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Jacek Grudny[1], Jacek Kołakowski[2], Marcin Kruszewski[3], Janusz Szopiński[1], Paweł Śliwiński[2], Elżbieta Wiatr[1], Jolanta Winek[1], Jolanta Załęska[1], Jacek Zych[1], Kazimierz Roszkowski-Śliż[1]

[1]Third Department of Lung Diseases, Institute of Tuberculosis and Lung Diseases, Warsaw
Head: Prof. K. Roszkowski-Śliż, MD, PhD
[2]Department of Diagnosis and Treatment of Respiratory Insufficiency, Institute of Tuberculosis and Lung Diseases, Warsaw
Head: Prof. P. Śliwiński, MD, PhD
[3]Radiobiology and Biological Dosimetry Centre, Institute of Nuclear Chemistry and Technology
Head: Prof. M. Kruszewski, PhD

Association of genetic dependences between lung cancer and chronic obstructive pulmonary disease

Uwarunkowania genetyczne koincydencji raka płuca i przewlekłej obturacyjnej choroby płuc

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Abstract

Introduction: Recent studies have shown an increased risk of lung cancer in patients with bronchial obstructive changes, including patients with COPD. It seems that there are common factors of pathogenesis of both diseases associated with oxidative stress. In the present paper the genes linked to the repair of oxidative damage of DNA, associated with cancer, of iron metabolism and coding proteolytic enzymes were assessed. **Material and methods:** The study was conducted in two groups of patients: 53 patients with non-small cell lung cancer and chronic obstructive pulmonary disease, and 54 patients only with chronic obstructive pulmonary disease. The polymorphisms of the single nucleotide were determined in the case of the majority of genes using the PCR-RFLP method. The statistical analysis of quantitative variables was executed using the Mann-Withney U-test and the test of medians; the analysis of genetic variables was executed using the χ^2 test. **Results:** Regarding the polymorphisms of genes involved in iron metabolism, statistically significant differences between the two groups have been demonstrated only in the case of haptoglobin gene HP1/2. A higher incidence of form 1/1 was found in patients with COPD and a higher incidence of form 1/2 in patients with lung cancer and COPD. Analysis of gene polymorphisms of proteolytic enzymes and inhibitors of the enzyme gene showed statistically significant differences between the two groups only for the MMP3 gene 6A/5A. In the case of the MMP12 gene polymorphism (A-82G) a tendency toward differences in the occurrence of specific alleles was identified. **Conclusions:** These results indicate that patients with coincidence of COPD and lung cancer have disorders of the genes involved in iron metabolism, and they have different genetic polymorphisms of proteolytic enzymes comparing to COPD patients.

Key words: lung cancer, chronic obstructive pulmonary disease (COPD), oxidative stress, genetic polymorphism, haptoglobin, metalloproteinases

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Streszczenie

Wstęp: Dotychczasowe wyniki badań wskazują na zwiększone ryzyko zachorowania na raka płuca u osób ze zmianami obturacyjnymi oskrzeli, w tym u chorych na przewlekłą obturacyjną chorobę płuc (POChP). Wydaje się, że istnieją wspólne

Adres do korespondencji: Jacek Grudny MD, PhD, Third Department of Lung Diseases, Institute of Tuberculosis and Lung Diseases, ul. Płocka 26 01–138 Warszawa, Tel.: +48 22 431 21 08, Fax: + 48 22 431 24 08, e-mail: j.grudny@igichp.edu.pl

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czynniki patogenetyczne obu chorób związanych ze zjawiskiem stresu oksydacyjnego. Przedmiotem oceny stały się geny związane z procesami naprawy oksydacyjnych uszkodzeń DNA, geny związane z nowotworami, z metabolizmem żelaza i geny enzymów proteolitycznych.

Materiał i metody: Badanie przeprowadzono w dwóch grupach pacjentów liczących łącznie 107 osób: 53 chorych na raka niedrobnokomórkowego płuca i POChP oraz 54 chorych tylko na POChP. W przypadku większości genów oznaczono polimorfizm pojedynczego nukleotydu metodą analizy długości fragmentów restrykcyjnych (RFLP). Analizy statystycznej zmiennych ilościowych dokonano testem *U* Manna-Whitneya i testem median, analiza zmiennych genetycznych została dokonana testem Chi-kwadrat.

Wyniki: W przypadku polimorfizmu genów związanych z metabolizmem żelaza istotne statystycznie różnice pomiędzy badanymi grupami wykazano jedynie w przypadku genu haptoglobiny Hp1/2. Stwierdzono częstsze występowanie formy 1/1 w grupie chorych na POChP i częstsze występowanie formy 1/2 w grupie chorych na raka płuca i POChP. Analiza polimorfizmu genów enzymów proteolitycznych i genu inhibitora tych enzymów wykazała istotne statystycznie różnice pomiędzy badanymi grupami jedynie w przypadku genu metaloproteinazy MMP3 6A/5A. W przypadku polimorfizmu genu metaloproteinazy MMP12 (A-82G) różnice w występowaniu poszczególnych alleli określono na poziomie tendencji.

Wnioski: Wyniki te wskazują, że u chorych z koincydencją POChP i raka płuca występowały różnice w stosunku do grupy chorych na POChP dotyczące genów związanych z metabolizmem żelaza oraz genów enzymów proteolitycznych.

Słowa kluczowe: rak płuca, przewlekła obturacyjna choroba płuc (POChP), stres oksydacyjny, polimorfizm genetyczny, haptoglobina, metaloproteinazy

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Introduction

The increased risk of lung cancer in patients with chronic obstructive pulmonary disease is more and more frequently becoming the subject of recent research projects. The results of research published in 1977 by Cohen et al. suggested an increased risk of the occurrence of respiratory tract obstruction in first line relatives of lung cancer patients and in relatives with COPD, regardless of tobacco smoking or other risk factors [1]. In 1986 a prospective research project was published. It consisted of monitoring for 10 years, from the point of view of lung cancer incidence, two groups of people who smoked cigarettes: patients with COPD ($FEV_1 < 70\%$) and a control group with $FEV_1 > 85\%$ of the predicted value. The incidence of lung cancer among patients with COPD was 8.8% and among the control group it was 2% [2]. The research carried out by Tockman et al. in 1987 showed an increased risk of lung cancer occurrence along with deterioration of lung function and a drop in FEV_1 [3]. More frequent lung cancer occurrence in patients with low values of FEV_1 and the increase of risk dependent on lowering of this parameter has also been shown in a study from 1994 that assessed the American population [4]. Similar conclusions have been drawn by other researchers [5, 7], who, additionally, showed an increased risk of mortality from lung cancer dependent on FEV_1 impairment degree. In The First National Health and Nutrition Examination Survey (follow-up research conducted on a group of 5402 people) it was established that former smokers with moderate COPD had a higher risk of lung cancer incidence (6.7%) in comparison with the whole group of former smokers in whom the risk was two times lower [6].

The above-mentioned data allow the conclusion to be drawn that COPD may be an independent factor that contributes to the development of lung cancer, and the frequency of the occurrence of this neoplasm probably depends on COPD progression. It has been revealed that diagnosis of COPD increases the risk of lung cancer incidence irrespective of tobacco smoking [8], and it has been suggested that it does not depend on age or sex, while in former smokers the probable pathogenic mechanism for both diseases is a chronic inflammatory process of the respiratory tract [9].

Among the pathogenic processes that are responsible for development of changes in respiratory tract in the course of COPD, neutrophilic inflammation, disorders of proteases/antiproteases system equilibrium, and oxidative stress caused by overproduction of oxygen free radicals and depletion of antioxidative reserves should be listed [10]. During the course of oxidative stress, DNA may be damaged due to nitrogenous base oxidation, DNA strand rupture and chromosomes injuries — it may consequently lead to disorders during the process of genetic material replication, which in turn may cause the death of cells or neoplastic mutation. The process of creation of oxygen radicals happens frequently in the presence of iron ions Fe_2+; therefore, disorders of homeostasis of this microelement may be of vital significance. It is supposed that in the cells that undergo oxidative stress, intercellular changes in iron and copper homeostasis occur.

The repair of damaged DNA, among others, with the help of BER enzymes, which remove the damaged base, or with the help of NER enzymes, which remove damaged DNA fragment, is an organism's natural defence mechanism against the effects of oxidative stress. Another known means of DNA repair is DSBR, which is connected with the repair of double-strand breaks [11, 12]. A key role in the repair of damaged DNA caused by oxidative stress in people exposed to tobacco smoke is attributed to the BER system [13]. Polymorphism of the genes that code these enzymes and their reduced activity may influence the development of neoplastic processes [14].

Transcription and growth factors such as proteins p53, p21 and TNF-α play a crucial role in carcinogenesis; the appearance of mutation or disturbances of gene expression of these factors influence the development of both lung cancer and COPD. Genes coding proteolytic enzymes of metalloproteinase (MMP) and the MMP inhibitors (TIMP) may be involved in COPD. MMP not only influences the alteration of stromal connective tissue in the course of inflammatory processes and necrosis, but also facilitate angiogenesis and the growth of neoplastic tumours. The MMP-1 overexpression is associated with tumour invasion and metastasis formation [16]; in research conducted by Sue et al. it was suggested that polymorphism of the MMP-1 gene, of certain MMP-3 and MMP-12 genes, haplotypes play a role in increased risk of lung cancer occurrence [17, 18].

The purpose of this study was to assess the influence of genetic factors on lung cancer and COPD occurrence. Polymorphism of genes connected with the repair processes of DNA oxidative damage and polymorphism of genes connected with neoplasms, iron and proteolytic enzymes metabolism have been assessed; subsequently the genetic variation in the group of patients with COPD and the group with COPD and non-small cell lung cancer were assessed.

Material and methods

The research was conducted on two groups of patients randomized in respect of sex, age, tobacco addiction and recognition of the disease. The patients were diagnosed and treated in the Institute of Tuberculosis and Lung Diseases in Warsaw in the years 2007-2009. The first group (Group I) consisted of patients with COPD and non-small cell lung cancer, whereas the second group (Group II) consisted of patients only with COPD. Only men at the age of the highest incidence of lung cancer and COPD, i.e. age 50–70-year-old, participated in the research. Tobacco addiction was measured in pack-years.

COPD diagnosis was established on the grounds of medical history, symptoms of disease and additional examinations including spirometric testing in accordance with established international criteria GOLD. Lung cancer diagnosis was made on the grounds of histopathological examination of: a) tumour specimens taken during bronchoscopy, b) punctate taken from mediastinal lymph nodes during EBUS, c) punctate taken from tumours during needle biopsy through the chest wall, d) mediastinoscopy and e) postoperative examination of removed tumours. The research was conducted in the Pathomorphology Department of the Institute of Tuberculosis and Lung Diseases.

Clinical assessment of respiratory tracts

All patients underwent spirometry with diastolic test, pulmonary function tests with DLCO, walk test, gasometry at rest, chest X-ray and computer tomography of the chest. Spirometric testing was conducted in the Lung Function Department of the Institute of Tuberculosis and Lung Diseases using MedGraphics apparatus. The research procedure was held in accordance with ERS/ATS international standards. Reversibility test was made after inhalation of salbutamol, administered in four separate doses of 100 micrograms, which were given at 30 seconds intervals. The increase in FEV_1 or FVC exceeding 200 ml and 12% of predicted value for these parameters was recognized as a positive result of the test. Body plethysmography was made using Master Screen Body apparatus. RV/TLC > ULN (upper limit of normal) was recognized as pulmonary hyperinflation, whereas VA/TLC < 0.85 was recognized as an index of disturbance of gas distribution. Assessment of DLCO impairment was based on the percentage predicted value of this parameter.

Gasometry was carried out in the Department of Laboratory Diagnosis in the Institute of Tuberculosis and Lung Diseases with the help of a pH and blood gases analyser 248 from Ciba Corning Diagnostic. Walk test was conducted in the Second Department of Lung Diseases in the Institute of Tuberculosis and Lung Diseases in accordance with ATS standards; it consisted of covering the longest possible distance during a six-minute walk in an indicated part of the hospital corridor. O_2 saturation was measured with the help of percutaneous method and pulse oximeter before testing, in the course thereof and after it; the covered distance was estimated.

Imaging studies included: chest radiological examination (RTG) and computer tomography (CT). All examinations were carried out in the Radiology Department of the Institute of Tuberculosis and Lung Diseases. Computer tomography of the chest was performed using CT Somaton Sensation 16 apparatus from Siemens, with the use of the spiral technique with intravenous administration of non-ionic contrast medium.

Evaluation of genetic polymorphism

Examination of gene polymorphism was executed in the Department of Radiobiology and Health Care in the Institute of Nuclear Chemistry and Technology in Warsaw. Single nucleotide polymorphisms (SNP) were determined in the majority of genes. The method of analysis of restriction fragment lengths, which uses restriction fragment length polymorphism (RFLP), was applied. This method consists of digesting amplified DNA fragments using the PCR technique, which includes the place of occurrence of polymorphism, with a suitable restrictive enzyme. The tested SNP occurs in a sequence that is recognized by the enzyme which cuts the DNA molecule only in the presence of one allele. Multiplex PCR technique, which uses another gene for positive checking, was used for polymorphism examination consisting of gene deletion. Gene promoter polymorphisms that consisted of d(CA) or d(GG) sequence duplication were tested using the PCR method (Tab. 1).

Statistical analysis

Statistical analysis of quantitative variables was carried out using the Mann-Withney *U* test (nonparametric test) and the test of medians. The value of $p < 0.05$ was taken as the significance level. Additionally, in order to evaluate the difference between distributions in the groups, the Kolmogorov-Smirnov test was used. Statistical evaluation of genetic variables was done with the use of the χ^2 test; the results include numerical and percentage values.

Results

Group I consisted of 53 patients. The average pack-years in this group was 49.72. Group II consisted of 54 patients. The average pack-years in this group was 42.28. The difference in tobacco exposure was not statistically significant. Spirometric examination showed more advanced changes in lung function in the course of COPD in Group II — the assessment of forced expiratory volume in 1 second in patients from Group II revealed significantly decreased parameters in comparison with Group I. Group II also had a lower average value of forced vital capacity FVC (Tab. 2). Squamous carcinoma was diagnosed in 10 patients from group I, adenocarcinoma in 6 patients, and large cell carcinoma in 3 patients. Non-small cell cancer without precise definition of histopathological type was diagnosed in the remaining 34 patients.

Greater hypoxemia (average values pO_2 60.94 mm Hg vs. 69.47 mm Hg) and hypercapnia (average values pCO_2 44.48 mm Hg vs. 37.52 mm Hg) were noted in Group II. More serious disorders of gas exchange were correlated with the highest COPD stage. The degree of pulmonary hyperinflation was highest in patients from Group II (Tab. 3).

Lung imaging in the group with cancer and COPD showed the presence of radiological features of COPD in 25 patients (47.17%), whereas in the group with COPD only, such features were noted in 45 patients (83.33%).

DNA repair genes, which remove the damage caused by genetic mutations, were assessed first. Single nucleotide polymorphisms (SNP) were determined in the majority of repair genes. Polymorphism of the following genes was determined: XRCC1 Arg194Trp, XRCC1 Arg399Gln, hOGG1 Ser326Cys, MYH Y165C, MYH G382D, XPA 5'UTR A(-4)G, XPD Lys751GlnA/C, XRCC3 Thr241Met C/T, XRCC4 Ile401Thr. No significant differences in the polymorphism of these genes between the groups were noted.

The next group of evaluated genes were protein genes connected with neoplasms. Polymorphism of the following genes were determined: TP53 Arg72Pro, CDKN1A Ser31Arg, CDKN1A Ln2 C/G, CDKN1A UTR C/T, TNF-α, G-308A. No significant differences in the polymorphism of these genes between the evaluated groups was noted.

Genes connected with iron metabolism, i.e. haptoglobin, hepcidin and ferroportin, were also included in the research (Tab. 4); disturbances of iron homeostasis, which catalyses reactions of creation of some oxygen radicals, may be of some significance from the point of view of oxidative stress. The presence of alleles of the haptoglobin gene was determined using the PCR method; the difference between the alleles Hp1 and Hp2 concerned the presence of a duplicated DNA segment with a length of 1,700 base pairs in the Hp2 allele.

The research results showed that in the case of the haptoglobin gene HP1/2 the occurrence of the 1/1 allele was more common in the group of patients with COPD, whereas the 1/2 allele was more frequently found in patients with lung cancer and

Table 1. Polymorphisms of genes

Gene	Gene's ID	Determined polymorphism
MMP1 Matrix metallopeptidase 1 (interstitial collagenase)	4312	-1607 1G/2G
MMP3 Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	4314	-1171 5A/6A
MMP9 Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	4318	C-1562T
MMP12 Matrix metallopeptidase 12 (macrophage elastase)	4321	Asn357Ser A-82G
TIMP2 TIMP metallopeptidase inhibitor 2	7077	G-418C
TNF-α Tumour necrosis factor (TNF superfamily, member 2)	7124	G-308A
SLC40A1 (alias: ferroportin) Solute carrier family 40 (iron-regulated transporter), member 1	30061	Q248H
XPA Xeroderma pigmentosum, complementation group A	7507	5' UTR A-4G
XPD (ERCC2) Excision repair cross-complementing rodent repair deficiency, Complementation group 2	2068	Lys751Gln
XRCC3 X-ray repair complementing defective repair in Chinese hamster cells 3	7517	Thr241Met
TP53 Tumour protein p53	7157	Arg72Pro
CDKN1A Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1026	Ser31Arg C/G intron 2 rs3176352 C/T 3'UTR rs1059234
MUTYH mutY homolog (E. coli)	4595	Tyr165Cys Gly382Asp
XRCC1 X-ray repair complementing defective repair in Chinese hamster cells 1	7515	Arg194Trp Arg399Gln
OGG1 8-oxoguanine DNA glycosylase	4968	Ser326Cys
XRCC4 X-ray repair complementing defective repair in Chinese hamster cells 4	7518	Ile401Thr
HAMP Hepcidin antimicrobial peptide	57817	Gly71Asp
HP Haptoglobin	3240	Hp1/Hp2

COPD. The 2/2 allele was slightly more common in patients with COPD; these differences were significant. The determined polymorphisms of the hepcidin gene HAMP (Gly71Asp) and ferroportin SLC40A1 (Q248H) demonstrated the occurrence of only one kind of genotype in all the investigated patients.

The last examined group of genes were genes of proteolytic enzymes MMP (metalloproteinases)

Table 2. Patients' characteristics with spirometry

Medical factors	Group I (lung cancer + COPD) n = 53	Group II (COPD) n = 54	p
Age (years)	60.60 ± 6.16	61.93 ± 5.74	NS
Height [cm]	173.08 ± 5.70	169.93 ± 7.60	NS
Weight [kg]	75.31 ± 15.35	82.43 ± 22.03	NS
BMI [kg/m^2]	25.10 ± 4.70	28.64 ± 7.87	$p < 0.05$
FEV_1 [L]	1.95 ± 0.75	1.10 ± 0.51	$p < 0.001$
FEV_1%	61.19 ± 21.98	35.98 ± 16.34	$p < 0.001$
FVC [L]	3.49 ± 0.98	2.58 ± 0.79	$p < 0.001$
FVC%	86.32 ± 23.77	65.04 ± 20.4	$p < 0.001$
FEV_1/FVC (%)	55.26 ± 10.79	43.32 ± 11.35	$p < 0.001$

Table 3. Gasometry at rest, gasometry after exertion and plethysmography

Medical factors	Group I (lung cancer + COPD)	Group II (COPD)	p
pH	7.43 ± 0.02	7.41 ± 0.03	$p < 0.001$
pO_2 [mm Hg]	69.47 ± 8.44	60.94 ± 8.93	$p < 0.001$
pCO_2 [mm Hg]	37.52 ± 4.23	44.48 ± 8.98	$p < 0.001$
HCO_3	24.67 ± 2.25	27.62 ± 3.66	$p < 0.001$
$satO_2$ (%)	94.08 ± 1.86	90.79 ± 3.88	$p < 0.001$
spO_2 max (%)	95.77 ± 2.13	92.16 ± 4.60	$p < 0.001$
spO_2 min (%)	90.0 ± 6.62	83.92 ± 6.68	$p < 0.001$
TLC [L]	7.57 ± 1.53	8.47 ± 1.48	$p < 0.05$
TLC (%)	113.22 ± 21.79	130.90 ± 22.26	$p < 0.001$
RV [L]	3.84 ± 1.27	5.34 ± 1.48	$p < 0.001$
RV (%)	163.10 ± 52.78	226.78 ± 61.07	$p < 0.001$
Raw [L]	0.34 ± 0.18	0.62 ± 0.29	$p < 0.001$
Raw (%)	113.57 ± 58.73	206.54 ± 100.15	$p < 0.001$
DLCO [L]	5.70 ± 1.91	4.23 ± 2.09	$p < 0.01$
DLCO (%)	62.17 ± 19.87	47.59 ± 22.17	$p < 0.01$

and the enzyme inhibitor thereof, TIMP2. In the group of metalloproteinases the polymorphisms of the 1st, 3rd, 9th and 12th metalloproteinase were determined (Tab. 5).

Significant differences were observed concerning the polymorphism of the MMP3 gene (6A/5A). The 5A/5A homozygote was present twice as frequently in patients from Group I as in group II. In this group the occurrence of the 6A/6A homozygote was also observed twice as frequently comparing with Group II (18 vs 9), while the 6A/5A genotype was present in Group II almost twice as frequently comparing with Group I. Analysis of the MMP3 gene polymorphism proved a statistical significance. Analysis of research results also revealed certain differences in the polymorphism of the MMP12 gene. In the case of MMP12 polymorphism (A-82G), a frequent occurrence of the A/G allele was shown in patients with lung cancer and COPD. The

Table 4. Polymorphism of genes connected with iron metabolism

Gene	SNP	Genotype	Group I n (%)	Group II n (%)	All patients	p
HP	Hp 1/2	1/1	5 (31.3)	11 (68.8)	16	0.03563
		1/2	27 (64.3)	15 (35.7)	42	
		2/2	21 (42.9)	28 (57.1)	49	
HAMP	Gly71Asp	Gly/Gly	53 (49.5)	54 (50.5)	107	
SLC40A1 (ferroportin)	Q248H	Gln/Gln	53 (49.5)	54 (50.5)	107	

Table 5. Polymorphism of genes of proteolytic enzymes and of their inhibitor

Gene	SNP	Genotype	Group I n (%)	Group II n (%)	All patients	P
MMP1	1607 1G/2G	1G/1G	12 (41.4)	17 (58.6)	29	NS
		1G/2G	26 (55.3)	21 (44.7)	47	
		2G/2G	15 (48.4)	16 (51.6)	31	
MMP3	6A/5A	5A/5A	16 (64.0)	9 (36.0)	25	0.00608
		6A/5A	19 (34.5)	36 (65.5)	55	
		6A/6A	18 (66.7)	9 (33.3)	27	
MMP9	C-1562T	C/C	42 (51.2)	40 (48.8)	82	NS
		C/T	11 (45.8)	13 (54.2)	24	
		T/T	0 (0.0)	1 (100.0)	1	
MMP12	Asn357Ser	Asn/Asn	51 (51.0)	49 (49.0)	100	NS
		Asn/Ser	2 (28.6)	5 (71.4)	7	
	A-82G	A/A	36 (43.9)	46 (56.1)	82	0.05376
		A/G	14 (63.6)	8 (36.4)	22	
		G/G	3 (100.0)	0 (0.0)	3	
TIMP2	G-418C	G/G	53 (49.5)	54 (50.5)	107	

A/A allele was more frequent in patients with COPD, whereas the G/G allele was present only in patients from Group I (cancer + COPD). These differences were not statistically significant. The determined polymorphism of the gene inhibitor of metalloproteinases TIMP2 (G/C) demonstrated the occurrence of only one G/G genotype in all the examined patients in both groups.

Discussion

In order to obtain maximum homogeneity of the investigated groups, patients qualified for the research were chosen in respect of sex, age, tobacco smoking addiction and diagnosis of a disease. Occupational exposure to harmful agents or family history of hereditary disease were not taken into account during the research. The examined patients were diagnosed with COPD in different stages; patients with mild or moderate degree of disease predominated in Group I, whereas patients with serious or very serious COPD predominated in Group II. However, for the evaluation of coincidence of genetic predisposition between lung cancer and chronic obstructive pulmonary disease (genotypic evaluation), such selection of patients seemed to be acceptable.

The main purpose of this study was to determine whether the polymorphism of genes that participate in the defence mechanism against oxidative stress influence the coincidence of lung cancer and chronic obstructive pulmonary disease. Genes connected with the repair of da-

maged DNA were analysed first. In the scope of the BER system the polymorphism of three genes: XRCC1, hOGG1 and MYH was assessed, the research did not reveal any statistically significant differences between the two compared groups. The multicentre research conducted by Matullo et al., published in 2006, suggested a protective effect of the Gln/Gln homozygote of the XRCC1 Arg399Gln gene in lung cancer [12], and similar results were obtained by Spanish authors in 2007 [19]. The present research did not prove such a dependence. Analysis of hOGG1 Ser326Cys gene polymorphism showed it to be the least frequent occurrence of the Cys/Cys homozygote in the two groups of patients; the research conducted among the Chinese population [15] showed an increasing risk of occurrence of COPD among patients with this genotype; such a dependence has also been suggested for the Gln/Gln genotype of the XRCC1 Arg399Gln gene among active tobacco smokers and for the Arg/Gln genotype among heavy smokers. Analysis of polymorphism of DNA repair genes of the NER system included two genes: XPA and XPD. The determined polymorphism of these genes did not show any statistically significant differences in the two examined groups of patients. Previous research projects have suggested a probable dependence of XPD Lys751Gln polymorphism on the risk of lung cancer occurrence in patients who smoke cigarettes (an increased risk of lung cancer occurrence in carriers of trait CC and CA vs. AA). Such a dependence was also observed in other research projects in former smokers who were carriers of the Gln/Gln homozygote and the Lys/Gln heterozygote [12, 21]. Analysis of DNA repair gene polymorphism connected with the DSBR repair system did not show any statistically significant differences either. XRCC3 Thr241Met gene polymorphism has been mentioned hitherto as a polymorphism that night be connected with the occurrence of lung cancer in smokers [21]. The mentioned study suggested a possible interaction of this gene with the XRCC--1-Arg399Gln and XPD-Lys751Gln genes.

The next group of analysed genes was connected with the process of neoplastic transformation. Evaluation has concerned genes coding the p21 protein, the p53 protein and tumor necrosis factor TNF-α. CDKN1A gene coding of the p21 protein demonstrated polymorphism of three codons: Ser31Arg, In 2C/G and UTR C/T. None of the determined polymorphisms was distinguished by statistical significance in the frequency of occurrence of individual genotypes. The p21 protein is a kinase inhibitor; it also influences division processes in cell growth cycle and participates in DNA repair regulation — disturbances of regulation of p21 concentration may influence the process of neoplastic transformation [22]. Several published studies concerning polymorphism of the p21 Ser31Arg gene did not find any dependence between the occurrence of definite genotypes or any increased risk of lung cancer occurrence [23, 24]. Yao-Ling et al. in their research of 2006 concerning polymorphism of the p21 and p53 genes in patients with COPD (vs. healthy humans) showed an increased risk of the COPD occurrence in tobacco smokers and patients with the Arg/Arg and Ser/Arg genotype of the p21 Ser31Arg gene [25].

The majority of studied mutations of the TP53 gene are point mutations that lead to a change in a single amino acid, or they are deletions of a gene fragment. The TP53 gene codes the p53 protein, which is called a guard of the genome due to its function of sensor of DNA damage. The research conducted by Yao-Ling et al. showed an increased risk of COPD occurrence in tobacco smokers with the Pro/Pro and Arg/Pro genotype of the TP53 Arg72Pro gene [25]. Studies concerning TP53 gene polymorphism have not proven any dependence between genotypes and the risk of occurrence of lung cancer, whereas a tendency toward the occurrence of the Pro/Pro allele has been shown in patients with adenocarcinoma [26]. Studies conducted subsequently seem to confirm an increased risk of lung cancer occurrence for the Pro/Pro homozygote — contrary to the Arg/Arg homozygote [27, 28]. In this study, analysis of the TP53 gene polymorphism and the other gene, TNF-α, did not show any statistically significant differences between the two groups. The TNF-α gene underwent evaluation of G-308 A polymorphism. The TNF-α is one of the main cytokines that participate in inflammatory and immune response, it shows antineoplastic properties and it may also influence the process of angiogenesis [29]. The role of TNF-α concerns, among others, the damage of epithelial cells caused by this pro-inflammatory cytokine in the presence of the stimulated T lymphocytes or by release of metalloproteinases from stimulated macrophages [30]. Research of TNF-α G-308A gene polymorphism in patients with COPD has shown that people with the A/A homozygote are predisposed to greater changes in obstructive respiratory tract and have worse prognosis in the course of this disease [31]. TNF-α G-308A polymorphism has also been investigated in patients with non-small cell lung cancer, and a higher risk of occurrence of this neoplasm has been connected with the A/A and G/A genotype [32].

A statistically significant difference was noted in genes connected with iron metabolism; the difference concerned the gene of the haptoglobin (HP) (HP). It was shown that the Hp1/1 form occurs more frequently in patients with COPD (twice as frequently as in the group of patients with lung cancer and COPD). While patients from Group I (cancer + COPD) more frequently had the 1/2 genotype (almost twice as frequently). The 2/2 homozygote was discovered with similar frequency in the two groups of patients (slightly more often in the group with COPD only). In the whole examined population of patients the genotypes 1/2 and 2/2 appeared most frequently; the 1/1 genotype appeared the most rarely, in accordance with the tendency that genotype 2/2 vs. 1/1 prevails in Europe [33]. Haptoglobin is a protein of the acute phase, which binds free haemoglobin and prevents the kidneys from losing free haemoglobin together with iron ions, which are precious for the organism [34]. There are 3 phenotypic forms of this protein identified in humans: Hp1-1, Hp1-2 and Hp2-2, which have different biological activity (they are built from α and β chains). Haptoglobin has the ability to inhibit synthesis of prostaglandins; therefore, it is estimated to have anti-inflammatory and anti-oxidative properties [35]. The anti-oxidative property of Hp1-1 in blood plasma is stronger than that of Hp2-2 as its affinity to bind with haemoglobin is stronger than that of Hp2-2. Another crucial function of haptoglobin is connected with angiogenesis — in blood serum Hp may be a promoter of growth of endothelial cells of the blood vessels [36]. Haptoglobin gene polymorphism has been the subject of several research projects into different chronic diseases, including neoplasms of the respiratory system; however, it has not been studied on a large scale with respect to occurrence of chronic obstructive pulmonary disease. Research carried out in 1996 showed a rare occurrence of the 2/2 homozygote in patients with diagnosed adenocarcinoma of the lung [33], and a study carried out in 2002 seemed to confirm this [37]. Recent research into new markers for the detection of lung cancer has proven an increase in haptoglobin levels in blood serum in patients with diagnosed lung cancer [38]. Furthermore, it has been shown that in this case haptoglobin with a β chain has a greater diagnostic value; the concentration of this form of protein is four times greater in patients with lung adenocarcinoma in comparison with healthy humans.

Proteolytic enzyme genes and inhibitor genes of these enzymes were analysed lastly. In the case of the MMP1 (1607 1G/2G), MMP9 (C-1562T) and MMP12 (Asn357Ser) gene polymorphisms, no statistically significant differences were noted in either group of patients. The results of analysis of MMP3 (6A/5) gene polymorphism appears to be statistically significant; in the case of MMP12 (A-82G) gene polymorphism, the differences between the genotypes showed a tendency toward significance. The results of hitherto conducted research projects into the role of MMP in neoplastic diseases suggests a higher probability of tumour invasion and metastasis formation in cases of overexpression of MMP1 [16, 39]. MMP-9 has been associated with pathogenesis of the development of pulmonary emphysema; this enzyme is also important in the progression of neoplastic changes in the course of lung cancer, especially the disturbed relation of MMP-9 to its natural inhibitor TIMP-1 [40]. Research conducted by Su et al. showed, in the evaluated MMP1(1607 1G/2G) polymorphism, an increased risk of lung cancer occurrence in patients with the 2G/2G homozygote and a lack of such dependence in patients with the 1G/1G and 1G/2G genotypes [39]. Subsequent studies have confirmed an increased risk of lung cancer occurrence in patients with the 2G/2G homozygote [41]. In the MMP gene group, a statistical significance was obtained only in the case of MMP3 polymorphism (6A/5A). The occurrence of the 6A/6A homozygote was revealed twice as often in the group of patients with cancer +COPD, and the occurrence of the 5A/5A homozygote was shown almost twice as often. The 6A/5A heterozygote was noted nearly twice as often in patients with COPD only. MMP3 may influence the process of carcinogenesis and the growth of neoplastic tumours [42]. It has been suggested that the 5A/6A genotype favours a higher risk of occurrence of certain neoplasms, including adenocarcinoma of the breast. However, there are only a few reports on lung cancer — research among the Chinese population has suggested an increased risk of the occurrence of non-small cell lung cancer in tobacco smokers with the 5A/5A homozygote [43]. Another study by Chinese researchers in 2005 showed a probable coexistence of a combination of genotypes of the MMP1, MMP3 and MMP12 genes, which give an increased risk of lung cancer occurrence [18]. In the case of MMP12, two polymorphisms of were evaluated, i.e. Asn357Ser and A-82G. No statistically significant differences were found between the groups in relation to the first polymorphism. The A/A homozygote occurred more frequently in patients only with COPD; the A/G heterozygote occurred more frequently in patients with lung cancer and COPD (statistical tendency). In the available lite-

rature the role of MMP12 gene polymorphism in the risk of development of lung neoplasms has not yet been defined. Polymorphism of the inhibitor gene of metalloproteinases TIMP2 G/C has shown the occurrence only of the G/G genotype in all the studied patients.

The small number of patients who participated in the research makes it difficult to draw definite conclusions. Therefore, it may be presumed that extension of the groups would make the obtained results more objective. Furthermore, continued observation of the patients with COPD only with regard to the possible occurrence of lung cancer could increase the reliability of the evaluation of polymorphic variants.

Conclusions

A comparative analysis of patients with lung cancer and COPD and patients with COPD only, showed statistically significant differences in the polymorphism of genes connected with iron metabolism. The gene of haptoglobin Hp 1/2 was connected with iron metabolism and showed differences in polymorphism. Patients with lung cancer and COPD only significantly more rarely had the 1/1 homozygote, and they had the 1/2 heterozygote more frequently than patients with COPD only. The above results might suggest some protective significance of the 1/1 homozygote in the case of evaluation of lung cancer risk; this may be connected with the greater antioxidative ability of this haptoglobin form in plasma.

In the scope of polymorphism of genes connected with proteolytic enzymes, statistically significant differences were noted concerning the MMP3 gene. The 5A/5A homozygote and the 6A/6A homozygote occurred more often in patients with lung cancer and COPD. Patients with COPD only were more often the 6A/5A heterozygote, which could suggest some protective significance of this genotype in relation to the risk of occurrence of non-small cell lung cancer. The A/G heterozygote and the G/G homozygotes of the MMP12 gene (A-82) occurred more frequently in patients with lung cancer and COPD, whereas the A/A homozygote was seen more frequently in patients with COPD only. These differences were non-significant; it is possible that further research conducted on a larger group of patients would allow verification of these data.

Differences in polymorphism of DNA repair genes and the genes connected with neoplasms proved to be insignificant to the risk of lung cancer occurrence in patients with COPD in the presented study.

Conflict of interest

The authors declare no conflict of interest.

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