Renal cancer makes up around 3-4% of human neoplasms. It may metastasise, infiltrate and colonise other tissues. Infiltration and metastasis may depend on changes in the oligosaccharide structures of glycoconjugates of the glycocalyx, basement membranes and extracellular matrix. Oligosaccharides are degraded by endo- and exoglycosidases. N-acetyl-β-glucosaminidase (NAG) produced mainly by the epithelial cells of proximal convoluted renal tubules, is the most active of all exoglycosidases. N-acetyl-β-hexosaminidase is a lysosomal exoglycosidase which hydrolyses β-glycosides of N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) from glycoconjugates. In human tissues it exists as two major isoenzymes: a thermolabile NAG A and thermostabile NAG B. NAG is a glycoprotein composed of two subunits α and β. Isoenzyme A has composition αβ, isoenzyme B ββ, respectively. Genes coding subunit α NAG are localised to chromosome 15, and those coding subunit β are localised to chromosome 5. The cancerous tissue revealed a significantly lower activity, of NAG and its isoenzyme A, and transitional tissue showed an intermediate activity in comparison to control renal tissue. A significant increase in NAG activity was observed in the serum and urine of renal cancer patients, in comparison to control subjects.
In conclusion we propose determination of NAG in urine as a potential marker of renal cancer.

Key words: renal cancer, renal tissue, serum, urine, N-acetyl-β-glucosaminidase, isoenzymes A and B.

The activity of N-acetyl--β-glucosaminidase and its isoenzymes in the renal tissue, serum and urine of patients with renal cancer

Aktywność N-acetylo-β-heksozoaminidazy i jej izoenzymów w tkance nerki, w surowicy krwi i w moczu pacjentów z rakiem nerki

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Introduction

In Poland renal cancer makes up some 3-4% of malignant neoplasms [1]. Renal cancer, (the most frequent kidney neoplasm in adults) is usually diagnosed when distant metastases have occurred. Up to now, the only effective treatment of renal cancer is surgical eradication [2]. Selective cell to cell adhesion of renal cells may be mediated by oligosaccharide chains of glycoproteins [3] present on the extracellular membrane. These oligosaccharide chains are recognised by cadherins and selectins on the cellular membranes of the neighbouring cells. The adhesion of the cellular membranes of renal cells to components of the extra cellular matrix (ECM), is mediated by integrins and integral membrane proteoglycans [3]. To infiltrate the neighbouring and colonise foreign tissues, a neoplastic cell has to detach from the neighbouring cells, (by reducing adhesion to them), invade local tissues and vessels by creating channels through the ECM, and establish new cellular colonies at distant sites. Endo- end exoglycosidases which degrade sugar chains of glycoconjugates (glycoproteins, glycolipids and proteoglycans) [4] may participate in reducing adhesion to the neighbouring cells and creating channels in the ECM. N-acetyl-β-glucosaminidase (NAG) is most active of the exoglycosidases and releases N-acetylglucosamine and N-acetylgalactosamine from the non reducing terminus of glycoconjugates [5]. NAG is a glycoprotein composed of two subunits α and β. Isoenzyme A has composition αβ, isoenzyme B ββ and isoenzyme S αα, respectively. Genes coding subunit α NAG are localised to chromosome 15, and those coding subunit α are localised to chromosome 5 [6].

In the kidneys, NAG is distributed along the whole nephron with the highest activity being found in lysosomes of proximal convoluted tubules [7]. It is worth noting that renal cancer cells derive from proximal convoluted tubules [8]. Physiological urine has traces of NAG activity. The urinary activity of isoenzyme A reflects the secretory activity of tubular cells (functional enzymuria) and isoenzyme B reflects the breakdown of tubular cells (lesional type enzymuria) [9].

The aim of our research is determination of the activity of NAG and its isoenzymes in renal tissue, serum and urine, to evaluate their diagnostic significance in renal cancer.

W Polsce nowotwór nerki, obejmujący 3–4 proc. wszystkich nowotworów złośliwych, stanowi trudny problem diagnostyczny. Rak nerki (najczęstszy nowotwór nerki u dorosłych) jest zwykle diagnozowany, gdy wystąpiły odległe przerzuty. Naciekanie okolicznych tkanek i powstawanie przerzutów zależy od zmian w strukturach oligosacharydowych glikokoniugatów glikokaliksu i substancji międzykomórkowej.
W degradacji łańcuchów oligosacharydowych glikokoniugatów biorą udział endo- i egzoglikozydazy.
Najbogatszym źródłem egzoglikozydaz w nerce są kanaliki nerkowe, skąd dostają się one do moczu. N-acetylo-β-D-heksozoaminidaza (NAG) to kwaśna egzoglikozydaza lizosomalna odszczepiająca reszty N-acetyloglukozoaminy (GlcNAc) lub N-acetylogalaktozoaminy (GalNAc) z nieredukującego końca łańcuchów oligosacharydowych glikokoniugatów. Jest najaktywniejszą ze znanych egzoglikozydaz. Substratami dla tego enzymu są gangliozydy, glikoproteiny i łańcuchy glikozoaminoglikanowe proteoglikanów, tj. siarczan chondrotyny, kwas hialuronowy, siarczan dermatynu i siarczan keratanu. N-acetylo-β -D-heksozoaminidaza jest glikoproteiną zbudowaną z łańcuchów polipeptydowych α i β. Izoenzym A ma budowę αβ, izoenzym B – ββ. Celem naszej pracy było oznaczenie aktywności N-acetylo-β-heksozoaminidazy i jej izoenzymów A i B w tkance nerki metodą kolorymetryczną w surowicy krwi i oraz N-acetylo-β-heksozoamindazy w moczu. Najniższe aktywności N-acetylo-β-heksozoaminidazy i jej izoenzymów stwierdziliśmy w tkance nowotworowej, istotnie większe w obszarze przejściowym, a największą w tkance zdrowej. Nasze wyniki sugerują, że proces nowotworowy powoduje zwiększone wydalanie N-acetylo-β-heksozoaminidazy do moczu. Pochodzenie guza nerki z komórek nabłonkowych proksymalnych cewek krętych czyni oznaczanie poziomu aktywności N-acetylo-β-D-heksozoaminidazy (NAG) w tkance i moczu szczególnie przydatną metodą diagnostyki tego nowotworu.

Słowa kluczowe: N-acetylo-beta-heksozoaminidaza, rak nerki, surowica krwi, mocz izoenzymy A i B N-acetylo-beta-heksozoaminidazy.

Materials and methods

Control, transitional and cancerous kidney specimens (consisting of the tissue from the resected kidney clinically and histopathologicaly free of cancer, transitional area and cancerous tissue, respectively) from 30 patients (18 men, 12 women) were obtained during surgery in the Department of Urology, and the diagnosis was confirmed in the Department of Pathological Anatomy Medical University in Bialystok. Resected specimens were cut into two parts. One part of the fresh tissue was subjected to biochemical investigations performed in the Department of Pharmaceutical Biochemistry, and the rest of the material was immersed in 10% formalin to perform histopathological and immunochemical investigations. The local Ethic Committee for Human Research approved the study (R-I-003/216/2003), performed according to guidelines for good clinical practice.

For determination and separation of NAG and its isoenzymes, renal tissue was suspended in 0.05 M citrate phosphate buffer pH 4.3 and homogenised in a Potter type homogeniser with a Teflon pestle. The homogenate was centrifuged for 60 min at 12 000xg at 4°C. The activity of NAG and its isoenzymes in supernatants were determined with p-nitrophenol-β-N-acetylglucosaminide as a substrate in 0.02 M citrate-phosphate buffer pH 4.7. The enzymatic reaction was stopped by addition of 0.2 M borate buffer pH 9.8 and released p-nitrophenol was determined at 410 nm [10].

We used packet Statistica 5.0 – test ANOVA and post hoc analysis calculated by test NIR, which indicated the least significance difference. The results were expressed as the mean and SD. P values less than 0.05 were considered significant

Results

In fig. 1 which shows total NAG activity in supernatant of renal tissue one can see that the cancerous tissue had a significantly lower activity than normal renal tissue, and transitional tissue also had a significantly lower activity than normal tissue.

In fig. 2 which shows activity of NAG-A one can see a significant decrease in NAG-A activity in transitional renal tissue (in comparison to normal tissue) and a further significant decrease in the cancerous tissue (in comparison to transitional tissue).

In fig. 3 which shows activity of NAG-B, one can see a significant decrease in NAG-B activity in transitional and cancerous tissue (in comparison to normal tissue).

Figs. 4, 5 and 6 show a significant increase in activity of NAG and its isoenzymes A and B, in the serum of the renal cancer patients in comparison to the serum of the control group.

Fig. 7 shows a significant increase in NAG activity in the urine of the renal cancer patients.

Discussion

Cells of renal cancer grow in the neighbourhood of other cells, basement membranes and the extracellular matrix. To infiltrate and colonise neighbouring tissues, the neoplastic cell has to detach from neighboring cells (i.e. reduce adhesion), invade local tissues and vessels by creating channels in the ECM, and establish new cellular colonies at distant sites. To create channels in the basement membranes and extracellular matrix, the cancer cells must degrade on their way the tri-dimensional network of basement membranes and extracellular matrix which is made up of proteins, and heterooligo- and heteropolysaccharide chains of glycoproteins and proteoglycans. Polypeptide chains of glycocalyx, basement membranes and the extracellular matrix are degraded by proteases (metalloproteinases, cysteine and serine proteases), but oligo-and heteropolysaccharide chains of glycoconjugates are degraded by endo- and exoglycosidases. Morell-

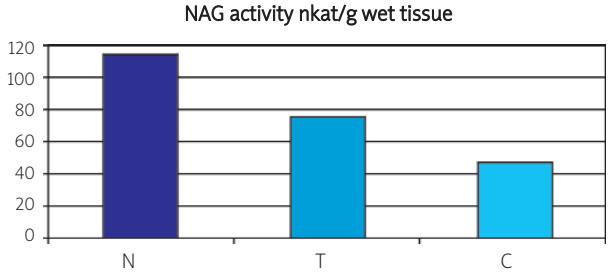

Fig. 1. NAG activity of renal tissue. **N** – normal tissue (clinically and histopathologicaly free of cancer); **T** – transitional tissue; **C** – cancer tissue, n=30
Ryc. 1. *Aktywność N-acetylo-β-heksozoaminidazy w tkance nerki*

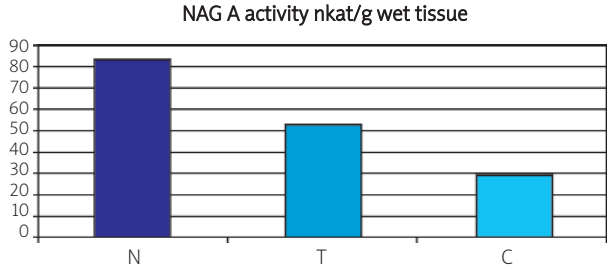

Fig. 2. The activity of NAG-A in renal tissue. For details see description to fig. 1.
Ryc. 2. *Aktywność izoenzymu A N-acetylo-β-heksozoaminidazy w tkance nerki*

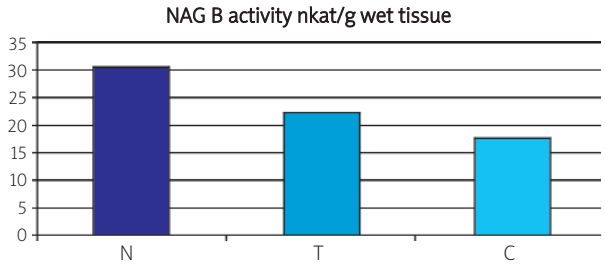

Fig. 3. The activity of NAG-B in renal tissue. For details see description to fig. 1.
Ryc. 3. *Aktywność izoenzymu B N-acetylo-β-heksozoaminidazy w tkance nerki*

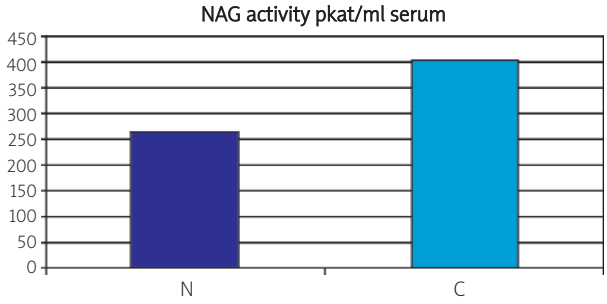

Fig. 4. The activity of NAG in serum of patients with renal cancer. N – control serum; C – serum of cancer patients; n=16
Ryc. 4. *Aktywność N-acetylo-β-heksozoaminidazy w surowicy pacjentów z rakiem nerki i osób zdrowych (surowica kontrolna)*

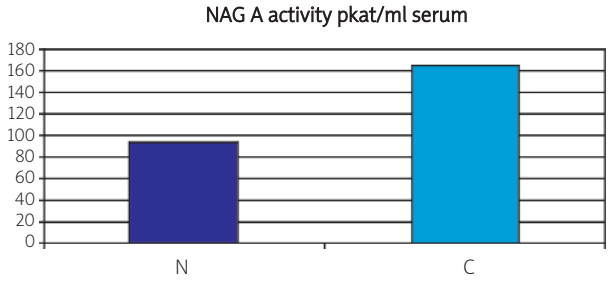

Fig. 5. The activity of NAG-A in serum of patients with renal cancer. For details see description to fig. 4.
Ryc. 5. *Aktywność izoenzymu A N-acetylo-?-heksozoaminidazy w surowicy pacjentów z rakiem nerki i osób zdrowych (surowica kontrolna)*

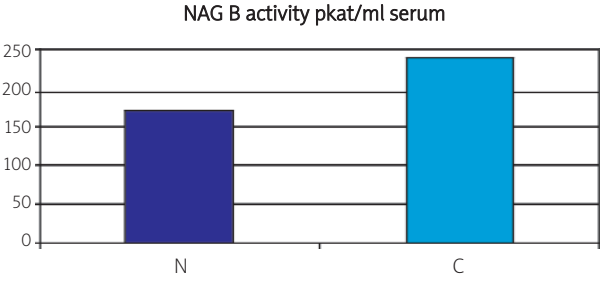

Fig. 6. The activity of NAG-B in serum of patients with renal cancer. For details see description to fig. 4.
Ryc. 6. *Aktywność izoenzymu B N-acetylo-β-heksozoaminidazy w surowicy pacjentów z rakiem nerki i osób zdrowych (surowica kontrolna)*

Quadreny [11] recently reported an inverse relationship between the concentration of E-cadherin, laminin and collagen IV, and the expression of metalloproteinases. In advanced stages of renal cancer Sherif [12] observed an increase in the activity of metalloproteinases in urine and a decrease in levels of proteins constituting the extracellular matrix [12]. Similar results have been presented by other authors [13-16]. We have not found any references of the distribution of endo- and exoglycosidases in basement membranes and extracellular matrix of normal and cancerous kidneys but it is known that without the activity of endo-and exoglycosidases, degradation of oligosaccharide chains of glycoproteins and

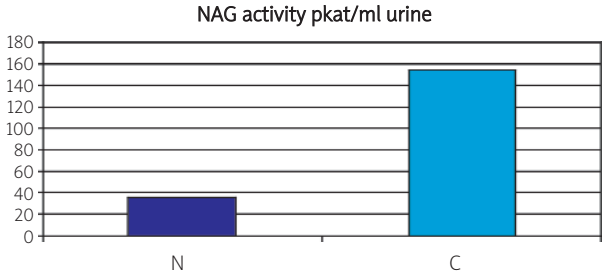

Fig. 7. The activity of NAG in urine of renal cancer patients. N – control urine; C – urine of patients with renal cancer; n=16
Ryc. 7. *Aktywność N-acetylo-β-heksozoaminidazy w moczu pacjentów z rakiem nerki i osób zdrowych (mocz kontrolny)*

glycosaminoglycans of glycocalyx, basement membranes and the extracellular matrix, which separate cells of renal tubules from each other and blood vessels is impossible.

Our results, presented in figs 1-3, show the influence of NAG and its isoenzymes A and B on the degradation of oligo-and heteropolysaccharide chains of glycoproteins and glycosaminoglycans in the control, transitional and cancerous tissue. In our material, the activity of NAG and its isoenzymes A and B was significantly the highest in control "healthy" tissues by comparison with the transitional and cancerous tissue. The significantly lower activity of NAG and its isoenzymes A and B in the cancerous tissue, may be because digestion by NAG sugar chains of glycocalyx, basement membranes and extracellular matrix glycoproteins and proteoglycans takes place not only in lysosomes but also during their transit from lysosomes to the cells of renal canalicules and the lumen of blood vessels. The results of determinations of exoglycosidases in normal and cancerous tissues are contradictory. Our results are in agreement with the data presented by Bosmann et al [17] and Kim et al [18] who reported a lower activity in colonic cancer than in neighboring tissues. Differences in the activity of NAG and its isoenzymes reported in different publications may result from differences in quality of cells taken for analysis. Brattain et al [19] obtained populations of cancer cells, varying 3-5 times in NAG activity, after differential centrifugation of homogenates in a Ficoll gradient. Differences in NAG activity between normal and cancerous renal tissue may depend on the degree of contamination of cancer cells by leucocytes, erythrocytes, cells of connective tissue, normal renal cells and focal points of necrosis [20]. The activity of NAG and its isoenzymes may be different in different cells of cancer tissue, as Kimball [21] reported a higher activity of NAG in colonic cancer cells, than cells of blood and connective tissue isolated from colonic cancer.

The results presented in figs 4-7 suggest that NAG and its isoenzymes A and B are excreted to serum and urine by "healthy" and cancerous kidneys. Up to now, we have had no data on NAG activity on the way between the lysosomes of the cells and the lumen of the blood vessel or the lumen of renal tubules. It may be assumed that NAG and other lysosomal exoglycosidases, may release particular sugars from ends of oligosaccharide chains of glycocalyx proteins, changing adhesive forces binding together cells, between cells and basement membranes, to the ECM, and the means of communication between cells end elements of extracellular matrix.

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