

Serum beta-glucuronidase in colon cancer patients dependent on alcohol and nicotine: preliminary report

Waszkiewicz N.¹, Zalewska-Szajda B.², Repka B.³, Szulc A.¹, Kępa A.⁴, Chojnowska S.⁵, Dadan J.⁶, Ładny JR.^{6,7}, Zwierz K.³, Szajda SD.⁷

¹ Department of Psychiatry, Medical University of Białystok, Poland

² Department of Imaging Diagnostics, Medical University of Białystok, Children Hospital, Poland

³ Medical College of the Universal Education Society, Łomża, Poland

⁴ Department of Biochemistry and Experimental Medicine, the Children's Memorial Health Institute, Warsaw, Poland

⁵ Medical Institute, College of Computer Science and Business Administration, Łomża, Poland

⁶ I Department of General Surgery and Endocrinology, Medical University of Białystok, Poland

⁷ Department of Emergency Medicine and Disasters, Medical University of Białystok, Poland

ABSTRACT

Introduction: Beta-glucuronidase (GLU) is a lysosomal exoglycosidase involved in the catabolism of glycoconjugates. Excessive GLU activity may be a primary factor in the etiology of colon cancer. The stimulation of glycosidases and other degradative enzyme activity has been noted in cancers as well as in alcohol and nicotine addiction.

Purpose: To compare the serum GLU activity between alcohol- and nicotine-dependent colon cancer patients and colon cancer patients without a history of alcohol- and nicotine-dependence.

Materials and methods: Material was the serum of 22 colon cancer patients, 11 of whom met alcohol and nicotine dependence criteria. The activity of serum GLU (pKat/ml) was determined by the colorimetric method. Carcinoembryonic antigen (CEA) concentration (ng/ml) in the serum was determined by the immunoenzymatic method. Comparisons between groups were made using the

Mann-Whitney "U" test. Spearman's rank correlation coefficient was used to measure the association between two variables.

Results: The activity of serum GLU was significantly higher in colon cancer patients with a history of alcohol and nicotine dependence, than in the colon cancer patients without a history of drinking and smoking ($p=0.003$). There was no significant difference in the CEA concentration between colon cancer patients with and without a history of drinking and smoking.

Conclusion: Alcohol and nicotine addiction may increase the serum GLU activity in all cancer patients, as already seen in colon cancer patients. This may potentially be important for the degradation of pericancerous matrix, tumor growth, invasion and metastasis.

Key words: Beta-glucuronidase, cancer, alcohol, nicotine

*Corresponding author:

Department of Psychiatry, Medical University of Białystok
16-070 Choroszcz, Poland

Tel.: /Fax: +48 85 7193977

Email: napoleonwas@yahoo.com (Napoleon Waszkiewicz)

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INTRODUCTION

More than 1 million individuals develop colorectal cancer in each year worldwide whereas the disease-specific mortality rate is nearly 33% in the developed world [1]. Risk factors of the colon cancer include increasing age, male sex, previous colonic polyps, previous colorectal cancer, as well as environmental factors such as red meat, high-fat diet, inadequate intake of fibre, obesity, sedentary lifestyle, diabetes mellitus, smoking, and high consumption of alcohol [1].

Earlier studies shown that increased activity of serum and urinary exoglycosidases may be a colon cancer markers [2,3]. A significant increase in the activity of β -hexosaminidase -HEX, α -fucosidase -FUC, β -galactosidase -GAL, and GLU was found in the serum of patients with colon cancer, in comparison to the healthy controls, which had a high diagnostic value [2-4]. However, in some studies, GLU appeared less sensitive and was significantly elevated only in patients with pancreas cancer but not in gastric, liver, or colorectal cancer [5]. Malignant tumors produce various hydrolases, including GLU, which degrade pericancerous matrix, favouring tumor growth, invasion and metastatic propagation [2, 3]. The proteolysis of matrix glycoproteins depends on the initial removal of the carbohydrate side chains. Generally, the stimulation of the activity of glycosidases and other degradative enzymes has been associated with the tumor development as well as with the alcohol drinking and smoking effects [2-8].

Beta-glucuronidase is a member of the lysosomal glycosidase family that catalyzes breakdown of complex carbohydrates (hydrolysis of β -D-glucuronic acid residues from the non-reducing end of mucopolysaccharides -also referred to as glycosaminoglycans- such as heparan sulfate [9]. The toxic and carcinogenic substances in the circulation are detoxified by the glucuronide formation in the liver and then enter the bowel via bile [10]. Generally, brush border GLU is an enzyme responsible for the hydrolysis of glucuronides in the lumen of the gut. Beta-glucuronidase is an inducible enzyme elaborated by anaerobic *E. coli*, *Peptostreptococcus*, *Bacteroides*, and *Clostridia*. By uncoupling glucuronides, beta-glucuronidase can deconjugate potential toxins, increasing the formation of carcinogens in the bowel and promoting the enterohepatic recirculation of toxins, hormones, and various drugs, in the body [11, 12]. Research correlated elevated levels of GLU with increased colon cancer risk, which suggested that excessive GLU activity may be a primary factor in the etiology of the colon cancer [10, 13]. Beta-glucuronidase may leak from the transduced tumor cells into the circulation. Leakage of the human GLU from the tumor cells

(where it is also produced) into the circulation has been demonstrated [14]. However, the release of GLU in the necrotic areas of the human tumor tissue biopsies has been also ascribed to the liberation by the activated monocytes/granulocytes [15].

Glucuronic acids are mainly incorporated into proteoglycans; therefore, the activity of GLU is a specific marker for the degradation of proteoglycans. Earlier studies found the increased activity of GLU in the plasma of alcohol-dependent patients after a heavy drinking period [16] and in the saliva of healthy persons after acute alcohol intoxication [17]. Generally, serum exoglycosidases such as GLU, HEX, FUC, and MAN, are increased in the serum of patients suffering from alcohol dependence, which seem to be due to the chronic liver damage [6-8, 16-19]. In persons addicted to alcohol, increased activity of lysosomal exoglycosidases was found also in the saliva [7]. The serum and urine levels of GLU were also found to be useful in the differentiation of patients depending on tobacco smoking [20]. In alveolar macrophages of smoking patients significantly more GLU was demonstrated, as compared to the controls [21]. Macrophages from smokers were reported to excrete twice as many enzymes and superoxides as cells from non-smokers [22]. It was also described a tendency to increase in the salivary GLU activity in smoking controls when compared to the non-smoking healthy subjects [2].

The aim of the study was to compare the serum GLU activity between alcohol- and nicotine-dependent colon cancer patients and colon cancer patients without a history of alcohol- and nicotine-dependence.

MATERIALS AND METHODS

The serum of 22 colon cancer patients (12 females and 10 males; mean age \pm SD 67 ± 11) was obtained in the Department of the General and Endocrinological Surgery of the Medical University of Białystok. All patients had histopathologically diagnosed colon adenocarcinoma. According to clinical-pathological classification of TNM and Duke's classification [23], tumors with cell differentiation G2 (n = 21) and G3 (n = 1) were found in patients operated on colon adenocarcinoma. Patients groups were selected in the extent of spread of colon adenocarcinoma: pT1 (n = 1), pT2 (n = 5), pT3 (n = 13) and pT4 (n = 3). In the macroscopic examination, groups were selected depending on the diameter of the resected tumor: 1-3.5 cm (n = 14), 4 cm (n = 4) and 5-7 cm (n = 4). Ten of colon cancer patients met the ICD-10 and DSM-IV criteria for the alcohol and nicotine dependence (three or more of the following manifestations should have occurred together for at least one month or if persisting for periods of less

than one month, then they have occurred together repeatedly within a twelve-month period: 1- a strong desire to take the drug, 2- difficulties in controlling its use, 3- persisting in its use despite harmful consequences, 4- a higher priority given to drug use than to other activities and obligations, 5- increased tolerance, 6- a physical withdrawal state). Colon cancer patients (dependent and not dependent on alcohol and smoking) had no any accompanying disorders or current treatment that might significantly influence the activity of serum GLU. The GLU activity in serum (pKat/ml) was assayed in duplicate by the colorimetric determination of p-nitrophenol released from p-nitrophenyl- β -D-glucopyranoside (Sigma, USA) by GLU [24]. The mixtures of enzyme and substrate was incubated for 60 min at 37°C. CEA concentration in the blood serum was determined by the immunoenzymatic method (MEIA) on Axym Analyzer of Abbot and expressed in ng/ml. The study was approved by the local Bioethical Committee of the Medical University of Białystok and was conducted in accordance with the Helsinki Declaration.

Statistical analysis

The comparisons between groups were made using the Mann-Whitney “U” test. Spearman’s rank correlation coefficient was used to measure the statistical association between two variables. Statistical analysis was performed with the Statistica 10.0. Statistical significance was assumed at $p < 0.05$.

RESULTS

Figure 1 shows, the activity of the serum GLU (pkat/ml) in colon cancer patients dependent on the alcohol and nicotine (337 ± 74 pkat/ml) was significantly higher than in the serum of the colon cancer patients without a history of drinking/smoking (219 ± 55 pkat/ml) ($p = 0.003^{**}$).

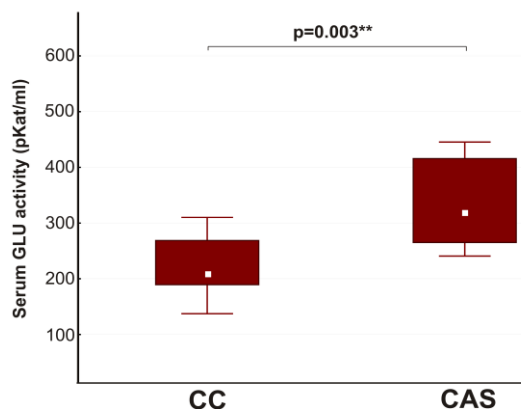


Figure 1. The activity of the serum β -glucuronidase (GLU) in the colon cancer patients without a history of alcohol drinking or smoking (CC), and in the colon cancer patients with alcohol and nicotine dependence (CAS).

The concentration of the serum CEA (ng/mL) in colon cancer patients with a history of drinking/smoking (9.4 ± 9.5 ng/mL) was not significantly different from the colon cancer patients without a history of drinking/smoking (8.2 ± 3.5 ng/mL) ($p = 0.743$) (Figure 2).

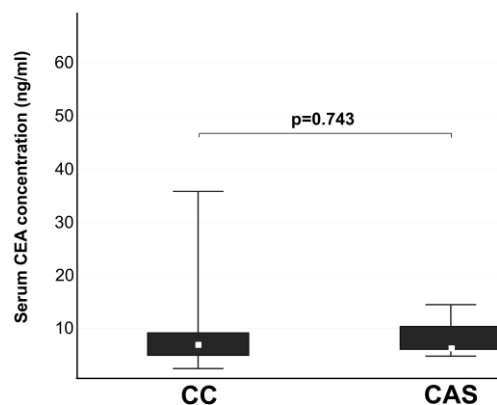


Figure 2. The concentration of the serum carcinoembryonic antigen (CEA) in the colon cancer patients without a history of alcohol drinking or smoking (CC), and in the colon cancer patients with alcohol and nicotine dependence (CAS).

There was no difference in age between colon cancer patients with and without a history of drinking/smoking ($p > 0.05$). There were no any significant differences in the cell differentiation -G, the extent of spread of colon adenocarcinoma -TNM, and in the diameter of the resected tumor (cm), between colon cancer patients with and without a history of drinking and smoking. There were no any correlations between the serum CEA concentration as well as the serum GLU activity and: stages of cell differentiation, the extent of spread of colon adenocarcinoma and the diameter of the resected tumor (cm).

DISCUSSION

It was found more than 7-fold higher risk for distal colorectal cancer in individuals who consume more than 20 g of ethanol a day and have subsequent low methionine and folate levels, as compared to occasional drinkers. Chronic inflammatory bowel disease, polyps, folate deficiency, alcohol dehydrogenase gene ADH1C*1 homozygosity and aldehyde dehydrogenase gene ALDH2*2 mutations (that increase acetaldehyde concentrations) are known risk factors for alcohol-associated colon carcinogenesis [25]. It is also known that alcohol and tobacco are the most abundantly consumed noxious compounds worldwide that act synergistically, resulting in an

increased cancer risk [26]. Even more than 80% of alcoholics smoke cigarettes [7]. Acetaldehyde found in alcoholics who smoke, comes from ingested ethanol and tobacco smoke. Acetaldehyde is known to be present in high concentration in the large intestine after alcohol consumption, corresponding to the blood level [25, 27]. Besides acetaldehyde, tobacco smoke is a source of oxidative stress and up to 3000 other toxic substances e.g. nicotine, nitrosamines, carbon monoxide, and other aldehydes, that may damage the tissues of the gastro-intestinal system. Moreover, reactive oxygen species (ROS) generated during drinking and smoking, as well as non-oxidative metabolites of ethanol (e.g., fatty acid ethyl esters -FAEEs) and the ethanol-water competition mechanism might be involved in the resulting damage of the alimentary tract tissues [7]. Acetaldehyde is responsible for the carcinogenesis, since it directly causes cellular injury and proliferation, decreases glutathione levels and ROS elimination, it is carcinogenic, mutagenic, binds to the DNA and proteins, destructs folate and results in secondary hyperregeneration [25,28]. Chronic alcohol consumption induces cytochrome P-450 2E1 in gastrointestinal mucosa cells and in the liver that results in the increased generation of ROS and activation of various dietary and environmental carcinogens such as those present in tobacco smoke and diet (polycyclic hydrocarbons, hydrazines and nitrozamines) [25]. Increased amounts of the lipid peroxidation products of ROS binding to DNA form highly mutagenic adducts [26]. Induced cytochrome P-450 2E1 decreases tissue levels of retinol and retinoic acid that have important functions in the regulation of the cell growth and differentiation. Nutritional deficiencies in alcoholics and disturbed methyl transfer, result in additional inadequate DNA synthesis and repair [25,26]. Although alcohol and smoking act synergistically, alcohol abuse has more than three times greater risk of developing cancer than smoking (data for oesophageal carcinoma) [26].

Here we have shown that patients suffering from a colon cancer addicted to nicotine and alcohol, have much higher GLU activity than colon cancer patients without addiction history ($p = 0.003$; Figure 1). We did not find any differences in the concentration of the serum CEA (Figure 2). However, CEA is not suitable for screening for early colorectal cancer, but is rather recommended for evaluation of prognosis and surveillance following curative resection and monitoring therapy in advanced disease [3]. It is widely known that the activity of glycosidases is higher in young tissue [23, 29]. Hence lysosomal exoglycosidases in tissues obtained during surgery procedures may be a helpful markers in distinguishing between benign/slowly-growing and malignant tumors, which is also crucial in the prognosis and strategy

of treatment of neoplastic diseases. The highest activities of exoglycosidases were observed in high-grade tumors, which correlated with the degree of malignancy, suggesting activity of exoglycosidases to be dependent on the phase of tumor development [2].

A significant increase in the activity of GLU, HEX, FUC, GAL in the serum of patients with colon cancer, when compared to the healthy controls, which had a high diagnostic value was reported [2-4]. Increased lysosomal exoglycosidases were also described in serum and urine of patients suffering from pancreatic cancer [3], in tissue of brain, lung, stomach, prostate, thyroid, cervical and breast cancer [29-32]. As glycoconjugates from the cell surface are involved in the important cellular and molecular processes such as cell adhesion, growth and proliferation, cell-cell interactions, division, differentiation, and signal transduction [31], glycohydrolases that act on glycoconjugates may be crucial in the cell transformation to the primary cancerous, localized tumor growth, and to the metastatic propagation. Malignancy process causes the increase in the cellular death, releasing lysosomal enzymes from the lysosomes, or causes also increase in the synthesis rate or disturb the packaging of the lysosomal enzymes in the lysosomes, thus increasing hydrolases activity in the body fluids [33]. The reported increase in the exoglycosidases in various body fluids in alcohol intoxication and smoking have been suggested to be due to the increased lysosomal membrane permeability/fragility and leakage of the enzymes to the cell, intercellular space, delayed removal of the enzymes from the fluid, impaired glycosylation and trafficking of lysosomal hydrolases to organelles, enhanced synthesis of the enzyme by activated leucocytes, or leakage from damaged cells [6-8, 34, 35].

As alcohol abuse has more than three times greater risk of developing cancer than smoking [26], much higher activity of GLU in smoking alcoholics suffering from colon cancer than colon cancer patients without a history of drinking/smoking, seems mostly to be due to the alcohol action. We have clearly shown that alcoholism, smoking, and colon cancer coincidence have significantly higher GLU activity than colon cancer patients without substance-dependence history. The lack of correlations between the serum GLU activity and stages of cell differentiation and of the extent of spread of colon adenocarcinoma may be due to the superimposed increase in the GLU activity induced by the action of alcohol and smoking together. As increased exoglycosidases are involved in the cancer propagation and metastases [2,3], alcohol drinking and smoking may potentially accelerate tumor growth, invasion and metastasis. Alcohol drinking and smoking metabolites such as

acetaldehyde and ROS are known factors of carcinogenesis. Therefore, additional decrease in the immunity while drinking and smoking superimposed on much higher activity of GLU, which is suggested to be a prime factor in the etiology of the colon cancer [9,13,34-37], might potentially favour tumor growth, invasion and metastases. As dietary modifiers, including the consumption of live bacteria (probiotics), polyphenols, lacto-vegetarian diet, high-fiber diet, are recognized modifiers of the numbers and types of microbes, reduced levels of beta-glucuronidase, and reduced colon cancer risk experimentally [38-40], their consumption while alcohol drinking and smoking might potentially reduce cancer risk/progression.

CONCLUSIONS

Chronic alcohol drinking and cigarette smoking, synergistically to the cancer process, increase the serum GLU activity. Further studies are needed to check if addiction to alcohol and nicotine, that additionally increase the serum GLU activity in colon cancer patients, can accelerate the degradation of pericancerous matrix by the cancer, tumor growth, invasion and metastasis.

Conflicts of interest

We declare that we have no conflicts of interest.

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