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From Cell Biology to Tissue Engineering

Apoptosis and proliferation of the prostate cells in men with benign prostatic hyperplasia and concomitant metabolic disorders

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Summary. Introduction. Apoptosis and proliferation of prostate cells are associated with both physiological increase and hyperplasia of the prostate. The aim of this study was to determine the contribution of metabolic syndrome to the processes of apoptosis and proliferation in gland epithelial cells and prostatic stromal cells in men with BPH. Materials and methods. The study involved 151 men, aged 52-89 years, receiving pharmacological treatment for BPH. The men were divided into two groups: those with and those without metabolic syndrome. The serum levels of the parameters were determined. Reactions for the identification of apoptosis (TUNEL) and proliferation (PCNA) in cells were also performed. Results. The relationships between the number of TUNEL(+) and PCNA(+) cells and metabolic syndrome were not observed. It was found that the total number of TUNEL(+) cells in the prostate stroma correlated negatively with the levels of high-density lipoprotein and insulin-like growth factor-1. The analysis of the correlations in BPH patients with and without metabolic syndrome demonstrated that the only parameter correlating with the number of PCNA(+) cells in the prostate stroma was insulin resistance. Conclusion. Metabolic syndrome in patients with BPH had no impact on the number of TUNEL(+) and PCNA(+) cells in the prostate gland. However, the disturbed levels of metabolic parameters, and deviations of anthropometric parameters from normal may influence the number of apoptotic and proliferating cells.

Key words: Apoptosis, Proliferation, Benign prostatic hyperplasia, Metabolic syndrome

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Introduction

Benign prostatic hyperplasia (BPH) belongs to the most common nonmalignant diseases found among aging men. It can involve progressive troublesome lower urinary tract symptoms (LUTS), which significantly worsen the quality of patients' lives. Despite numerous clinical and experimental studies, the etiology of BPH has not yet been thoroughly elucidated (Moss et al., 2017).

Apoptosis and proliferation of prostate cells are processes associated with both physiological increase and hyperplasia of the prostate. Imbalance between cell proliferation and apoptosis can lead to a constant growth of epithelial and stromal cells, contributing to the development of BPH (Zhang et al., 2009; Roehrborn, 2008). Researchers discern a relationship between age-related prostatic hyperplasia and the level of androgens. Androgen deficiency can lead to many conditions, among them obesity, vascular dysfunction, and inflammation (Cheung et al., 2015).

The connection between metabolic disorders and the processes of cell apoptosis and proliferation, occurring in the course of BPH, requires further research. However, seeking common etiology of prostatic hyperplasia and

cardiovascular disease gives a new look at the epidemiology of BPH (Ejike and Ezebuiro, 2011). A diagnosis of diabetes, the level of insulin-like growth factor, and obesity are regarded as independent risk factors for developing BPH (Vikram et al., 2010a; Antunes et al., 2014). Metabolic syndrome (MetS) is one of BPH etiological factors. An element that appears to be critical to the interaction between these conditions is insulin resistance (Hammarsten et al., 2009), correlating with more dynamic BPH development (Ozden et al., 2007). Moreover, the level of insulin has been proved to be a positive and the level of high-density lipoprotein (HDL) - a negative predictor of BPH (Nandeesha et al., 2006).

According to some studies, Body Mass Index (BMI) and waist size positively correlate with the prostate gland volume in patients having no abdominal obesity, but suffering from other concomitant diseases potentially suggesting MetS, such as diabetes, a disturbed glucose level, dyslipidemia, and hypertension (Lee et al., 2006).

It seems that a key element linking metabolic disorders with BPH is hyperinsulinemia. A mechanism of this relationship is associated with the influence of hyperinsulinemia on the sympathetic nervous system, whose activation can contribute to the increased tone of prostate muscles (He et al., 2014). Initially such a state can result from LUTS progression, leading as a consequence to BPH (He et al., 2014; Ferreira et al., 2015).

Other factors taken into account in MetS diagnosis - such as a decreased HDL level and an increased level of triglycerides (TG) - are also related to BPH through the paths leading to inflammatory conditions in this gland. All these associations may suggest that the coexistence of MetS, BPH and LUTS should be taken into consideration in targeted therapy for prostatic hyperplasia.

The aim of our study was to determine the contribution of MetS to the processes of apoptosis and proliferation in gland epithelial cells and prostatic stromal cells in BPH patients.

Materials and methods

Patients

The study involved 151 men, aged 52-89 years (67.33±8.28, mean ± SD), receiving pharmacological treatment for BPH. The patients were qualified for planned transurethral resection of the prostate (TURP) at the Clinic of Urology and Urological Oncology at the Pomeranian Medical University in Szczecin, Poland. The BPH patients were treated with 5-alpha reductase inhibitor (finasteride, orally, 5 mg daily). The patients with active alcoholism, thyroid or liver diseases, and active cancerous disease were excluded from the study. The participants were informed about the purpose and course of the study, and gave a written consent to take part in it.

The study was conducted with the consent of the Bioethical Commission of Pomeranian Medical University in Szczecin (permission number KB-0012/132/12).

Clinical trials

Blood pressure and such anthropometric parameters as weight, height, age, and waist size were measured in the patients. The participants were asked to complete the following questionnaires: the International Prostate Symptom Score (IPSS), which is a standardized tool applied to track the symptoms of the prostate gland diseases, and the respondent's particulars.

The men were divided into two groups: those with and those without MetS diagnosed on the basis of the guidelines of the International Diabetes Federation (IDF) from 2005. The diagnostic criteria assumed in our study were: abdominal obesity (waist size ≥94 cm for European men), and the coexistence of at least two selected deviations from normal: triglycerides ≥150 mg/dl or treatment for dyslipidemia, HDL cholesterol ≤40 mg/dl or treatment for dyslipidemia, blood pressure ≥130/85 mm Hg or treatment for hypertension, fasting plasma glucose ≥100 mg/dl or treatment for type 2 diabetes (Alberti at al., 2005). We calculated BMI, and in patients without diabetes - also insulin resistance (HOMA-IR) index according to the formula: HOMA-IR =fasting plasma glucose (mmol/l) x fasting plasma insulin level (μU/ml) / 22.5 (Matthews et al., 1985; Esteghamati et al., 2010; Radziuk, 2014).

Laboratory analysis

Blood for laboratory analysis was taken from a cubital vein on fasting between 7.30 and 9.00 o'clock. The blood was collected to blood collection tubes containing a gel separator and clot activator. Next it was centrifuged. Serum was kept at a temperature of -20°C. The levels of the following biochemical parameters: fasting plasma glucose (FPG), total cholesterol (TC), low-density lipoprotein (LDL), HDL, and TG were determined in serum by a spectrophotometric method using reagent kits (Biolabo, Aqua-Med, Łódź, Poland). The level of insulin-like growth factor-1 (IGF-1) was determined by ELISA reagent kits (DRG Medtec, Warsaw).

Morphological studies

Specimens of the prostate with hyperplasia were routinely fixed in 4% buffered paraformaldehyde, and embedded in paraffin. The paraffin blocks were sliced into 3 μm sections.

Immunohistochemical analysis

The analysis was performed on the sections taken from 60 men (30 BPH patients without MetS, and 30 BPH patients with MetS). To determine immuno-localization and immunoexpression of PCNA in the prostates of the

BPH patients, the prostate sections were deparaffinized and hydrated with various concentrations of alcohol, in descending order. The section preparations were then heated for 30 min in a water bath at a temperature of 96°C in Target Retrieval Solution buffer of pH 9.0 (Dako). Endogenous peroxidase activity was blocked with Peroxidase Blocking Solution (Dako, Glostrup, Denmark) for 10 min in a humidified chamber at room temperature. Next, the sections were incubated for 30 min in a humidified chamber with the monoclonal mouse antibody IgG against PCNA (clone PC10; Dako Denmark A/S, Glostrup, Denmark) diluted in the ratio of 1:200.

Then, the sections were incubated with a secondary antibody linked to horseradish peroxidase (Dako REAL™ EnVision™ Detection System Peroxidase/DAB+, Rabbit/Mouse, Dako). Diaminobenzidine (DAB) reagent was used to visualize the immunohistochemical reaction (Dako Denmark A/S). The sections were counterstained with Mayer's hematoxylin. After each step, the slides were rinsed with phosphate-buffered saline (PBS). The sections were dehydrated and closed in the medium Histo kit (Mar-Four, Lodz, Poland). The preparations were assessed using a light microscope (BX 41, Olympus Optical, Tokyo, Japan). All reactions were performed simultaneously under the same conditions.

Histochemical analysis

The analysis was performed on sections taken from 60 men (30 BPH patients without MetS, and 30 BPH patients with MetS). The TUNEL method was used to detect apoptosis (ApopTag® Peroxidase In Situ Apoptosis Detection Kit; Millipore™ , USA). The prostate sections were deparaffinized, hydrated, and digested with proteinase K (Dako, Dania). Endogenous peroxidase activity was blocked with Peroxidase Blocking Solution (Dako, Dania) for 10 minutes. Next, the sections were incubated with terminal deoxynucleotidyl transferase (TdT) for 60 minutes, and with digoxigenin antibodies for 30 minutes in a humidified chamber. A diaminobenzidine (DAB) reagent was used to visualize the immunohistochemical reaction (Dako Denmark A/S). The sections were counterstained with Mayer's hematoxylin, dehydrated, and closed in the histo kit. The sections were rinsed with phosphate-buffered saline (PBS) between consecutive steps of the procedure. The preparations were assessed using a light microscope (BX 41, Olympus Optical Co., Ltd., Tokyo, Japan).

Quantitative computer image analysis

Preparations were subjected to a scanning procedure (200× magnification, 0.25 μm resolution) with a ScanScope AT2 scanner (Leica Microsystems, Wetzlar, Germany). The digital images of the slides were analyzed on a computer screen using an Image Scope viewer (Version 11.2.0.780; Aperio Technologies, Inc., Vista, CA, USA). For the automatic computer analysis of the nuclear immunohistochemical PCNA and histochemical TUNEL reactions, nuclear v.9 algorithm (Version 11.2.0.780; Aperio Technologies, Inc.) was used.

To obtain reliable and repeatable values, the total number of PCNA and TUNEL positive cells was counted in 40 random fields with each reaction preparation. The average area of the fields is shown in Table 1.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0. The group was characterized by means, standard deviations, as well as minimum and maximum values. The normality of the distribution was examined using a Shapiro-Wilk test. The differences between the groups were assessed using the Mann-Whitney U test and Student's t-test. Correlations between the quantitative variables were calculated using Pearson's correlation coefficient. The level of significance was set at $p \leq 0.05$.

Results

In the analysis of differences between the parameters, MetS was accepted as a division criterion (Table 2). Statistically significant differences between MetS diagnostic parameters were demonstrated. The differences were observed in the patients' weight, BMI, and WC, as well as the serum levels of FPG, HDL, and TG. No differences were found in systolic and diastolic blood pressure (p=0.114 and p=211).

The analysis of lipid profile disorders showed that the patients with BPH and MetS had significantly higher LDL levels (p=0.05) than those with BPH and without MetS. It should be also emphasized that MetS in BPH patients had no influence on the severity of symptoms accompanying prostatic hyperplasia (p=0.182).

There were no statistically significant relationships between age and the levels of TC, HOMA-IR, and IGF-1.

The incidence of selected pathological entities was analyzed in the study sample, with MetS accepted as a division criterion. The study involved 38 patients (24.17%) diagnosed with and receiving treatment for

Table 1. Average surface area in which computer image analysis was performed (mm^2).

Reaction	Part of the prostate	Patients with BPH and with MetS	Patients with BPH and without MetS
PCNA(+)	Epithelial cells	0.86	1.17
	Stromal cells	2.26	1.96
TUNEL(+)	Epithelial cells	0.90	1.37
	Stromal cells	1.51	2.18

BPH, benign prostatic hyperplasia; MetS, metabolic syndrome; PCNA, nuclear proliferative protein; TUNEL, reaction to identification of apoptosis.

type 2 diabetes, 84 (55.63%) patients with hypertension, and 47 (31.13%) patients with lipid disorders.

In the study group, we analyzed how the presence of MetS in BPH patients was related to the number of TUNEL(+) and PCNA(+) cells (Table 3, Figs. 1, 2). However, the relationships between these parameters were not observed.

Correlations between the total number of apoptotic cells, and the values of selected anthropometric and metabolic parameters were analyzed (Table 4). It was found that the total number of TUNEL(+) cells in the prostate stroma correlated negatively with the levels of HDL (P=-0.39, p=0.03) and IGF-1 (P=-0.48, p=0.02).

No significant correlations were found between the number of TUNEL(+) cells and such variables as age, weight, BMI, WC, FPG, TG, TC, LDL, and a HOMA-IR index.

There was no statistically significant relationship between the number of PCNA(+) cells in the prostate and the values of the tested parameters.

Detailed correlations were also analyzed in the patients with regard to MetS presence (Table 5). In the group of patients with MetS, the number of TUNEL(+) cells in the prostate epithelium correlated negatively with the waist size (P=-0.53, p=0.02). It should be underlined that this relationship, being close to the level of statistical significance, was also observed in stromal cells (P=-0.50, p=0.06). In the same group of patients, the number of apoptotic cells in the epithelium correlated negatively with serum HDL level (P=-0.56, p=0.02).

It was demonstrated that IGF-1 correlated negatively

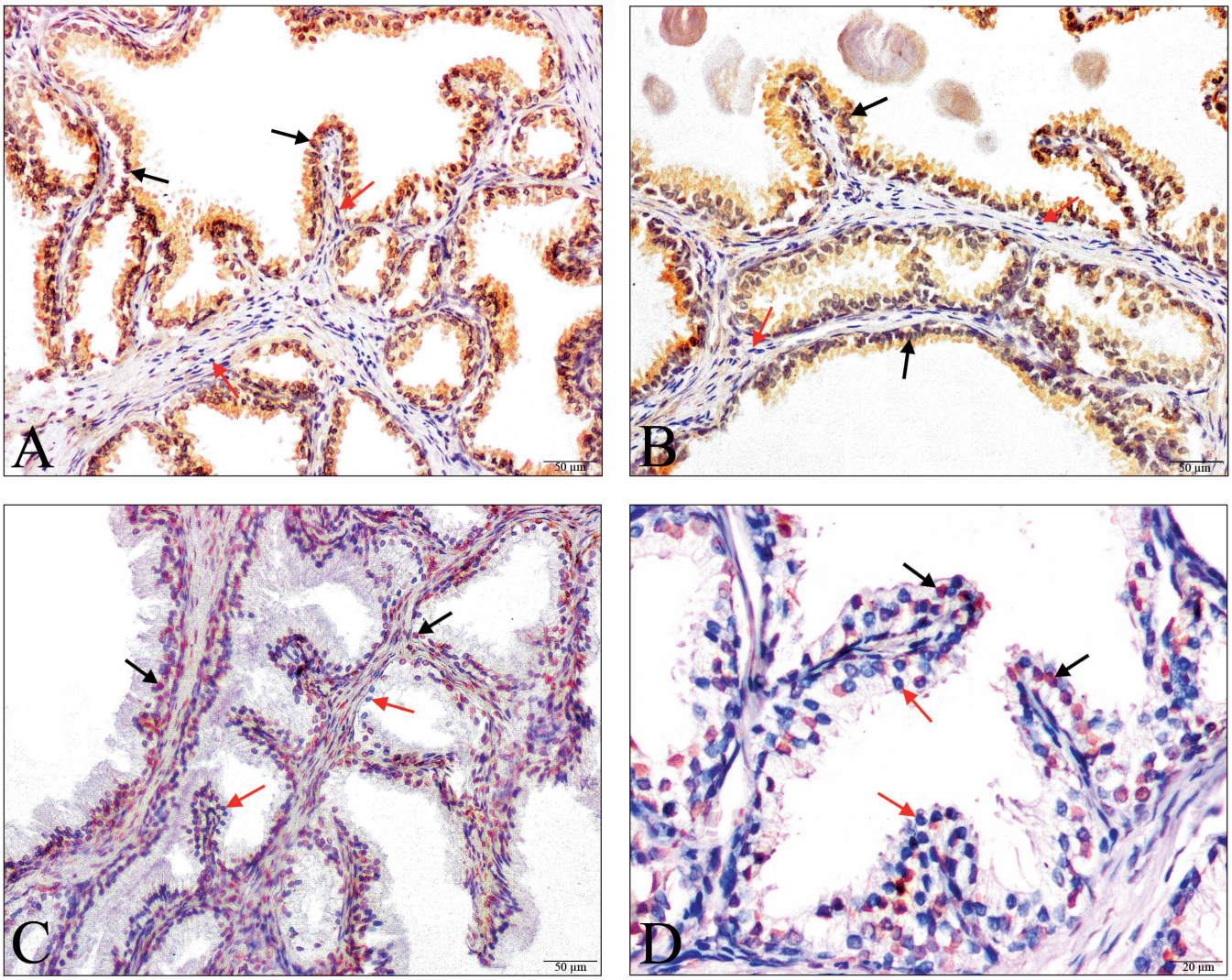

Fig. 1. Location of apoptosis marker in prostate cells (TUNEL) in men with BPH. **A and B.** Prostate from patient without MetS. **C and D.** Prostate from a patient with MetS. TUNEL(+) cells (black arrow), TUNEL(-) cells (red arrow). Scale bars: A-C, 50 μm; D, 20 μm.

Table 2. The relationships between anthropometric and biochemical parameters and the IPSS results in BPH patients with and without MetS.

Parameters	Patients with BPH and without MetS (n=60)				Patients with BPH and with MetS (n=91)				p
	X̄	SD	Min	Max	X̄	SD	Min	Max	
Age [Years]	67.66	8.41	55.00	85.00	67.12	8.24	52.00	89.00	0.729
Weight [kg]	76.52	15.03	51.50	165.00	88.14	12.76	60.00	120.00	<0.001*
BMI [kg/m^2]	25.21	6.26	0.00	60.61	29.39	3.96	21.26	39.18	<0.001*
WC [cm]	92.47	6.82	79.00	115.00	103.73	8.12	87.00	125.00	<0.001*
FPG [mg/dl]	98.25	11.87	79.00	148.00	105.95	16.03	74.00	164.00	0.015
APB-s [mmHg]	117.75	13.29	90.00	160.00	121.37	13.95	90.00	160.00	0.114
APB-d [mmHg]	76.25	9.55	60.00	90.00	78.20	10.00	55.00	90.00	0.211
HDL [mg/dl]	39.44	12.61	21.43	74.97	32.17	7.09	20.70	51.11	<0.001*
TG [mg/dl]	115.91	34.23	61.82	214.35	166.32	84.40	57.42	609.09	<0.001*
TC [mg/dl]	183.28	56.03	83.79	353.12	201.32	61.99	119.81	454.86	0.095
LDL [mg/dl]	118.53	51.26	18.10	287.19	138.70	61.77	38.99	405.31	0.050*
IPSS	19.27	9.83	0.00	34.00	18.51	8.61	0.00	34.00	0.182
HOMA-IR	4.22	5.14	0.11	23.86	6.55	6.88	0.23	27.90	0.060
IGF-1 [ng/ml]	117.47	36.68	41.58	207.70	132.17	56.70	41.97	351.10	0.387

BPH, benign prostatic hyperplasia; MetS, metabolic syndrome; BMI, body mass index; APB-s, systolic blood pressure; APB-d, diastolic blood pressure; WC, waist circumference; FPG, fasting plasma glucose; HDL, high-density lipoproteins; LDL, low-density lipoprotein; TC, Total cholesterol; TG, triglycerides; IPSS, International Prostate Symptom Score; HOMA-IR, homeostasis model assessment; IGF-1, insulin-like growth factor 1; SD, standard deviation; X̄, arithmetic average; Min, minimum; Max, maximum; p, statistical significance; *, parameter statistically significant.

Table 3. Comparison of the localization of apoptotic cells (detected by TUNEL method) and proliferating cells in the prostate in BPH patients with and those without MetS.

Parameters	Percentage of positive cells in prostate from patients with BPH and without MetS (n=30)				Percentage of positive cells in prostate from patients with BPH and with MetS (n=30)				p
	X̄ (%)	SD	Min	Max	X̄ (%)	SD	Min	Max	
TUNEL(+) reaction in epithelial cells	39.92	15.23	12.32	60.52	41.83	20.14	5.27	76.89	0.930
TUNEL(+) reaction in stroma cells	20.26	10.08	6.84	43.45	16.83	13.86	3.06	60.71	0.150
PCNA(+) reaction in epithelial cells	61.31	12.31	30.95	77.53	55.56	14.97	31.81	80.92	0.260
PCNA(+) reaction in stroma cells	34.11	9.92	16.02	48.18	28.47	11.42	13.00	45.34	0.120

BPH, benign prostatic hyperplasia; MetS, metabolic syndrome; SD, standard deviation; X̄, arithmetic average; Min, minimum; Max, maximum; p, statistical significance; PCNA, nuclear proliferative protein; TUNEL, reaction to identification of apoptosis.

Table 4. Correlations between anthropometric, metabolic and protein parameters, and the number of TUNEL(+) and PCNA(+) cells in prostatic stromal cells and gland epithelial cells in BPH patients.

Parameters	Prostate sections of patients with benign hyperplasia (n=30)			
	TUNEL(+) reaction in epithelial cells	TUNEL(+) reaction in stroma cells	PCNA(+) reaction in epithelial cells	PCNA(+) reaction in stroma cells
Age	-0.03 (0.90)	0.27 (0.16)	-0.13 (0.55)	-0.29 (0.14)
Weight	-0.09 (0.66)	-0.17 (0.40)	0.12 (0.56)	0.18 (0.36)
BMI	-0.22 (0.26)	-0.18 (0.36)	0.12 (0.56)	0.15 (0.45)
WC	0.15 (0.42)	0.13 (0.48)	0.10 (0.64)	0.16 (0.40)
FPG	0.01 (0.96)	-0.11 (0.63)	-0.16 (0.54)	-0.12 (0.59)
HDL	-0.28 (0.13)	-0.39* (0.03)	-0.20 (0.33)	-0.36 (0.06)
TG	-0.19 (0.33)	-0.31 (0.10)	-0.30 (0.13)	0.05 (0.78)
TC	-0.15 (0.43)	0.11 (0.58)	-0.29 (0.15)	0.02 (0.90)
LDL	-0.03 (0.86)	0.22 (0.25)	-0.21 (0.30)	0.08 (0.69)
HOMA-IR	-0.34 (0.14)	-0.23 (0.30)	0.38 (0.13)	0.35 (0.12)
IGF - 1	-0.24 (0.26)	-0.48* (0.02)	0.18 (0.42)	-0.02 (0.93)

P, Pearson correlation coefficient; (p), statistical significance; BPH, benign prostatic hyperplasia; PCNA, nuclear proliferative protein; TUNEL, reaction to identification of apoptosis; BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; HDL, high-density lipoproteins; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment; IGF-1, insulin-like growth factor 1.

Table 5. Correlations between selected anthropometric, metabolic and protein parameters and the number of TUNEL(+) and PCNA(+) cells in prostatic stromal cells and gland epithelial cells in BPH patients with and those without MetS.

Parameters	Prostate sections of patients with benign hyperplasia (n=30)							
	Patients without MetS		Patients with MetS		Patients without MetS		Patients with MetS	
	TUNEL(+) cells in epithelial	TUNEL(+) cells in stroma	TUNEL(+) cells in epithelial	TUNEL(+) cells in stroma	PCNA(+) cells in epithelial	PCNA(+) cells in stroma	PCNA(+) cells in epithelial	PCNA(+) cells in stroma
Age	0.16 (0.60)	0.46 (0.10)	-0.20 (0.46)	0.15 (0.61)	-0.44 (0.20)	-0.39 (0.21)	0.27 (0.33)	-0.12 (0.67)
Weight	-0.06 (0.84)	-0.20 (0.49)	-0.16 (0.57)	-0.45 (0.10)	-0.5 (0.27)	-0.41 (0.18)	0.27 (0.32)	-0.12 (0.67)
BMI	-0.31 (0.30)	-0.19 (0.51)	0.35 (0.18)	0.07 (0.81)	0.33 (0.34)	0.16 (0.60)	-0.05 (0.85)	-0.09 (0.73)
WC	-0.11 (0.70)	-0.22 (0.42)	-0.53* (0.02)	-0.50 (0.06)	-0.10 (0.77)	0.05 (0.86)	-0.03 (0.93)	-0.05 (0.86)
FPG	-0.21 (0.48)	-0.31 (0.26)	0.54 (0.21)	0.46 (0.29)	-0.21 (0.53)	-0.26 (0.39)	0.07 (0.90)	0.03 (0.94)
HDL	-0.20 (0.49)	-0.36 (0.19)	-0.56* (0.02)	-0.40 (0.14)	-0.14 (0.68)	-0.27 (0.38)	-0.10 (0.73)	-0.38 (0.15)
TG	-0.54 (0.07)	-0.42 (0.12)	-0.04 (0.88)	-0.27 (0.34)	-0.46 (0.16)	-0.05 (0.86)	-0.43 (0.11)	-0.06 (0.83)
LDL	0.06 (0.84)	0.33 (0.23)	-0.15 (0.60)	0.14 (0.63)	0.05 (0.89)	0.24 (0.44)	-0.41 (0.13)	-0.03 (0.91)
HOMA-IR	-0.38 (0.188)	-0.22 (0.44)	-0.30 (0.51)	-0.24 (0.60)	0.35 (0.29)	0.64* (0.04)	0.79 (0.06)	-0.03 (0.94)
IGF-1	-0.69* (0.01)	-0.47 (0.10)	0.03 (0.92)	-0.61* (0.03)	0.01 (0.97)	0.03 (0.92)	0.34 (0.28)	-0.18 (0.56)

P, Pearson correlation coefficient; (p), statistical significance; BPH, benign prostatic hyperplasia; MetS, metabolic syndrome; PCNA, nuclear proliferative protein; TUNEL, reaction to identification of apoptosis; BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; HDL, high-density lipoproteins; TG, triglycerides; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment; IGF-1, insulin-like growth factor 1; *, parameter statistically significant.

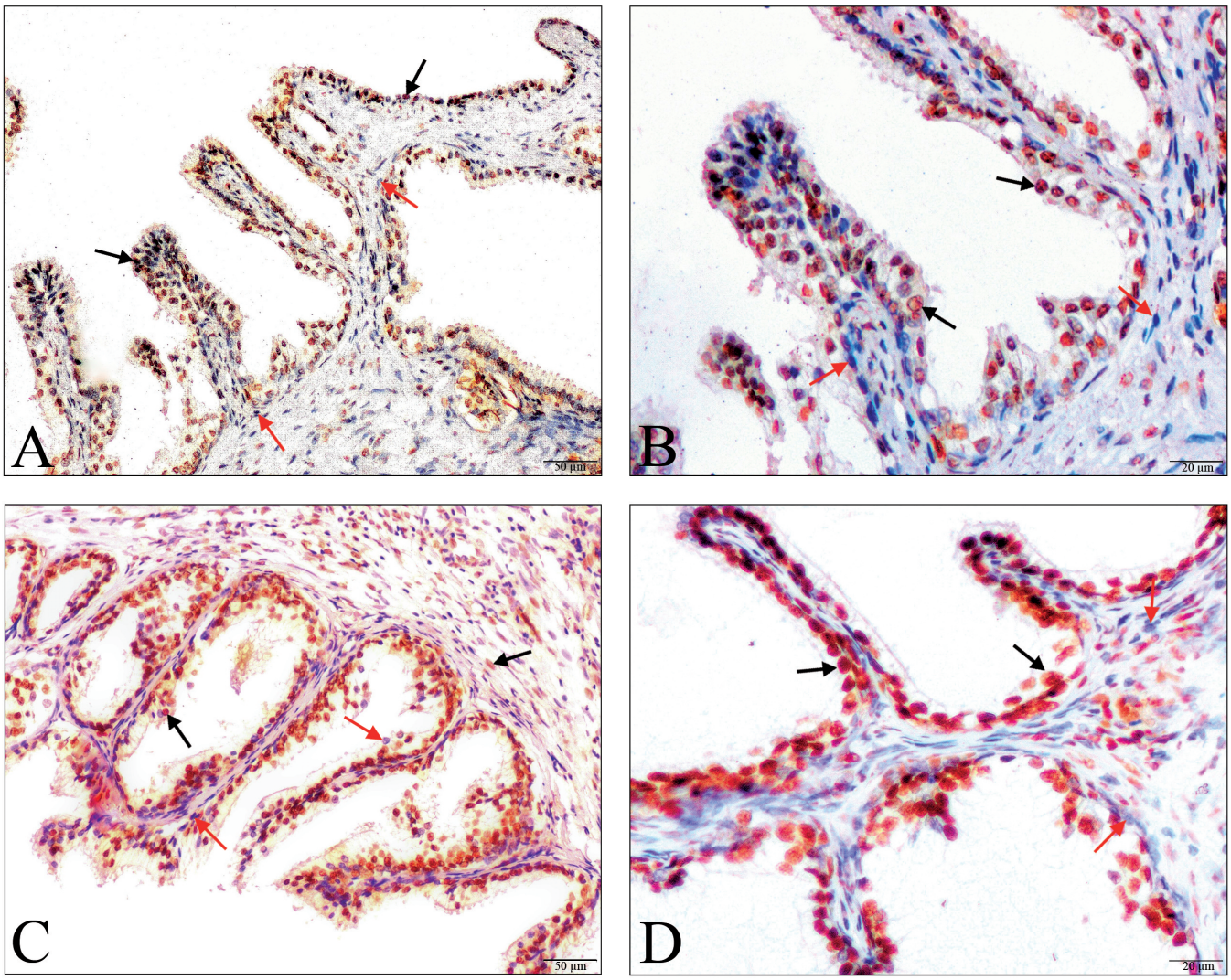

Fig. 2. Localization of cell proliferation markers (PCNA) in men with BPH. **A and B.** Prostate from patient without MetS. **C and D.** Prostate from a patient with MetS. PCNA(+) cells (black arrow), PCNA(-) cells (red arrow). Scale bars: A-C, 50 μm; D, 20 μm.

with the number of TUNEL(+) cells in the epithelium in patients without MetS (P=-0.69, p=0.01), and with the number of TUNEL(+) cells in the prostate stroma in patients with MetS (P=-0.61, p=0.03).

The analysis of the correlations in BPH patients with and without MetS (Table 5) demonstrated that the only parameter correlating with the number of PCNA(+) cells in the prostate stroma was a HOMA-IR index (P=0.64, p=0.04).

Discussion

Apoptosis plays an important role in the regulation of cellular growth, and maintaining tissue homeostasis. It involves a number of changes at the cellular level, leading as a consequence to the death of functionally impaired cells (Sebastiano et al., 2012). Analysis of the mechanism of apoptosis can be a vital part of BPH treatment. It is important because prostatic hyperplasia is a consequence of the disturbed ratio of the number of apoptotic cells to the number of proliferating cells (Alberto et al., 2009). BPH arises from proliferation of cells in the transition zone of the prostate gland, especially smooth muscles and prostate epithelial cells (Minutoli et al., 2014).

Epidemiological and clinical research has provided new evidence concerning a potential role that MetS and its components play in BPH and LUTS (Nunzio et al., 2012; Vanella et al. 2014). However, the relationship between metabolic disorders in BPH patients and the presence of apoptotic cells has so far not been clearly elucidated, and the available literature offers very few scientific publications on this subject. Our research demonstrated that in BPH patients, MetS had no impact on the expression of TUNEL(+) cells in the prostate gland cells. We found, however, that the number of TUNEL(+) cells in the prostate stroma correlated with the serum levels of HDL and IGF-1. This association was not observed in the analysis of the number of apoptotic cells in the prostate glandular epithelium. Since they show the connection between the HDL level and the etiology of BPH, these findings may confirm one of the hypothetical causes of BPH (Nandeesha et al., 2006). Nevertheless, there are also reports suggesting that hyperlipidemia is not related to the histological picture of the prostate (Zucchetto et al., 2005) and the severity of LUTS (Lekili et al., 2006).

Our study did not demonstrate the connection between a HOMA-IR index and the total number of TUNEL(+) cells in the epithelium and stroma of the prostate. Nonetheless, the literature provides evidence that insulin resistance is related to stronger expression of the receptors for IGF-1, which is a strong mitogen and has anti-apoptotic effects. This seems to be essential for analysis of factors raising the risk of BPH in patients with MetS (Ngo et al., 2003). What is more, animal model-based studies confirm increased cell apoptosis and prostatic atrophy symptoms in rats with hyperinsulinemia (Arcolino et al., 2010; Vikram et al., 2010b).

It is worth emphasizing that in our study, the number of TUNEL(+) cells in the patients with BPH and MetS correlated negatively with the patients' abdominal circumference. In another analysis of this relationship, obesity was taken into account as a factor predisposing to insulin resistance (De Pergola and Silvestris, 2013). It may support the theory that abdominal circumference contributes to apoptosis in the prostate cells in BPH patients. Abdominal obesity and hyperinsulinemia were also analyzed as factors associated with an increase in the circulating IGF-1 level. Gallagher and LeRoith (Gallagher and LeRoith, 2010) assert that the number of growth hormone receptors can rise due to elevated blood insulin levels. This state has an impact on the transmission of signals by receptors to IGF-1 in the cells in patients with hyperinsulinemia. A result of these changes is inhibition of cell apoptosis (De Pergola and Silvestris, 2013).

In our study, MetS diagnosed in BPH patients had no effects on the localization and expression of PCNA(+) cells in the prostate. However, there was a positive correlation between a HOMA-IR index and the number of PCNA(+) cells in the prostatic stroma of BPH patients with MetS. The animal-model-based research performed by Bahey et al. (2014) revealed that diabetes in animals induced a visible increase in the cell apoptosis index, and was also associated with the reduced height of the epithelium and the lower number of androgen receptors. Nonsignificant changes in the proliferation pace were observed in epithelial cells, as compared to the cells in rodents without diabetes.

Studies of PCNA(+) cells are based on evaluation of the links between prostate neoplastic cells and cells found in the prostate of BPH patients. In these studies, BPH patients usually constitute control groups.

The most popular markers used to determine the level of cell proliferation in the prostate tissue are PCNA and Ki-67. Ki-67 is considered to be a classic marker of cell proliferation and is routinely applied in pathological examinations (Oka et al., 2011; Salehinejad et al., 2011). We decided to employ the PCNA marker in our study owing to the available literature reports proving its usefulness as a first line marker for proliferation of non-malignant cells. This marker is closely related to the clinical stage of the disease, and its expression can also suggest extensive epithelial remodeling, which is associated with the histopathological picture of BPH (Zhong et al., 2008).

Nonetheless, there are few reports on the relationship between PCNA markers and the levels of biochemical parameters in patients with BPH. Such research was conducted by Zhong et al. (2008), who demonstrated that expression of Ki-67 and PCNA markers was stronger in patients with tumors and those with BPH, than in healthy prostate gland tissues. At the same time, the ratio of the expression of both investigated markers in the tissue of cancerous prostate was higher than in the prostates of men with BPH.

MetS in BPH patients had no impact on the number

of TUNEL(+) and PCNA(+) cells in the prostate gland. However, disturbed levels of metabolic parameters, and deviations of anthropometric parameters from normal may influence the number of apoptotic cells and the number of proliferating cells. Therefore, analysis of the processes associated with the TUNEL(+) and PCNA(+) cells with regard to metabolic disorders in BPH patients seems to be essential, and potentially contributes to better understanding of the pathomechanism of BPH. Education of patients and a healthy change in their lifestyles, manifested by improvement of basic metabolic parameters, might also be of benefit in the prevention of hyperplastic lesions in the prostate.

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