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KRYSTYNA OLCZYK[1], EUGENIUSZ J. KUCHARZ[2], ANDRZEJ GŁOWACKI[1]
PIOTR SONECKI[1] and EWA KOŚCIELNIAK-KOCUREK[1]

PROTEIN-BOUND GLYCOSAMINOGLYCANS IN SERUM OF PATIENTS WITH LUNG CANCER AND PATIENTS WITH DIABETES MELLITUS

[1]Department of Clinical Chemistry and Laboratory Diagnostics,
[2]Fourth Department of Internal Medicine, Silesian School of Medicine,
ul. Jagiellońska 4, 41-200 Sosnowiec, Poland

Glycosaminoglycans (GAG) are the important constituents of the connective tissue extracellular matrix and are suggested to be involved in a number of pathophysiological phenomena.

Cancer growth and spreading are closely related to penetration of the connective tissue matrix. Interaction between the cancer cells and the matrix is a complex phenomenon (for review see: [1, 2]). It is clear that local growth and development of metastases is preceeded by the neoplastic cell adhesion to and degradation of the matrix barriers (e.g. basement membranes, vascular wall).

The lungs are built up of a large amount of the connective tissue. The tissue appearing in the pulmonary system is composed of a variety of matrix forms, the loose connective tissue, vascular wall and cartilage of the bronchi [3]. Thus, the progress of the lung cancer seems to affect the local, and subsequently, the systemic metabolism of the connective tissue.

The majority of the already published reports has been focused on the collagen metabolism in patients or animals with cancer (for review see: [4]). The present study describes the preliminary data on the serum glycosaminoglycans pattern in patients with the lung cancer.

Diabetes mellitus is a metabolic disease characterized by hyperglycemia secondary to relative or absolute insulin deficiency. The progress in the treatment of diabetes mellitus resulted in the increased incidence of the complications, including the vascular changes. Macro- and microangiopathy is common in diabetic patients. The vascular wall is composed of the connective tissue components. Changes in GAG metabolism in patients with diabetes mellitus or in alloxan-induced diabetic animals have been reported in a few papers [5 - 8]. An increase in GAG level in tissues has been found. Ceriello *et al.* [9] described a decreased sulphated GAG/hyaluronic acid ratio in patients with diabetes mellitus.

The present study was designed to determine the protein-bound glycosaminoglycan pattern in serum of patients with the lung cancer and of patients with diabetes mellitus.

Twenty patients (18 male, 2 female) with planocellular lung cancer were investigated. The mean age of the patients was 58.3± 6.2 years. All patients had no detectable metastases and were before the application of pharmacological or X-ray treatment. Control values were obtained from 15 healthy, age- and sex-matched individuals. Fifteen patients with insulin dependent diabetes mellitus were investigated, as well. Compensation of the diabetes mellitus were measured with the fructosamine test. All patients were women, mean age 48.2 ± 5.7 years. Control values were obtained from 20 age-matched healthy women. Blood samples were taken at 7.00 a.m. after overnight fasting.

Serum protein-bound GAG were isolated and determined with the method of Antonopoulos and co-workers [10], as described by Seethenathan & Kurup [11]. In brief, serum samples were defatted and dried and subsequently suspended in phosphate buffer, pH, 6.5. Papain digestion was applied in order to separate carbohydrate moiety. Undigested proteins were eliminated with trichloroacetic acid treatment. Several dialyses and ethanol precipitations were used for purification of GAG mixture. Individual GAGs were separated chromatographically. The concentration of GAG were measured as uronic acid (assayed according to Bitter and Muir [12]) or galactose (assayed according to Carney [13]). Uronic acid and galactose values were converted to glycosaminoglycans by multiplying by the factors calculated from the polymer repeating units as described by Ghosh and co-workers [14].

A shift in glycosaminoglycan pattern in blood serum of patients with lung cancer was found. It was caused by a marked elevation of keratan sul-

Table 1

Protein-bound glycosaminoglycans in serum of patients with lung cancer and patients with diabetes mellitus (μg of disaccharide units/g of dry mass)

Values are expressed as mean ±S.D. Statistical significance ($\alpha = 0.05$) was estimated with Student's t test

	Total	Keratan sulphate	Hyaluronic acid	Heparan sulphate	Chondroitin-4-sulphate	Chondroitin-6-sulphate	Dermatan sulphate	Heparin
Controls	492.760	41.950	20.321	16.987	37.489	306.381	26.729	42.903
n = 15	±20.321	±8.310	±2.307	±2.143	±2.122	±20.143	±3.253	±3.130
Lung cancer	470.920	162.850	54.660	31.610	25.530	110.130	44.320	41.790
n = 20	±18.375 @	±23.400	±9.890	±4.020	±2.410	±20.140	±6.800	±4.510 @
Controls	539.141	52.960	28.730	13.580	24.350	340.750	35.420	43.351
n = 20	±12.823	±8.101	±6.310	±5.290	±6.320	±18.231	±7.114	±6.215
Diabetes mellitus	629.203	196.990	98.735	49.503	12.300	169.521	11.803	90.351
n = 15	±21.122	±39.854	±8.704	±6.407	±2.297	±50.101	±4.112	±8.214

@, statistically insignificant

phate as well as increase in hyaluronic acid, heparan sulphate and dermatan sulphate levels. Significant decrease in chondroitin-6-sulphate level was observed and a lesser one in chondroitin-4-sulphate. No changes in total GAG content were shown. On the other hand, an increase in total GAG serum level was found in diabetic patients. It was accompanied by an increase in keratan sulphate, hyaluronic acid, heparan sulphate and heparin concentration. A decrease in chondroitin-4/6-sulphates and dermatan sulphate level was observed. The ratio of sulphated GAG to hyaluronic acid was 4.457 ± 0.121 in diabetic patients as compared to 16.257 ± 2.322 in healthy controls.

The lung cancer and diabetes mellitus are disorders characterized by cellular proliferation and vascular degeneration, respectively. Connective tissue matrix is mainly involved in these diseases. Serum changes are believed to reflect tissue alterations.

The obtained results indicate that serum GAG pattern is altered in patients with the lung cancer. The mechanism of these phenomena is unclear and is probably related to several factors directly and indirectly caused by the growth and progression of the cancer. Such mechanisms as degradation of the pulmonary parenchyma, bone metastases (and also micrometastases) and systemic acute-phase response to the neoplastic growth can be considered to be the mechanisms changing the serum GAG pattern. Changes in serum GAG are consistent with an increased level of collagen metabolites in serum and urine of patients with the lung cancer [15].

An enhanced level of elastin-derived peptides in serum of those patients was also shown (Dróżdż *et al.* unpublished data). In the light of these alterations, it is clear that all components of the connective tissue are affected by the neoplastic growth.

The preliminary results indicate the need of further investigations on the relationship between the stage and cellular type of the cancer, therapeutical approaches and the amount of the neoplastic mass in the lungs and an abnormal serum GAG pattern. It is possible that determination of serum GAG pattern will be useful in estimation of spreading potential of the lung cancer.

Vascular changes are believed to be caused, at last partially, by the non-enzymatic glycosylation of collagen and other structural proteins [16]. The involvement of GAG into process of the matrix diabetic alterations remains unclear. It may be the secondary phenomenon to collagen structural changes or may resulted from hyperanabolic effect of hyperglycemia.

Systemic mechanism of the connective tissue regulation (e.g. hormonal factors, for review see: [17]) may be also involved in this process. The present data are concomitant with results of Ceriello *et al.* [9], who described similar changes in the GAG pattern. It is of interest to mention that similar shift in GAG was reported in vascular wall (mainly aorta) of diabetic rats by Malathy & Kurup [6]. It is possible that this alterations facilitates the development of atherosclerosis in diabetic patients.

The obtained preliminary results indicate for need of further studies on pathomechanism of the matrix involvement in diabetes mellitus and diagnostic value of serum GAG level.

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