Thyroxine-Binding Globulin as an Indicator of Body Exposure to Unfavorable Environmental Factors

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Affinity chromatography and radioimmunoassay revealed enhanced nonspecific glycosylation of blood plasma proteins, in particular, thyroxine-binding globulin in oncopathies of different ethiology, during pregnancy, and in adolescents living under the conditions of high background radiation. This phenomenon can be of diagnostic and prognostic values.

Key Words: *thyroxine-binding globulin; protein glycosylation; molecular markers; euthyroiditis; affinity chromatography*

Thyroxine-binding globulin (TBG) is the major blood protein binding thyroid hormones 3,5,3'-L-triiodothyronine (T_3) and 3,5,3',5'-L-tetraiodothyronine (thyroxine, T_4). Increased blood content of TBG due to activation of its synthesis in the liver is a physiological response to excessive release of thyroid hormones. Changes in TBG plasma content observed under some extreme physiological (stress, elevated estrogens) or pathological (liver diseases, myeloma, carcinoma) conditions can result from posttranslational modification of its carbohydrate component. These changes in the plasma TBG are largely due to changes in the lifetime of glycosylated protein in the circulation. Additional glycosylation of TBG has practically no effect on the affinity of its binding center for thyroid hormones. For instance, TBG containing only triantennary N-acetylglucosamine oligosaccharide chains does not differ from standard TBG in hormone-binding properties and the majority of physicochemical parameters [1], but is characterized by 1.4 times longer lifetime [10]. Desialylated TBG with exposed galactose residues is characterized by a high clearance rate due to rapid binding to surface hepatocyte receptors which are capable of binding many asialoglycoproteins [9]. Glycosylated proteins enriched with fucose residues are used as markers for differential diagnosis of hepatoma and nonmalignant liver diseases (cirrhosis, hepatitis) with similar symptomes [5-7]. Fucosylated albumin was proposed as a marker for early diagnosis of stomach cancer and the preoperative assessment of tumor spread [11].

The aim of this study was to clarify the prognostic value of posttranslational glycosylation of proteins in the assessment of the risk of oncologic disease.

MATERIALS AND METHODS

Serum samples of healthy adolescents (12 girls and 16 boys) were obtained from the Clinical Institute of Radiation Medicine and Endocrinology. All subjects were born in 1986 and lived in the town of Khoiniki (^{137}Cs contamination level 185-555 kBq/m^2 according to the Republic Register). Blood samples of oncologic patients were taken at the Institute of Oncology and Medical Radiology (Minsk). First year students of A. D. Sakharov International Ecological University, Minsk residents comprised the control group.

The following media and reagents were used: concanavalin A-sepharose and lentil lectin-sepharose 4B (Pharmacia LKB Biotechnology AB), tris(hydroxymethyl)aminomethane (Serva), 1-O-methyl-α-D-glucopyranoside (Sigma), polyethylene glycol 6000 (Serva). ^{125}I-TBG (30-160 kBq), RIA-T3-ST and RIA-T4-

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TABLE 1. Serum Content of Thyroid Hormones in Clinically Healthy Adolescents *(M±m)*

Index	Control		Adolescents from Khoiniki	
	girls (n=10)	boys (n=10)	girls (n=12)	boys (n=16)
T_3, nmol/liter	1.70±0.09	1.63±0.12	2.71±0.56*	2.32±0.68
T_4, nmol/liter	89.31±1.60	83.23±1.53	153.7±19.82*	146.8±25.77*
TBG, μg/ml	18.96±0.71	15.04±0.37	26.46±1.76*	24.95±3.50*

Note. Here and in Tables 2 and 3; *p<0.05 in comparison with the control.

ST kits and rabbit polyclonal antibodies to TBG (abTBG) were obtained from the Institute of Bioorganic Chemistry, Belarus National Academy of Sciences.

Plasma content of T_3 and T_4 was determined using RIA-T3-ST and RIA-T4-ST radioimmunoassay kits. The total pool of TBG molecules was evaluated with a laboratory test-system based on competitive immunospecific binding of the antigen to ^{125}I-labelled abTBG. Standard solutions (0.02 ml) containing 0; 0.5, 1.5, 2.5, and 5.0 μg/ml TBG for calibration curve and individual plasma samples diluted 1:10 (0.02 ml) were mixed with 0.1 ml ^{125}I-TBG and 0.1 ml rabbit abTBG (1:1000) and incubated for 2 h at room temperature. The antigen-antibody complexes were precipitated with 1 ml 25% polyethylene glycol and centrifuged for 20 min at 2000g. Radioactivity of the sediment was measured on an LKB Wallac RIA Gamma 1274 scintillation counter. TBG concentration in individual samples was determined using the calibrating curve.

Plasma concentration of TBG-1 (TBG isoform with triantennary N-acetyl-lactosamine olygosaccharide chains) was determined according to [2].

The ratios between fucosylated and unfucosylated total blood proteins and TBG were determined by affinity chromatography on lentil lectin-sepharose 4B. Serum sample (0.2 ml) was applied to a microcolumn (0.2×3 cm) with the affinity sorbent and incubated for 15 min at room temperature. Two buffers were used for further treatment: buffer A, pH=7 (20 mM Tris-HCl buffer containing NaCl, 150 mM, Ca^{2+}, Mg^{2+}, and Mn^{2+}, 1 mM each) and buffer B composed of buffer A with 50 mM 1-O-methyl-α-D-glucopyranoside. Unbound plasma proteins were eluted with buffer A until the disappearance of optical density at 280 nM (2 fractions, 0.7 ml each were collected). Lentil lectin-reactive fraction was eluted with buffer B (4 fractions, 0.7 ml each were collected). The optical density at 280 nM was determined for each fraction. The TBG concentration in both reactive and nonreactive fractions was measured by competitive immunoassay. Rabbit abTBG and ^{125}I-TBG (0.1 ml each) were added to test tubes containing 20% (0.28 ml) nonreactive plasma fraction, 50% (1.4 ml) lentil lectin-reactive fraction, or standard TBG solutions (0, 0.5, 1.5, 2.5, and 5 μg/ml). The samples were incubated for 3 h at room temperature, 1.2 ml 25% polyethylene glycol were added, shaken, and centrifuged at 3000g for 20 min. The supernatant was discarded, and TBG concentration in the sediment was determined by counting radioactivity and referring the data to the calibrating curve.

RESULTS

The content of T_3 and T_4 in adolescents from the town of Khoiniki exceeded the normal (Table 1). In girls, serum content of T_3 and T_4 almost 2-fold surpassed the control. In boys, the mean content of T_3 did not exceeded the normal, but approximated its upper limit. The level of T_4 1.5-fold surpassed the normal.

Serum TBG in adolescents was significantly higher (p<0.05) than in the control group. Similar changes in TBG content in response to T_4 elevation were observed in euthyroidite syndrome and they were not accompanied by changes in the content of free hormones responsible for metabolic effects. The content of T_4-binding proteins, mainly TBG, increases during estrogen therapy and pregnancy, in innate TBG excess

TABLE 2. Serum Concentration of TBG-1 in Clinically Healthy Adolescents and Oncologic Patients *(M±m)*

Group	TBG concentration, μg/ml
Control	
boys	0.22±0.04
girls	0.27±0.02
Adolescents from the town of Khoiniki	
boys	0.25±0.03
girls	0.43±0.04*
Patients with cancer of	
stomach (n=15)	0.92±0.14
prostate (n=5)	2.17±0.10
breast (n=10)	1.18±0.07

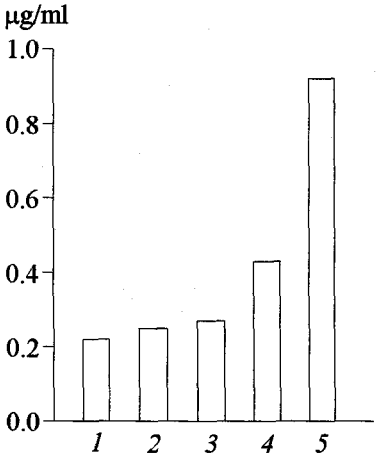

Fig. 1. Serum concentration of thyroxine-binding globulin in control group (*1,3*), children living under conditions of high background radiation (*2,4*), and patients with stomach cancer (*5*). *1,2*: boys; *3,4*: girls.

and liver diseases. Malignant neoplasms are also accompanied by activation of TBG biosynthesis. Euthyroid syndrome revealed in clinically healthy children can result from increased body demand for thyroid hormones during adaptation to specific environment. At the same time, these children can form a risk group for oncologic diseases not related to thyroid status.

Changes in plasma protein concentration under different physiological and pathological conditions can result from activation of selective biosynthesis of the molecular variants of both glycoproteins (cholinesterase [8], α-fetoprotein [14]) and nonglycoproteins (albumin [11]). These minor isoforms differ from standard proteins by the level of posttranslational glycosylation. Although the biological meaning of increased microheterogeneity of plasma proteins is not completely understood, quantitative assessment of their molecular variants in patients turned out to be helpful for early diagnosis [12]. In this connection, it was of interest to evaluate the proportion of minor glycoprotein variants (TBG-1) in the TBG pool of adolescents living in ecologically unfavorable environment.

Activation of TBG-1 biosynthesis is an adaptive response to some molecular factors (hormones, drugs) and some pathological conditions (cirrhosis, hepatitis, neoplasm) [3,4]. To assess the prognostic value of TBG-1 we compared plasma content of this molecular isoform in adolescents and oncologic patients (Fig. 1, Table 2).

In the examined children, the blood concentration of TBG-1 was much lower than during pregnancy (1.42±0.41 *vs.* 3.8±0.53 μg/ml [3]) and in patients with neoplasms. A higher level of TBG-1 in girls could result from enhanced adaptive response to unfavorable ecological conditions during pubertant period or from psychoemotional stress.

In patients with different tumors, the level of total protein fucosylation approximately 1.5-fold exceeded the control. At the same time, thyroid diseases are not accompanied by marked increase in the content of fucosylated proteins. Therefore, considerable activation of posttranslational fucosylation of serum proteins in the adolescents from the town of Khoiniki warrants special attention (Table 3).

The same tendency was noted for fucosylated TBG (Table 3). These findings confirm published data that activation of proliferative processes increases the content of minor serum proteins differing from standard proteins by the extent of fucosylation.

We found that the enhanced TBG fucosylation is typical of malignant neoplasms irrespective of their histogenesis. The selective stimulation of biosynthesis of fucosylated TBG at the stage of posttranslational modification can represent a response to increased TBG demands. From this point of view, the increased content of fucosylated TBG in adolescents living in unfavorable environment can represent a physiological adaptation. These changes can result from enhanced α-1-6-fucosyl transferase and N-acetylglucosamine transferase-5 activities. About 20% blood proteins can

TABLE 3. Serum Concentration of Nonfucosylated and Fucosylated Total Proteins and TBG in Different Pathological Conditions (*X*±*m*)

Group	Protein, rel. units			TBG, μg/ml		
	nonfucosylated	fucosylated		nonfucosylated	fucosylated	
		abs.	% of the total		abs.	% of the total
Control (*n*=10)	1.48±0.03	0.227±0.006	13.32±0.30	10.54±0.15	1.54±0.02	12.70±0.16
Adolescents from Khoiniki (*n*=10)	1.61±0.05*	0.341±0.004*	17.44±0.35*	11.65±0.65*	1.92±0.24*	14.14±1.15*
Patients						
hypothyroidism (*n*=8)	1.66±0.09*	0.267±0.030*	13.45±0.94	10.65±0.24	1.49±0.06	12.28±0.50
hyperthyroidism (*n*=8)	1.74±0.12*	0.290±0.025*	14.18±0.39*	10.76±0.16	1.58±0.04	12.80±0.35
sarcoma, melanoma (*n*=5)	1.77±0.16*	0.385±0.065*	17.88±0.54*	11.41±0.25*	2.35±0.23*	17.00±1.24*
breast cancer (*n*=5)	1.94±0.10*	0.442±0.008*	18.64±0.90*	10.90±0.13*	2.00±0.08*	15.48±0.54*

be fucosylated due to high activity of these enzymes, which prolongs the time of their circulation in the blood. It can be assumed that posttranslational glycosylation of blood proteins is a response to activation of biosynthetic and/or proliferative processes in the organism. This modulation does not depend on the nature of this process which can be triggered by changes in physiological conditions (pregnancy, increased level of estrogens) or some pathological changes. The peculiarities of posttranslational protein glycosylation in the norm and pathology can be used as markers in early diagnosis of pathological conditions and, especially, oncologic diseases.

Thus, enhanced nonspecific glycosylation of blood protein in adolescents living in ecologically unfavorable environment indicates considerable shifts in posttranslational glycosylation system rather than changes in hormonal mechanisms.

The increased glucosylation of both TBG and total plasma protein results primarily from natural activation of biosynthetic processes.

Selective activation of biosynthesis of TBG molecular isoforms at the stage of posttranslational modification allows to assign the examined adolescents to a risk group for oncologic diseases which necessitates careful diagnostic monitoring.

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