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Original Article

Changes in membrane organization of blood erythrocytes from men with different forms of prostate tumors

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Abstract: The *protein spectrum* and sorption capacity of *erythrocyte membranes have been studied in* blood of men with Benign hyperplasia of prostate (BHP), BHP with High Grade Prostatic Intraepithelial Neoplasia (BHP with HGPIN) and Cancer of prostate (CaP). Isolation of the erythrocyte membranes was performed by the method of Dodge. Separation of the membrane proteins was performed by the SDS-PolyAcrilamide Gel Electrophoresis using the "Laemmli" system. Sorption capacity of erythrocytes was determined spectrophotometrically at 630 nm. The amount of Middle Molecular Weight Substances (MMWS) was determined in erythrocytes and in blood plasma spectrophotometrically at 242, 246, 254, 258, 266, 274, 282 and 298 nm. Specific changes have been revealed in protein composition of erythrocyte membranes from men with prostate tumors. Proteins with molecular weights of 180 kD, 70 kD, 60 kD, 36 kD and 120 kD have been detected in erythrocyte membranes from the different forms of prostate tumors. Sorption capacity of erythrocyte membranes was abnormally increased in patients with BHP with HGPIN and CaP. The amount of MMWS and coefficient of intoxication of blood was especially raised in case of BHP with HGPIN. Obtained data suggests the role of erythrocytes as one of the components that demonstrate the functional state of men with prostatic neoplasia, and substantialy reflects the changes that take place in the course of tumor pathology with disrupted homeostasis.

Keywords: Prostate tumors, erythrocytes, membrane proteins, adsorption capacity

Introduction

Determination of the main homeostatic indices of cancer patients is an active area of research in recent oncology. According to literary data investigation of the peripheral blood erythrocytes is one of the promising approaches for solving the problem [1]. Since the erythrocyte membrane is easy to be gained and studied, it represents an ideal model for membrane investigations and also substantively reflects the changes taking place on the background of disrupted homeostasis in the case of tumor pathology [2].

In our early experiments significant changes of some functional and morphological characteristics of erythrocytes, which were reflected on their structure as well, have been revealed in patients with benign hyperplasia of the prostate (BHP) and cancer of the prostate (CaP) [3]. Whether the change occurring in erythrocytes is a concomitant process of tumor formation, or whether it is a reaction leading to the suppression of immune response is still unclear.

The purpose of the presented work was to study the changes that occur in membrane proteins of blood erythrocytes from patients with BHP, prostate benign hyperplasia with High Grade Prostatic Intraepithelial Neoplasia (BHP with HGPIN) and CaP, and to evaluate the specificity of the changes as far as possible.

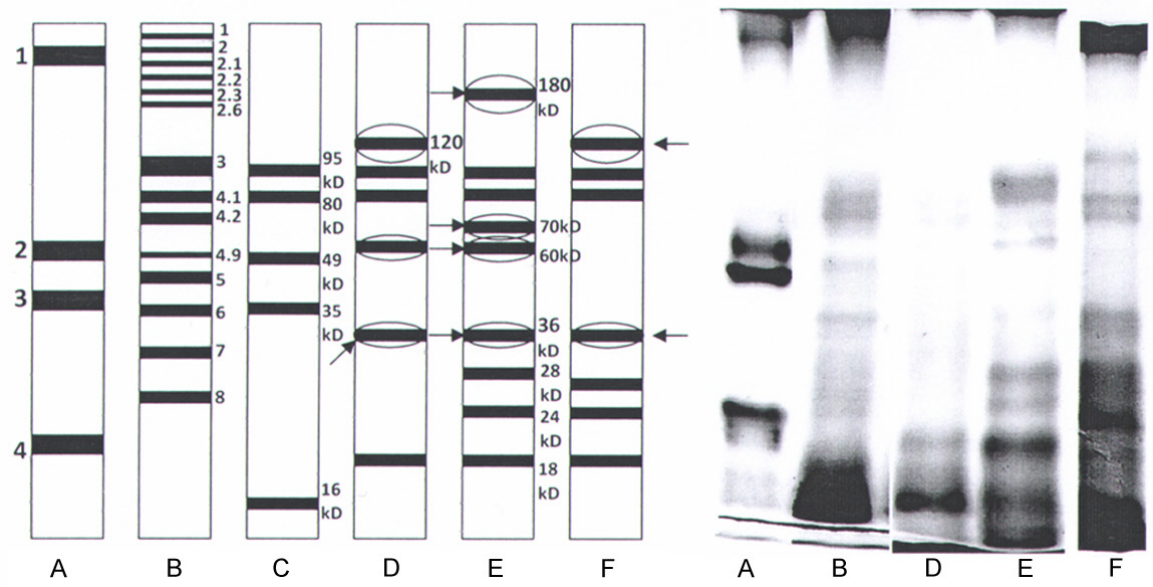

Figure 1. Electropherogram of erythrocyte membrane proteins in SDS -polyacrylamide gel. A. Standard proteins: 1. Thyroglobulin (330 KD); 2. Catalase (60 KD); 3. Lactate dehydrogenase (36 KD); 4. Ferritin (18,5 KD); B. Electrophoregram of membrane proteins of donor blood erythrocytes; C. Electrophoregram of membrane proteins of control group erythrocytes; D. Electrophoregram of erythrocyte membrane proteins of the patients with Benign Hyperplasia of Prostate with HGPIN; E. Electrophoregram of erythrocyte membrane proteins of the patients with Benign Hyperplasia of Prostate (BHP); F. Electrophoregram of erythrocyte membrane proteins of the patients with adenocarcinoma of prostate (CaP).

Material and methods

Subjects

The erythrocytes and blood plasma of the patients with benign hyperplasia of the prostate (BHP), BHP with HGPIN and Cancer of Prostate (CaP) served as materials for the studies. Each study group consisted of 15 patients of ages 60-75. The control group consisted of 15 practically healthy men of the same age group. Patients were not receiving any type of treatment during sample collection for this investigation.

The Ethics Committee of Georgia approved the study and informed consent was obtained from each patient

The blood samples were obtained before 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 examination of the prostate gland. Histological analyses of TURP derived specimens were performed by the uropathologist of the National Centre of Urology.

Methods

Gaining of the erythrocyte membranes was performed by the method of Dodge [4]. Protein concentration was detected by the method of Lowry [5]. Electrophoresis of the proteins in dissociation conditions was performed at 10-25% gradient of polyacrilamide gel, with 0, 1% *sodium dodecyl sulfate* (SDS) using the "Laemmli" system [6]. Electrophoresis was performed by "Hoefer scientific instrument SE-200" device for 3.5 hours.

Sorption capacity of erythrocytes was determined spectrophotometrically at 630 nm according to Dobrotyna [7]. The amount of Middle Molecular Weight Substances (MMWS) were determined in erythrocytes and in blood plasma by the Gabrielian method of spectrophotometry at 242, 246, 254, 258, 266, 274, 282 and 298 nm, respectively [8]. Endogenous Intoxication Coefficient (EIC) was calculated by the ratio of MMWSs in blood plasma and erythrocytes.

Statistical analysis

Statistical analyses of experimental data were processed using the variance method, by

Table 1. The changes of MMWS content, sorption capacity of erythrocytes and alteration of coefficient of intoxication in blood of men with prostate tumors

Objects	Sorbtion capacity of erythrocytes (%)	MMWS* in plasma (E total)	MMWS* in erythrocytes (E total)	Coefficient of Intoxication (rel. un)
Control group	37.12 ± 1.43	1.76 ± 0.32	5.25 ± 1.17	2.38 ± 0.48
Benign hyperplasia of prostate (BHP)	38.53 ± 1.72	2.97 ± 0.59	7.67 ± 1.67	4.15 ± 0.87
BHP with HGPIN**	59.40 ± 2.01	3.69 ± 0.88	9.62 ± 2.01	5.06 ± 1.03
Prostate Cancer (CaP)	67.60 ± 2.83	3.31 ± 0.74	8.86 ± 1.84	4.50 ± 0.94

*MMWS-Middle Molecular-Weight Substances; **HGPIN- High Grade Prostatic Intraepithelial Neoplasia. $P \leq 0.05$.

means of computer program (Graphpad prisma 6). $P < 0.05$ was regarded as statistically significant.

Results

Proteins with molecular weights 180 kD, 120 kD, 70 kD, 60 kD and 36 kD were revealed on the electropherogram of the erythrocyte membrane proteins of patients with prostate tumors (**Figure 1**).

It should be noted that the presence of these proteins is not typical for normal erythrocyte membranes.

Among them, the 180 kD and 70 kD proteins were only found in samples from BHP patients (**Figure 1E**). As for the 60 kD protein, it was detected in BHP and BHP with HGPIN cases (**Figure 1D**, **1E**). The 120 kD molecular weight protein was revealed among BHP with HGPIN and CaP patients by the electrophoresis of blood erythrocyte membranes (**Figure 1D**, **1F**). And the last one, the 36 kD molecular weight protein was observed in samples from all studied groups of prostate tumors (**Figure 1D-F**).

Investigation of the membrane destruction process has revealed two groups of patients with prostate benign tumors according to sorption capacity. The mean values of these groups differed significantly such that patients with BHP and BHP with HGPIN were clearly distinguished. Our data futher demonstrated that the sorption capacity of erythrocytes from BHP patients was slightly higher than those from the control group, or was in normal ranges (**Table 1**). The sorption capacities of erythrocytes form BHP patients with HGPIN and especially of CaP patients were significantly higher compared to patients with BHP (**Table 1**).

According to our results, in blood plasma MMWS was increased in all studied groups compared to controls. In particular, the content of MMWS was increased ~1.68-times in BHP patients, ~2.01-times in BHP patients with HGPIN and ~1.88-times in CaP patients. The clear tendency of MMWS growth was sharply expressed in blood plasma of BHP patients with HGPIN and this seems to be maintained in CaP patients (**Table 1**).

As for MMWS content in erythrocytes, we have shown that the amount is increased in BHP (~1.46 times), and CaP (~1.65 times) patinets compared to the control group. The index reached its maximal level in erythrocytes from the BHP patients with HGPIN (~1.83-times) (**Table 1**).

For the evaluation of the toxic products in circulation, the coefficient of intoxication, which depends on the amounts of MMWS in plasma and erythrocyte membrane, was used. Our investigations have demonstrated that the coefficient of intoxication significantly rose in cases of BHP with HGPIN (about 2.12 times), compared to the control group, while in CaP and BHP patients it increased, but to a lesser degree than in HGPIN cases (**Table 1**).

Discussion

It is known that normally the 180 kD molecular weight protein is a component of erythrocyte membrane, and has been identified as ankirin [9]. In our observations, the presence of the 180 kD protein in BHP and its absence in control group erythrocytes is unlikely to be due to the protein ankirin, but rather a different structural protein with the same molecular weight. Formation of the 180 kD protein in samples from BHP patients may be a reactionary pro-

cess in these men due to the development of benign tumors.

As for the 70 kD molecular weight protein, it is not specific to erythrocyte membrane. According to some authors, this 70 kD protein in blood may indicate the presence of both, benign or malignant tumor, and may be caused by circulating α-fetoprotein [10]. Since the same protein is detected during pregnancy and inflammatory processes, the opposite point of view exists as well [11]. According to clinical data, BHP occurs on the background of acute inflammation. Therefore, the presence of this protein in samples from men with BHP may be accounted for by the accompanying inflammation and not by tumor transformation.

The 60 kD molecular weight protein revealed by the electrophoresis of the blood erythrocytes membrane of BHP and BHP with HGPIN patients (**Figure 1E**, **1D**), is not specific for normal erythrocyte membrane. No literary data exist on the given molecular weight protein.

It is known that usually the 120 kD protein is not a characteristic of the cytoskeleton of erythrocytes. The presence of the mentioned protein in erythrocyte membranes from these patients indicates its evolution with malignant tumor progression [3].

It is established that tumor growth is accompanied by absorption of blood plasma globulins on erythrocyte membranes, resulting in a solid, practically indestructible complex of erythrocyte and protein [12]. It is known as well, that the formation of pathogenic globulin molecules, the so-called pre-albumin or post-gamma globulin fractions, takes place in plasma during tumor progression, which does not occur in healthy organisms [12]. Accordingly, synthesis of the 120 kD protein in CaP cases may be accounted for by formation of the mentioned complex. This fact once more suggests the formation of erythrocyte-protein complex in prostate cancer patients [13].

It is established that 36 kD protein takes an active part in tumor transformation of a cell [11]. It must also be noted that one of the dimers of the mentioned protein has been identified as the Nuclear Antigen causing cell proliferation (PCNA). It is encoded in the nucleus and moves to the cytoplasm very fast [11]. PCNA does not exist in the cytosol of healthy cells and is found only in the cytosol of tumor cells. If we suppose that PCNA is encoded in cell nucleus of all tumor tissues and in the nucleus of prostate tumor cells as well, it may be brought to circulating blood and later adsorbed by erythrocytes, since one of the functions of erythrocytes is adsorption of antigens [14]. Appearance of the 36 kD protein in the protein spectrum of the erythrocyte membranes from patients with prostate tumors may be the result of this process.

Thus, according to our results and on the basis of data from the literature, we have attempted to reveal the changes that occur in the protein spectrum of the erythrocyte membrane of patients with prostate tumors. We will be pursuing further characterization of the differentially expressed or processed proteins in the future.

Presumably, the described changes have a significant impact on membrane destruction processes of erythrocytes. Accordingly, the adsorption capacity of erythrocyte membrane, an important *parameter of erythrocyte function* and integrity was addressed in this study as well. Sorption capacity of erythrocytes is a part of adsorption-transport function of red blood cells, as they have the ability to adsorb endotoxins from plasma and thus ensure detoxication of blood [7]. It is also known that the extent of erythrocyte membrane disorganization depends on the concentration of endotoxins as well as the period of their presence in blood [15]. Hence, we've also tested the quantity of MMWS both in plasma and erythrocytes, and also the coefficient of endogenous intoxication.

Increased sorption capacity of erythrocytes on the background of the various pathologies may be explained by the "buffer" functions of erythrocytes involved in the neutralization of different endogenous toxins, products of protein proteolysis, fatty acids, biologically active amines, etc [16]. It is known that in parallel with enhanced accumulation of metabolites in blood, the transport as well as adsorption functions of erythrocytes increase, so does the adsorption of different organic substances on their membranes [15]. As the erythrocytes adsorb endotoxins from plasma they become a "target" loaded with extra organic substances. Thus,

enhanced adsorption-transport function of erythrocytes is a very important adaptive and compensatory reaction directed against endotoxicosis [17].

In parallel with high levels of endotoxins in plasma, the sorption capacity of erythrocytes increases on the background of malignant growth (**Table 1**). This indicates the increase in the circulating levels of toxins in patients with CaP and correspondingly to the increase of erythrocyte membrane damage. Increased sorption capacity of erythrocytes may lead to saturation of the cell membrane with toxic products, later resulting in increases in toxic substances in the blood plasma as well [18]. Long term effects of endotoxins may lead to conformational changes in membrane proteins (**Table 1**) and phospholipids, increasement of membrane viscosity and as a result formation of rigid membranes [19, 20]. Enhanced sorption capacity of erythrocytes both in the case of BHP patients with HGPIN and CaP unambiguously suggest changes in membrane organization of circulating erythrocytes during tumor development in toxic enviroment.

Thus the obtained results show that erythrocytes from men with BHP normally perform adsorption-transport function and yet actively carry out detoxification and neutralization of different endogenous toxins (sorption capacity of BHP group is only slightly higher than or is the same as the control group (**Table 1**).

Our findings suggest that the adsorption-transport function of erythrocytes in BHP with HGPIN and CaP patients is reduced due to the membrane-destructive processes in erythrocytes caused by the excess concentrations of toxins. At the same time a clearly expressed changes in protein spectrum by erythrocytes from prostate cancer patients are revealed.

In summary, our data suggest the role of erythrocytes as one of the components that demonstrate the functional state of men with prostatic neoplasia, and substantialy reflects the changes that take place in the course of tumor pathology with disrupted homeostasis.

Conclusions

Specific changes have been revealed in protein composition of erythrocyte membranes from men with prostate tumors. The 36 kD MW protein that was observed in erythrocyte membranes of all studied groups (BHP, BHP with HGPIN, CaP) does not exist in normal red blood cell membranes. It is likely that this 36 kD protein plays an active role in tumor transformation. The structural proteins with molecular weights 180 kD, 70 kD, and 60 kD can be considered as characteristic features of erythrocytes from BHP patients.

The 120 kD protein found in samples from BHP with HGPIN and CaP patients may be the result of pathogenic erythrocyte-protein complex formation. The appearance of the mentioned complex indicates the high degree of malignant tumor progression. Our supposition is that the 120 kD protein is specific to BHP with HGPIN and this fact once more provides evidence to the opinion that HGPIN is a rick factor or precursor lesion for CaP, and generally predicts poor prognosis.

Increased sorption capacity of erythrocytes in samples from BHP with HGPIN and CaP has been revealed by our study, and this can be explained by the “buffering” functions of erythrocytes that is involved in the neutralization of different endogenous toxins. The given fact indicates the toxic load increase and correspondingly to increase in the extent of erythrocyte membrane damage during tumor progression and toxins poduction.

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Disclosure of conflict of interest

None.

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