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Alterations in physicochemical characteristics of blood plasma in men with prostate tumors

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Prostate cancer is one of the most common cancers in men worldwide. Therefore, identification of specific biomarkers for early dignosis are crucial prerequisites for efficient treatment of patients. We investigated alterations in the physicochemical characteristics of blood plasma proteins from men with prostate tumors, and its diagnostic significance with the identified alterations. Blood plasma of patients with benign hyperplasia of the prostate (BHP), BHP with high-grade prostatic intraepithelial neoplasia (HGPIN) regions and Cancer of Prostate (CaP) were used. Spectroscopic and SDS-PAGE methodologies were utilized for examining alterations in the physicochemical characteristics of blood plasma proteins. Relationship between alterations of the first-peak fluorescence intensities and prostate tumor progression was observed. Also, increased intensity of the second-peak corresponding to nicotinamide co-enzymes (NADH and NADPH) was noted in BHP-with-HGPIN and CaP specimens. The main peak maximum (Td) was observed at 66-67°C in BHP plasma and at 63-64°C in BHP-with-HGPIN regions and CaP. In BHP and BHP-with-HGPIN regions, an arm was noted at 70-71°C. The portions of thermostable proteins (acute phase proteins) were increased and modified proteins were formed in plasmas during the malignant transformation. Recorded fluorescence spectra allowed to differentiate prostate tumors and determine the disease progression. Differential scanning calorimetry markedly differentiated benign BHP-with-HGPIN regions and CaP, which shows the diagnostic importance of the method.

Keywords: Differential scanning calorimetry, Hyperplasia, Neoplasia, Plasma proteins

Cancer of prostate (CaP) is the most common cancer and one of the leading causes of death in men worldwide[1]. Prostate cancer incidence and mortality rates are strongly related to the age with the highest incidence being seen in elderly men (>65 years of age)[2]. However, recent statistics point to an increased incidence of the disease in men aged under 55 years[3,4]. In this group, presumably specific type of energy metabolism of prostate peripheral epithelial cells should also be taken into account. Prostate cancer is a disease model of great interest from a metabolic perspective as prostatic tissue exhibits unique metabolic activity under baseline conditions[5]. Notably, prostate cancer is a heterogeneous disease characterized by different clinical behaviour, from indolent to aggressive tumors with lethal progression. Therefore, early diagnostics and identification of prostate cancer aggressiveness are crucial prerequisites for efficient treatment of patients[6]. Early detection of prostate cancer using prostate-specific antigen (PSA) in blood reduces death among the unscreened men. However, due to the relatively modest specificity of PSA at commonly used cut-offs, there are urgent needs for additional and more specific biomarkers contributing enhanced risk classification among men with modestly elevated PSA[7].

Thus, early detection/diagnosis of the disease is not optimal yet, since complete and complex diagnostic tests, which can detect prostate tumors on the initial stage are not available yet. As a result, up to 60-80% of the CaP patients are diagnosed with progressed and metastatic cancer by the time of its clinical manifestations[8]. As noted, the development of newer

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diagnostic methods for detection and therapy of early and progressed CaP remains one of the major issues in modern urologic medicine. The clinical and fundamental research in prostate cancer is currently entering the proteomic and genomic era. The focus has switched from single marker (e.g., PSA) to panels of biomarkers (including heat shock proteins and blood plasma proteins). A number of comparative proteomic studies have been carried out to find specific diagnostic biomarkers able to distinguish prostate cancer from benign prostatic hyperplasia (BPH) as well as indolent from aggressive cancers. Novel genetic markers [such as transmembrane protease serine 2 (TMPRSS2)-ERG fusion gene mRNA] or prostate cancer gene 3 (PCA3) had already entered the clinical practice[6]. A new trend in serum/plasma biomarker search is to go beyond protein, in search of circulating tumor cells or circulating genetic material (DNA, miRNA). However, due to their abundance, proteins still hold the main focus on biomarker research[6].

Human blood plasma is a complex biological system, containing thousands of proteins and peptides, which have been isolated and studied using various physical and biochemical methods, including differential scanning calorimetry (DSC)[9], The study of blood plasma/serum of the patients with tumors showed strong differences in plasma/serum protein thermostability compared to the normal, as confirmed by investigators using DSC method[10].

Therefore in this study, we examined potential alterations in the physicochemical characteristics of blood plasma proteins in men with prostate tumors and also tried to establish the specificity and possible diagnostic significance of the detected changes.

Material and Methods

In order to study the above objective we used three methods viz. differential scanning calorimetry (DSC)[10], fluorescence spectroscopy[11], which allowed us to differentiate prostate tumors and discuss the degree of cancer progression; and gel electrophoresis[12] (for protein fragmentation in polyacrylamide gel), which allowed us to detect and analyze tumor specific proteins, and also to demonstrate basic alterations in the plasma proteins in blood of men with prostate tumors.

Samples

Blood plasma of the patients with benign hyperplasia of the prostate (BHP), the BHP with high-grade prostatic intraepithelial neoplasia (HGPIN) regions and Cancer of Prostate (CaP) served as materials for this study. Each study group consisted of 15 patients of ages 60-75 year. The control group consisted of 15 healthy men of the same age group. Patients were not receiving any type of medication/treatment during the sample collections for this investigation.

The Ethics Committee of Georgia approved the study and informed consent was obtained from each patient with BHP, the BHP-with-HGPIN regions, Cap, and the control groups. The blood specimens were obtained before the transurethral resection of the prostate (TURP) at the National Centre of Urology (Georgia). Clinical stage of the disease was diagnosed by the same Center by means of rectal, histological, and echographic examinations of the prostate gland. Histological analyses of the TURP-derived specimens were performed by the uropathologist at the Urology National Centre, Georgia.

Blood samples (10 mL each) were rinsed with 1.0 mL of heparin (5000 ME; 1 ME=0.0077 mg of heparin). The blood was centrifuged at 3000 rpm for 15 min to obtain the clear plasma.

Blood plasma fluorescence spectroscopy

Optical spectroscopic methods are widely used in cancer research today[13]. For example, fluorescence spectroscopy, which can be used in the evaluation of pre-cancerous and cancer processes *in vivo*, deserves special attention since it is non-invasive, quick and highly informative. Analysis of fluorescence spectra allows quantitative and qualitative evaluation of various substances as well as detection of their conformational changes. Application of fluorescence method may improve diagnosis and therapeutic approaches for many diseases[11,13].

For blood plasma fluorescent measurements, blood samples were taken before the surgery. A 0.5 mL plasma was diluted with 2.0 mL distilled water, and the diluted plasma sample was placed in a spectroscopic quartz minicuvettes. These cuvettes were free of absorption and emission in the spectral range of interest (300-600 nm). Fluorometric measurements were made using a Spectrofluoro-photometer (model MPF-3), using the excitation wavelength of 316 nm[13].

Differential scanning calorimetry (DSC)

The measurements were performed using a DSC with sensitivity of 0.1 μW[10]. Volumes of the measuring vessels varied from 0.03 to 0.1 cm^3; the

chosen heating rate was 0.75°C per min, and the temperature range of measurements was from 40 to 100°C. The accuracy of the temperature measurements was ≤0.05°C. The error in the determination of the melting enthalpy (ΔH_d) and heat capacity dQ/dT (ΔC_d) did not exceed 10%. The microcalorimetery data processor was equipped with the software needed for determination of the melting thermodynamic parameters of blood plasma specimens, and the calorimetric curves were thus plotted and deconvoluted with Origin 9.0.

Gel electrophoresis

Electrophoresis was carried out according to the method of Laemmli[12,14], using 10-25% polyacrylamide gel (2 mm) and 0.1% sodium dodecyl sulphate (SDS), with an SE-200 (Hoefer Scientific Instruments) over a period of 3.5 h with 2 mA electrical current. High molecular weight standard proteins were used as markers for electrophoresis: thyroglobulin (330 kD), catalase (60 kD), lactate dehydrogenase (36 kD), and ferritin (18.5 kD). The gels were stained with 0.2% Coomassie Brilliant Blue G-250 Dye[14].

Results

Fluorescence studies of blood plasma

First, we investigated the physical and chemical alterations occurring in the blood of patients with BHP, the BHP-with-HGPIN$_{4-5}$ regions and CaP. This includes the assessments of alterations in the first- and second-fluorescence peak intensities corresponding to dysproteinemia and the reduced redox co-enzymes (NADH and NADPH) in plasma samples using the spectro-fluorometric method to establish the specificity of alterations occurring in plasma, and also to determine whether any metabolic alterations specific to prostate tumors could be reflected in the composition of blood plasma.

Fluorescence spectra of plasma in the ultraviolet and visible regions (340-470 nm) showed a clear difference between the control group and patients with prostate tumors. Two peaks of fluorescence characteristics to plasma were observed in the control group (Fig. 1) as well as in men with prostate tumors (Fig. 2). However, in relation to prostate tumors, the intensity of the first peak increased in the following order: control group (Fig. 2A) → BHP (Fig. 2B) → BHP-with-HGPIN regions (Fig. 2C) → CaP (Fig. 2D). The intensity of the first peak was found to be highest in case of CaP (Fig. 2D). As for the second-peak, its intensity was sharply decreased in plasma of BHP patients as compared to the control group (Fig. 2A). In case of BHP-with-HGPIN regions, the second peak clearly expressed increased intensity (Fig. 2C). In plasma of CaP patients, the second peak's fluorescence had increased (Fig. 2D), but shifted in the direction of the short wave length (~440-460 nm) and plateaued.

Differential scanning calorimetry studies

We utilized the DSC method to study the thermodynamic parameters of the plasma proteins (albumins, 'acute phase' proteins) in patients with prostate tumors. In particular, temperature corresponding to the maximum of the main peak on the denaturation curve (Td), existence of the peaks and arms, and denaturation heat (Q) were examined. Based on the results, we attempted to evaluate the specificity of the detected alterations and possible diagnostic significance of the differential scanning microcalorimetry to determine

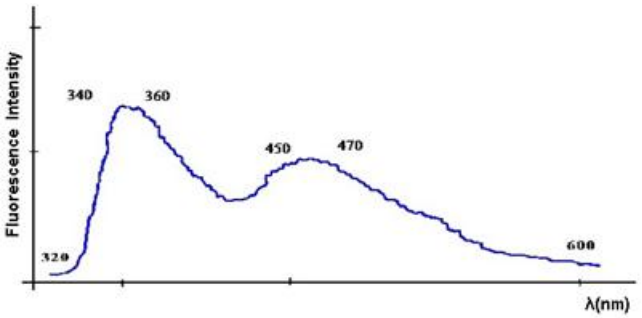

Fig. 1 — Fluorescence spectrum of the blood plasma of the control group[13]

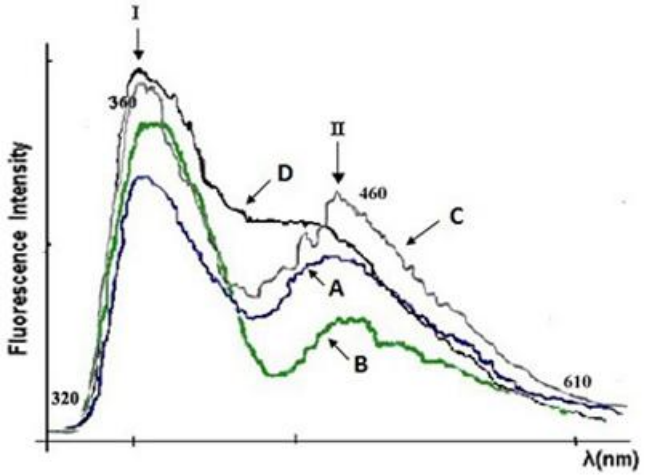

Fig. 2 — Fluorescence spectra of the blood plasma of the men with prostate tumors. (A) Control group; (B) Benign hyperplasia of prostate (BHP); (C) BHP with high-grade prostatic intraepithelial neoplasia (HGPIN) regions; and (D) Cancer of prostate (CaP)

the degree of prostate tumor progression. We found that the temperature corresponding to the plasma main peak maximum was 60-61°C on the calorimetric curve of the control group (Table 1; Fig. 3A). The Q or change of enthalpy (ΔH) was determined to be 18±0.5 J in the given case (Table 1).

On the calorimetric curve of BHP patients, temperature of the main peak (T_d) was shifted in the direction of high temperature range (66-87°C), with a small arm in the range of 70-71°C (Fig. 3B; Table 1). The denaturation heat of plasma albumins (Q) was also studied in BHP patients, which did not change (19±0.5 J) compared with the control group (Table 1).

In the patients with BHP-with-HGPIN regions, altered temperature of the main peak maximum (T_d) on the calorimetric curve of blood plasma was noted (the observed value was higher than the control group but lower than the BHP group; 63-64°C). Interestingly, an additional arm, was absent in the control and BHP groups, which was detected in the range of 58-59°C of patients with BHP-with-HGPIN regions, as well as the arm in the range of 70-71°C (Fig. 3, Table 1). The Q value in patients with BHP-with-HGPIN regions was 20 ±0.5 J, which changed slightly compared to the same index in BHP patients, and was more distinctly expressed as compared to the control group (Table 1).

Table 1 — Thermodynamic parameters of blood plasma proteins in men with prostate tumors

Objects	Shoulder (°C)	Peak (°C)	Peak Maxim (°C) (dT)	Q (J) (ΔH)
Control group	-	-	60-61	18±0.5
BHP	70-71	-	66-67	19±0.5
BHP with HGPIN	58-59; 70-71	-	63-64	20±0.5
Prostate cancer (CaP)	-	58-59	63-64	24±0.5

[BHP, Benign hyperplasia of prostate; HGPIN, High-grade prostatic intraepithelial neoplasia; dT, temperature change; ΔH, melting enthalpy; Q (J), heat (Joule). Accuracy of temperature measurements was ≤0.05°C]

On the calorimetric curve of the plasma of patients with CaP, the temperature interval (corresponding to the main peak maximum) was 63-64°C (Fig.3D), similar to that of patients with BHP-with-HGPIN regions. However, this arm was not found in temperature interval of 70-71°C. On the calorimetric curve of patients' plasma with CaP (Fig. 3D), instead of the arm in temperature interval 58-59°C, a visible peak was detected (Table 1). The Q value in this case was 24±0.5 J, which was increased as compared to all other experimental groups.

SDS-PAGE studies

We investigated the qualitative and quantitative alterations in protein fractions in blood plasma of men with prostate tumors utilising gel-electrophoresis (SDS-PAGE). The following protein fractions were observed on the electropherogram (Fig. 4) of control group, BHP, the BHP-with-HGPIN and CaP patients: 220, 135, 98, 88, 68-70, 53-62, 46, 41, 38, 32, 27, 25 and 14 kD.

We found an increase of 220 kD protein fraction in all experimental groups as compared to the control group; however, the difference was relatively smaller in case of CaP. The amount of 135 and 88 kD proteins increased in parallel with the progression of the disease (control group → BHP → BHP-with-HGPIN → CaP) (Fig. 4). While the 98 kD fraction was not found in the control or BHP and CaP groups, but it was revealed in the BHP-with-HGPIN regions. The analysis also showed an increase of 88 kD fraction with the progression of the disease as compared to the control group. The levels of 68 kD fraction were increased in the following order: control group → CaP → BHP → BHP-with-HGPIN regions. The sharpest increase of the given fraction (i.e., 68 kD) was found in case of BHP-with-HGPIN. The fraction of the 63-53 kD mass proteins was the largest among

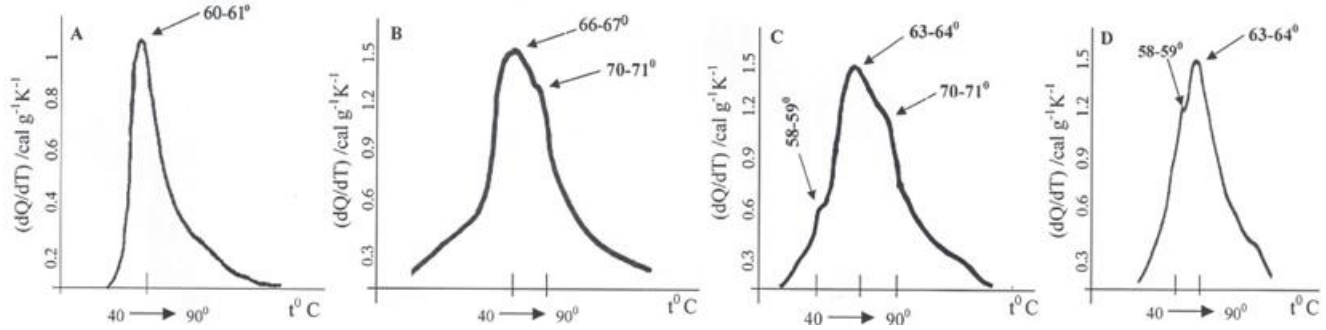

Fig. 3 — Calorimetric curves of the blood plasma of men with prostate tumors. (A) Control group; (B) Benign hyperplasia of prostate (BHP); (C) BHP with high-grade prostatic intraepithelial neoplasia (HGPIN) regions; and (D) Cancer of prostate (CaP)

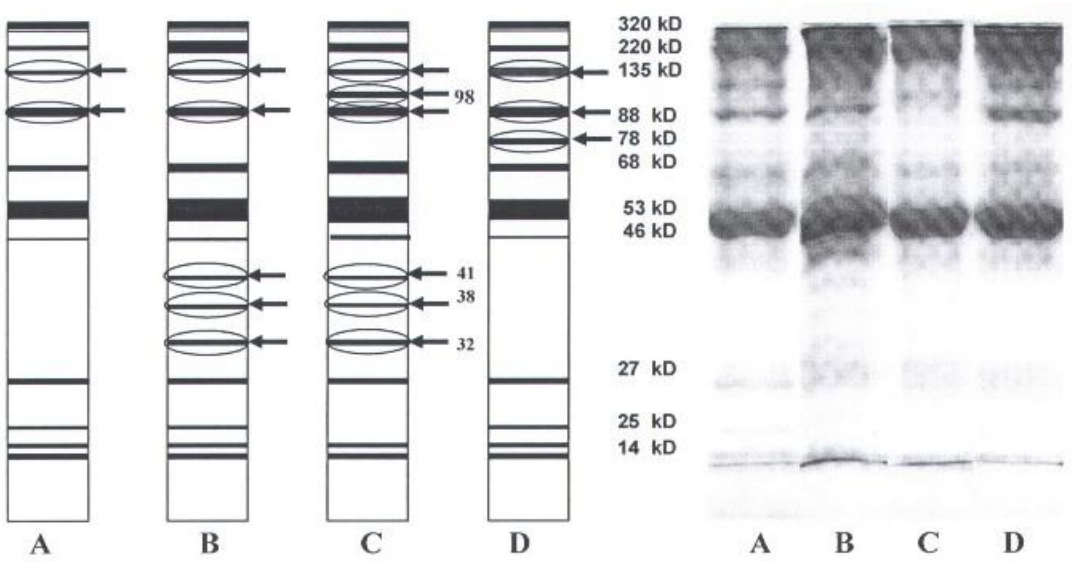

Fig. 4 — Electropherogram of blood plasma proteins in SDS–polyacrylamide gel in men with prostate tumors. Standard proteins: (i) Thyroglobulin (330 kD); (ii) Catalase (60 kD); (iv) Lactate dehydrogenase (36 kD); and (iv). Ferritin (18.5 kD). (A) Electropherogram of the membrane proteins of the control group erythrocytes; (B) Electropherogram of erythrocyte membrane proteins of the patients with benign hyperplasia of prostate (BHP); (C) Electropherogram of erythrocyte membrane proteins of the patients with BHP with high-grade prostatic intraepithelial neoplasia (HGPIN); and (D) Electropherogram of erythrocyte membrane proteins of the patients with cancer of prostate (CaP)

the plasma proteins, which was increased with the following pattern: control group → BHP-with-HGPIN → CaP → BHP. As for the low molecular weight fractions (such as 41, 38 and 32 kD), these were not found in the control group and CaP patients, but were detectable in smaller amount in cases of patients with BHP and the BHP-with-HGPIN regions (Fig. 4).

Discussion

Charateristically, it is recognized that there are two fluorescence peaks to blood plasma of healthy men (Fig. 1). The first peak corresponds to blood plasma proteins (at 340-360 nm) and the second peak corresponds to the reduced nicotinamide co-enzymes (at 450-470 nm)[13]. The ratio of the intensity of these peaks is an important characteristic of the functional state of the organism[15]. Increase in the first fluorescence peak intensity in patients with prostate tumors may be the result of dysproteinemia, which implies the formation and increased concentration of cancer embryonic, or ”acute phase” proteins (e.g., orosomucoid, α1-antitrypsin, haptoglobin and C-reactive protein) associated with tumor growth[16]. The increase in the peak intensity may also be caused by the modified forms of the mentioned proteins[9], increase of α- and β- globulin concentrations, decrease of native albumin concentration and increase of the so called modified albumin levels in blood plasma[16]. Thus, the increase in the tumor-specific proteins in blood of patients with prostate tumors fairly coincides with the literarture data, which may indicate the tumor-specificity of the obtained results.

The formation (or increased level) of all the above mentioned proteins is considered from two points of views: (i) the response of an organism to the tumor growth; and (ii) as a localized reaction in response to the infiltration of the normal tissue by the tumor cells. We presume that the tumor progression together with the increased concentration of ’acute phase’ proteins may be caused by the release of lysosomal hydrolases, which stimulate the formation of blocking anti-proteases (e.g., α1-antitrypsin, α1-antichymotrypsin) from the destructed tumor and non-tumor cells. The other cause may be due to inflammation process, which accompanies the tumor cell proliferation[16]. Thus, a direct relationship between the alterations of the first peak fluorescence and prostate tumor progression was observed in our assessments.

The decrease of the second peak intensity in patients with BHP (Fig. 2B) compared to the control group (Fig. 2A) may be caused by the increased enzyme activity on the background of BHP (glucose-6-phosphate dehydrogenase, NADH-oxidase, and cytochrome P450, etc.) and subsequent increased utilization of nicotinamide coenzymes[17]. It is possible

that the synthesis and metabolism of these substances might be impaired in pathological conditions (even in BHP)[13,15]. In cases of BHP-with-HGPIN regions and CaP, the increased intensity of the second-peak corresponding to nicotinamide co-enzymes may be caused by the tumor development and malfunction of the enzyme systems in parallel with the disease progression, and as a result, nicotinamide co-enzymes accumulated in the blood plasma[17]. In addition, the increased intensity of the second-peak may also be caused by the alterations in the energy metabolism of tumor cells. It is established that unlike other hormone-dependent tumors, prostate epithelial cells have specific energy metabolism[5]. According to the peer-reviewed literature data, changes of nicotinamide co-enzyme containing enzyme activities may also be caused by the altered intensity of lipid peroxidation in blood[18]. Thus, the fluorescence study of the blood plasma allowed us to study the living system *in vivo* without disturbing the structure-functional integrity and interrupting natural processes occuring in the organisms. Moreover, the given spectra allowed us to differentiate prostate tumors and discuss the extent of the disease progression.

On the calorimetric curve of the control group plasma (recorded by DSC), main peak maximum was revelead in the interval between 60-61°C, which coincides with the literature data and corresponds to the denaturation temperature of native albumin (60.5°C)[13,23]. In the case of plasma of BHP patients, the shift of the main peak maximum towards the higher temperature range (66-67°C) may be a result of the increased 'acute phase' protein levels during the tumor development[16,18]. Formation of the small arm at 70-71°C is presumably associated with the increased immunoglobulin concentrations in blood plasma[16], which fully corresponds to the histomorphological diagnosis of prostate benign hyperplasia with inflammatory changes. Thus, the formation of this small arm may be associated with the enhanced protective function of an organism, and intensification of the immunoglobulin synthesis during the inflammation process. In case of the BHP-with-HGPIN reigons, shift of the main peak maximum (T_d) towards the higher temperature range of 63-64°C may be caused by the decreased level of native albumin. Appearance of an additional arm (58-59°C) in this case may also be associated with the progression of the disease, formation of HGPIN regions, and increased level of the modified albumin[16].

In case of CaP patients, the peak in the temperature interval of 58-59°C on the calorimetric curve of plasma (Table 1) may be the result of an even sharper increase in the modified albumin portion in the overall albumin fraction. Additionally, it is worth noting that the arm at 70-71°C range was not detectable in the given case, which may be a result of tumor metastasis [as supported by the tumor, node, metastasis (TNM) staging/clinical data] and supressed immune system. Thus, we presume that in blood plasma of men with BHP and BHP-with-HGPIN, the arm in temperature interval of 70-71°C is specific and points to the inflammatory processes on the background of the noted disease. As for the temperature interval 63-64°C corresponding to the main peak (Td) maximum in BHP-with-HGPIN and CaP plasmas, it should point to high risk of malignant transformation in men. Thus, the increased ratio of the thermostable proteins (acute phase proteins) and appearance of the modified forms of these proteins in blood plasma occurs along with the disease progression. This is reflected in the increased thermostability of the given system, which points to the degree of orderliness in the system. Therefore, the method of DSC makes it possible to differentiate healthy men from the patients with prostate tumors and to evaluate the degree of disease progression.

Based on the peer-reviewed literature, 220 kD protein fraction may be represented by the α1-globulin fraction (α1-lipoproteins) and γ-globulins. Moreover, the mentioned protein levels are also increased during the carcinogenesis and inflammatory processes[15,16]. It is known that BHP and the BHP-with-HGPIN regions develop and progress on the background of inflammatory process. This hypothesis is supported by the emergence of the small arm in the temperature range of 70-71°C on the calorimetric curve of BHP plasma (Fig. 4B), which may be due to the result of enhanced protective function of the organism and corresponding intensification of immunoglobulin synthesis[16,20]. We presume that the decrease of 220 kD protein fraction in case of CaP as compared with prostate benign tumor may be the result of inflammatory process being overriden by the malignant tumor, which causes the inhibition of γ-globulin synthesis and deterioration of the immune system of the organism. This hypothesis is supported by the disappearance of the small arm in the temperature interval of 70-71°C corresponding to

immunoglobulins (on the calorimetric curve of CaP patients, Fig. 3D).

It is reported that the tumor specific marker kallikrein-2 (hK2), with the molecular weight of 30 kD, is released from the tumor tissue and circulates in blood as a free form or bound to the plasma proteins (such as α1-macroglobulin, protein C1-inactivator, and α-antitrypsin, etc.)[21]. It is also known that hK2 forms a 135 kD fraction with blood plasma C_1-inactivator protein. We presume that the observed 135 kD fraction is a complex of hK2-protein and C_1-inactivator. Moreover, it is known that hK2 protein levels are increased in case of CaP, alongside the mentioned complex concentrations[7,21], which could be the reason for the increased 135 kD fraction in case of CaP in our study. Thus, 135 kD fraction, observed in our experiments, may serve as one of the specific/prognostic markers for prostate tumors.

It is known that haptoglobin belongs to 98 kD proteins that binds to cathepsins B, H, T and hemoglobin (released as a result of erythrocyte hemolysis) and transport them out of the organism[22]. It is established that BHP-with-HGPIN regions contain cancerous, as well as necrotic sections, and suggested that BHP-with-HGPIN regions are the precursor of prostate transitory cell type cancer[23]. It is known that in case of BHP-with-HGPIN regions, necrotic sections are formed alongside the enhanced blood hemolysis. As a result, hemoglobin is released in high concentrations and haptoglobin synthesis is intensified in response[22]. This process is responsible for the withdrawal of any unneccessary hemoglobin from the organisms. In case of prostate BHP-with-HGPIN regions, 98 kD mass protein fraction, as observed in our study, may be the manifestation of intensive haptoglobin synthesis.

It is demostrated that hK2 also forms another 80 kD complex with other blood plasma proteins (protein C inhibitor and α2-antiplasmin)[22]. Thus, it may be hypothesized that the 88 kD fraction observed in our study consists of the referred complexes of hK2 and plasma proteins. Moreover, the literature data shows that prostate-specific antigen (PSA) forms 90 kD mass complex with α1-antichymotrypsin[24]. Accordingly, the 88 kD fraction may contain both hK2-protease inhibitors and PSA protease inhibitors. Considering that increased PSA level is characteristic of CaP, it is clear that the 88 kD fraction is also increased in case of prostate tumors as compared with the control group. It is likely that the increased portion of the 88 kD fraction is a manifestation of the increased PSA complex concentration, rather than the hK2 concentration.

According to the literature reports, a large part of 68 kD protein fraction belongs to plasma albumin, which may be similar to the molecular-weight protein α1-antichymotrypsin (68 kD) in this fraction[22]. Moreover, albumin (a ~66 kD protein) overrides other proteins in 50-70 kD fraction, which has been observed on the electropherogram of albumin-free plasma proteins[17,25]. In our case of plasma of patients with BHP and the BHP-with-HGPIN regions, several peaks were detected on the protein electropherogram (67-70 kD fractions), presumably these peaks correspond to several protein fractions, which were not revealed in our control or CaP groups. This finding supports our hypothesis that proteins, other than albumin, may exist in this fraction. Thus, in cases of BHP and the BHP-with-HGPIN regions, an increased 68 kD fraction is not caused by albumin (it must be taken into account that in case of tumor, albumin levels decrease insignificantly or do not change at all[16]), but with other proteins of the blood plasma (e.g., α1-antichymotrypsin, etc.) having similar molecular-weight[20]. The decrease of the noted fraction in CaP patients, compared to the patients with benign tumors, may be caused by the reduced levels of albumin[16] and α1 antichymotripsin, which may be a result of the overriding inflammatory processes by tumor. It is also shown that modified albumin is found, and its portion is often increased in the albumin fraction of plasma in case of different pathologic conditions, including cancer[15]. Existence of the modified albumin in plasma of patients with BHP and CaP was verified by our data obtained by calorimetry determinations of plasma proteins (Fig. 3).

It is noted that albumin found in blood of oncologic patients binds to carbohydrate components, such as fucose[25]. We presume that the appearance of 78 kD fraction in case of CaP, which is absent in both the control and BHP groups, is caused by the formation of the modified albumins. We also speculate that the 78 kD fraction may cause the decrease of 68 kD fraction in case of CaP. Based on the peer-reviewed literature, 63-65 kD fraction consists of light- and heavy-chains of immunoglobulins - lgA and lgG1, G2, G3 (lgA, α1: 56-58 kD, α2: 52-54 kD), as well as of α-2-HS-glycoprotein (MW=58 kD, heavy chain: 53 kD, light chain: 5 kD)[26]. This assumption is supported

by our data obtained by the calorimetric method as well (arm at 70-71^{0}C; Fig. 3B, 3C). Increased immunoglobulin concentrations in patients with BHP and the BHP-with-HGPIN regions compared to the control may be a result of the enhanced immune reponse of an organism to the inflammatory processes that accompany carcinogenesis. Decrease in 63-65 kD fraction on the plasma electropherogram in case of CaP, as compared to prostate benign tumors, may be a result of immunodeficiency caused by the pathology, as well as of the decrease in acute phase protein levels (e.g., α2-HS-glycoprotein, prealbumin) in 63-53 kD fraction[26].

Clonclusion

In this study of the plasma of prostate tumor patients, we observed: (i) direct relationship between the alterations of the first peak fluorescence inensities and prostate tumor progression; (ii) increased first peak intensity in case of prostate tumors, which may be caused by the elevated levels of 'acute phase' proteins associated with tumor growth, as well as by the appearance of cancer embryonic antigen, and formation of modified albumin in the albumin fraction of blood plasma; and (iii) increased intensity of second peak corresponding to nicotinamide co-enzymes (NADH and NADPH)in case of benign hyperplasia of the prostate (BHP) with high-grade prostatic intraepithelial neoplasia (HGPIN) and Cancer of Prostate (CaP). This may be the result of enzyme system malfunction in parallel with the disease progression, which results in the accumulation of the mentioned co-enzymes and alteration of energy metabolism in cancer cells.

Conflict of interest

Authors declare no conflict of interests.

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