring reactions of glucuronide to other acceptors, most frequently: phenols, alcohols and carboxylic acids. The creation of such conjugates is considered to be one of the detoxification methods [6].

Despite being a part of the detoxification process, GLU controls regulation and guarantees required concentration of the important endogenous and exogenous substances, including medications. It should be mentioned that the excess amounts of several simple endogenous and exogenous substances are removed from the human body as glucuronides, most frequently with urine. The increase of GLU activity reduces production of glucuronides by the combination of glucuronic acid with toxins, hormones, steroids, medicaments and carcinogens [17].

The aim of this study was to estimate the serum activity of GLU and evaluate its applicability in the diagnosis of colon adenocarcinoma.

Material and methods

the blood was taken from the cubital vein of 21 patients (13 women and 8 men) aged 39-81 years (average age 68 ± 11.26) with histopathologically diagnosed colon adenocarcinoma with the grade of cell maturity G2 (low or moderately differentiated, n=19) and G3 (low-differentiated or non-differentiated, n=2) and clinical grading pT1 (tumor infiltrates submucosal membrane, n=1), pT2 (tumor infiltrates mucosal layer, n=8), pT3 (tumor infiltrates through muscular layer to subserous layer or to pericolonic or perianal tissues not covered by peritoneum, n=9) and pT4 (tumor infiltrates by continuity surrounding tissues and organs or infiltrates visceral peritoneum; infiltration per continuum also concerns other regions of the colon occupied after infiltration of serous membrane (e.g. infiltration of sigmoid colon loop by rectal cancer, n=3), who did not undergo chemo- and radiotherapy and were treated at the 1st Department of General and Endocrine Surgery, Medical University of Bialystok. The control group consisted of 17 healthy persons (9 women and 8 men) aged 34-62 (average age 48 ± 9.06) who did not suffer from any conditions influencing the activity of GLU (Table 1).

Table 1. Characteristics of the examined persons

GROUP	N	SEX		AGE
		Women (N)	Men (N)	Years
Healthy persons	17	9	8	48 ± 9.06
Colon adenocarcinoma	21	13	8	68.11.26

Consent of the Bioethics Committee of the Medical University of Białystok no. RI-003/300/006 was obtained.

Blood, after coagulation, was centrifuged for 10 minutes at 4000 x g at 4°C. The supernatant (serum) was transferred to Eppendorf (safe-lock) tubes and frozen at -80°C.

GLU activity was determined by the method of Marciniak et al. [5] as follows: 10 µL of serum, 40 µL of 200 mM acetate buffer, pH 4.5, and 30 µL of 75 mM 4-nitrophenyl-β-D-glucuronide (Sigma, St. Louis, MO, USA) solution were applied into each well on a microplate. The micro-

plate was incubated at 37°C for 60 minutes. The reaction was terminated by addition of 200 µL of 0.2 M borate buffer, pH 9.8. The absorbance of released p-nitrophenol was measured at 405 nm using the microplate reader ELx 800 and the program KC junior (Bio-Tek Instruments, Winooski, VT, USA).

Statistical analysis

For the statistical analysis, SPSS 8.0 for Windows PL (SPSS, Chicago, Il, USA) was used. The differences between groups were evaluated using the Mann-Whitney U test. Statistical significance was assumed at $p<0.05$.

Results

The average activity of GLU in the serum of patients with colon adenocarcinoma (284.52 ± 88.35 pKat/mL) was significantly greater ($p<0.0001$) in the serum of the whole group of patients with colon adenocarcinoma in comparison to the healthy individuals (151.27 ± 61.53 pKat/mL) (Figure 1). Also detailed results concerning cell maturity (Figure 2) and clinical grading (Figure 3) were higher in the serum of patients with colon carcinoma than in healthy persons. It was proved that the Marciniak et al. assay [5] of GLU activity in serum allows differentiation of examined persons as healthy or unhealthy (AUC: 0.8956; p (AUC=0.5): 0.0000) (Table 2). Assay of the GLU activity in the serum is highly sensitive (80%) and specific (82.35%) for colon cancer, at the limit value of >208.10 pKat/mL (Figure 4).

Discussion

GLU is a lysosomal exoglycosidase whose activity usually increases in different catabolic (e.g. inflammatory) conditions [6,17]. In mild oxidative stress, some lysosomes fracture and release hydrolytic enzymes into the cytosol, which is accompanied by apoptosis and further release of the hydrolytic enzymes from the cells [17].

Human serum GLU derives from the tissues and enteric bacteria (*Escherichia coli*, *Peptostreptococcus*, *Bacteroides* and *Clostridia*) [17]. It was reported that the activity of bacte-

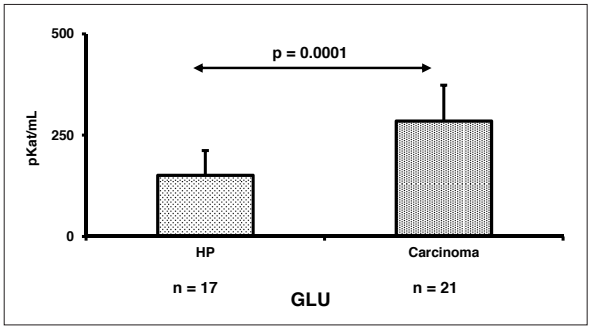

Fig. 1. GLU activity in serum of patients with colon adenocarcinoma as compared to healthy persons. HP - healthy persons, n - the amount of cases in the analysis

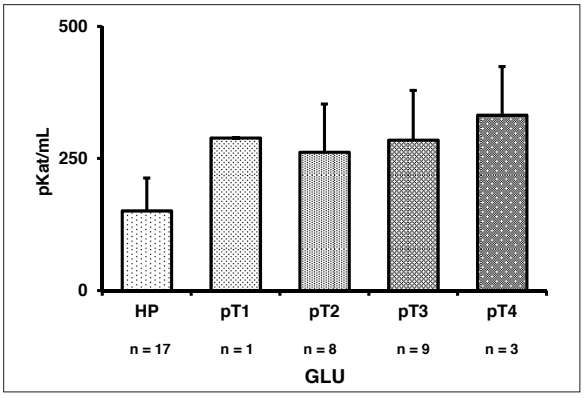

Fig. 2. GLU activity in serum of patients with colon adenocarcinoma as compared to clinical grading. HP - healthy persons, pT - clinical grading, n - the amount of cases in the analysis

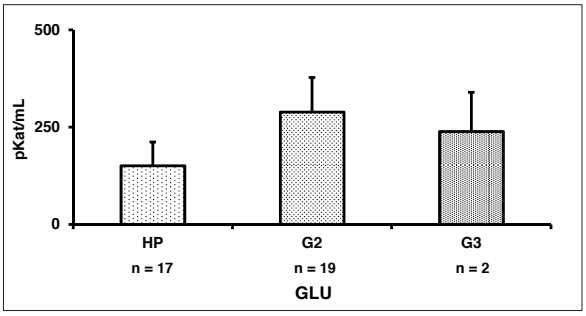

Fig. 3. GLU activity in serum of patients with colon adenocarcinoma as compared to grade of the cell maturity. HP - healthy persons, G - grade of the cell maturity, n - the amount of cases in the analysis

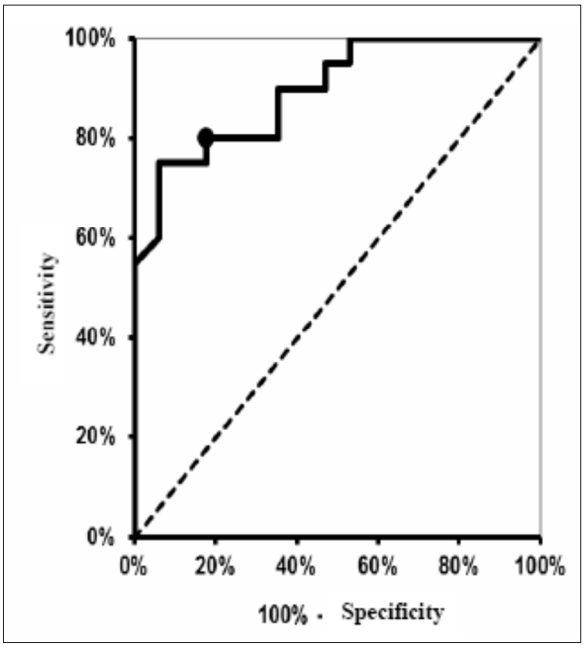

Fig. 4. Sensitivity and specificity of GLU activity in serum of patients with colon adenocarcinoma as compared to healthy individuals

Table 2. Diagnostic value of GLU activity assay in serum of patients with colon adenocarcinoma, as compared to healthy individuals

Serum [pKat/mL]	n	n(-)	n(+)	AUC	SE	95% C.I.(AUC)	p (AUC=0.5)
GLU	38	17	21	0.8956	0.0498	(0.798-0.993)	0.0000

n – the amount of cases in the analysis , **n(-)** – the amount of negative cases, **n(+)** – the amount of positive cases, **AUC** – Area Under the Curve ROC (Receiver Operating Characteristic), **SE** – standard error for estimated AUC, **95% C.I.(AUC)** – 95% confidence interval for AUC, **p(AUC=0.5)** – p value for the test analyzing diagnostics value of the method

rial GLU in intestines of patients eating large quantities of meat is significantly higher than in patients on a vegetarian diet [7,17]. In this study, a significant increase of GLU activity in the serum of patients with colon adenocarcinoma, in comparison to the control group, was proved (Figure 1). Also detailed results of GLU determination concerning cell maturity (Figure 2) and clinical grading (Figure 3) were higher in the serum of patients with colon carcinoma than in healthy persons. Determination of serum GLU activity has high sensitivity (80%) and specificity (82.35%) at the limit value >208.10 pKat/mL (Figure 4). Increased tissue activity of GLU has also been observed in central nervous system neoplasms [12,17]. GLU released to the urine by gall bladder tumors hydrolyzes the urinary glucuronides of aromatic amines and liberates active carcinogens, affecting tissues of the gall bladder [1,17]. In previous studies we found that the serum activity of another lysosomal enzyme, N-acetyl-β-D-hexosaminidase (HEX), and its isoenzymes A (HEX A) and B (HEX B), is a useful marker in differential diagnostics of thyroid and renal cancers as well as pancreatic adenocarcinoma [2,10,16]. We proved that the estimation of HEX, HEX A and HEX B activity concentration in the serum and urine, as well as the urinary activity calculated per 1 mg of creatinine, is diagnostically valuable in patients with colorectal cancer. We have observed that assay of specific activity of HEX and HEX A in urine is also highly valuable in the diagnosis of colorectal cancer [8]. Recent research suggests that the assay of GLU activity in the serum of patients with colon adenocarcinoma may also have significant diagnostic value (Figure 4, Table 2). However, our results should be confirmed on larger groups of subjects.

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The authors have no potential conflicts of interest to declare.