Immunohistochemical Expression of Matrix Metalloproteinase-1 and Cyclooxygenase-2 in Cutaneous Squamous Cell and Basal Cell Carcinoma

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ABSTRACT The most common nonmelanoma skin cancers (NMSC) are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The incidence of NMSC is 18-20 times higher than the incidence of melanoma. The Cyclooxygenase-2 (COX-2) and Matrix Metalloproteinase-1 (MMP-1) enzymes have both been linked to the development of these diseases but their exact significance is unknown. We conducted a retrospective analysis on 148 adult patients with cutaneous BCC and SCC. Cases were divided according to the sub-types of BCC and the degree of SCC differentiation. Immunohistochemical staining for COX-2 and MMP-1 was performed and analyzed to determine if the expression of these biomarkers were associated with BCC subtypes and the degree of SCC. differentiation.
We did not find a significant association of the level of differentiation of SCC with the immunohistochemical expression for MMP-1 or COX-2. There was a significant association between BCC subtypes and immunohistochemical expression for MMP-1; positive expression of this enzyme reduces the odds for the infiltrative subtypes by 90%. A marginally significant association between BCC subtypes and immunohistochemical expression for COX-2 was also found. This enzyme was highly expressed in non-infiltrative basal cell carcinoma types (94%) compared with infiltrative types (71%). In conclusion, we did not find a significant predictor for SCC expression levels for either of two biomarkers, while the expression of MMP-1 in BCC was significantly inversely associated with the infiltrative type (moderate sensitivity and high specificity). Further research with larger sample sizes is needed to precisely determine the role these enzymes have in these diseases.

KEY WORDS: squamous cell carcinoma, basal cell carcinoma, matrix metalloproteinase-1, cyclooxygenase-2, biomarker

INTRODUCTION

Malignant skin neoplasms comprise a heterogeneous group of diseases with variable disease course and treatment prognosis. These malignant neoplasms appear in the daily practice of most dermatologists and surgeons. In most cases, they require significant investment of human and technical resources, such as complex diagnostic and surgical procedures, radiological examinations, and regular checkups.

The most common nonmelanoma skin cancers (NMSC) are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The incidence of NMSC is 18-20 times higher than the incidence of melanoma (1). Compared with melanoma, the epidemiology of NMSC has not been sufficiently investigated. There are significant limitations in the study of the incidence of NMSC, which is mainly attributed to its significant geographical variability as well as the fact that large cancer registries usually exclude NMSC from their records or have incomplete records (2).

BCC is the most common malignant neoplasm in whites, followed by SCC. It is characterized by slow growth but if left untreated can become locally invasive and destructive (3-8).

It most commonly occurs between the ages of 50 and 80, regardless of sex. Metastases have rarely been described (3-8). The incidence of metastatic BCC and SCC ranges from 0.00281-0.05% and 0.5-16%, while the age-adjusted mortality rate is estimated at 0.12 per 100,000 for BCC and 0.3 per 100,000 for SCC (9-11). However, despite its relatively small malignant potential, NMSC is associated with outstanding morbidity and significant cost for the healthcare institution (12,13).

SCC is a malignant neoplasm originating from suprabasal epidermal keratinocytes. Unlike BCC, it also occurs on mucous membranes, especially at the transition of skin to mucous membranes. It most commonly occurs around age 70, but can also occur in younger populations, which have a higher risk of developing SCC compared with the general population (14-16). Over the last 30 years, the incidence of SCC has been growing at 3-10% per year (17). The incidence rate of BCC is estimated to have increased between 20% and 80% in the United States over the same period (17). BCC is more common than SCC, and the standardized ratio is approximately 4:1.2 (18).

Cases of cutaneous SCC manifest as a wide range of tumor changes ranging from easily curable superficial changes to highly infiltrating, fatal metastatic tumors (16).

Matrix metalloproteinase (MMPs) are enzymes that specifically degrade certain parts of the extracellular matrix and are expressed during various physiological and pathological conditions. The ability of MMPs to cope with molecules such as growth factors, adhesion molecules, other proteinases, and proteinase inhibitors allows them to act as a control of molecular processes within the microenvironment. Altered values of MMP expression play an important role in tumor progression and invasion of surrounding tissue, but large clinical trials have not yet determined which of these molecules may be effective in the prevention or treatment of BCC (Petrella and Margolin, 2012) and SCC (19).

In tumor progression, the formation and activity of MMPs itself may change under the influence of various factors such as cytokines, tissue inhibitors of MMPs, and other proteases produced from tumor cells, fibroblasts, and / or inflammatory cells (20,21).

In their studies, Zlatarova *et al.* 2012, Variani *et al.* 2000, Brennan *et al.*, 2004, showed that MMP-1 is involved in extracellular matrix degradation and tumor invasion in BCC of the head and neck and that it is regulated in tumor cells and the surrounding stroma to varying degrees in all BCC subtypes included in published studies (22-24). MMP-1 mRNA was detected in tumor cells and / or stromal cells in all cases of SCC, and its expression may be an early indicator in the development of SCC [52].

Cyclooxygenase (COX) is a key enzyme that mediates the production of prostaglandins from arachidonic acid. So far, two COX isoforms have been identified – COX-1 and COX-2. COX-1 is expressed constitutively, while COX-2 is induced by growth factors, mitogens, tumor promoters, and cytokines (25).

COX-2 has been shown to play an important role in the development of various tumor types. Recent studies have reported an association of COX-2 expression with tumor invasion (26,27), apoptosis suppression (28), and tumor angiogenesis (29,30). An association with COX-2 expression in various tumors such as ovarian cancer (31), breast (32), stomach (33), kidney (34), and squamous cells of the scalp and neck (35) was found. All these previous studies suggest that cellular regulation of COX-2 may be a critical event in carcinogenesis (36).

PATIENTS AND METHODS

A retrospective analysis of the histologic materials of 148 adult patients who received surgery for a primary skin tumor (SCC and BCC) were included in the present study. The patients all underwent primary surgical treatment between 2007 and 2008 at the Department of Surgery, University Hospital Center Sisters of Mercy in Zagreb, Croatia. The study was approved by the hospital's Ethical Committee.

In the group of patients with SCC, there were a total of 89 patients. They were divided into groups according to the degree of tumor differentiation (grade I, II, III, IV) (37,38). The first group (grade I) consisted of 30 patients, the second group (grade II) of 31 patients, and the third group (grade III) of 27 patients. Grade IV consisted of 1 patient. Additionally, a total of 59 patients with skin BCC were treated, and their

samples were categorized into a non-infiltrative subgroup (superficial, nodular, fibroepithelial subtype) and infiltrative subgroup (infiltrative, morpheaform, sclerosing, micronodular, and basosquamous subtype) (39,40). The non-infiltrative subgroup consisted of 17 patients, while the infiltrative subgroup consisted of 42 patients. In the control group, there were 30 samples of peritumoral skin (15 samples from patients with basal cell carcinoma and 15 samples with squamous cell carcinoma).

The material was treated by a standard pathohistological method, which included fixation of the material in 10% buffered formalin, tissue incorporation into paraffin, cutting into 5 µm thick sections, and staining with hematoxylin-eosin. BCC subtypes and the degree of SCC differentiation were determined based on preparations in which the pathohistological diagnosis of BCC and SCC was confirmed.

Two additional incisions were made from each tumor for immunohistochemical treatment for COX-2 using a mouse monoclonal antibody (Santa Cruz; sc-58344; 1:100) and for MMP-1 using a mouse monoclonal antibody (Santa Cruz; sc-21731 (3B6); 1:200). The immunohistochemical treatment procedure was performed with an immunohistochemical staining apparatus (DAKO autostainer, Universal Staining System) according to the manufacturer's recommendation.

The results of immunohistochemical analysis are presented semiquantitatively, based on the intensity of staining according to the percentage of stained cells, with the following classification:

0 – negative reaction;

1 – weakly positive reaction (<10% of tumor cells);

2 – moderately strong positive reaction (10-50% of tumor cells) (Figure 1);

3 – strong positive reaction (>50% of tumor cells) (Figure 2).

In the statistical analysis, immunohistochemical expressions 0 and 1 were considered a negative result, and 2 and 3 a positive result.

Immunohistochemical expression of MMP-1 and COX-2 was analyzed and statistically processed for each tumor group separately, for SCC according to Broder's classification, and for BCC according to

Table 1. Baseline characteristics of patients and location of skin cancer according to type (squamous cell or basal cell skin carcinoma) (N=148)

		All	Squamous cell carcinoma (n=89)	Basal cell carcinoma (n=59)	Statistics	*P*-value
Sex (%)	Men	68 (46.0)	43 (48.3)	25 (42.4)	$\chi2=0.504$	0.478
	Women	80 (54.0)	46 (51.7)	34 (57.6)		
Age, years, mean (SD)		73.0 (11.3)	76.8 (10.1)	67.3 (10.6)	t=5.504	<0.001
Tumor location	Head	112 (75.7)	73 (82.0)	39 (66.1)	$\chi2=6.334$	0.042
	Trunk	24 (16.2)	9 (10.1)	15 (25.4)		
	Extremities	12 (8.1)	7 (7.9)	5 (8.5)		
Detailed tumor location*	Nose	26 (17.6)	14 (15.7)	12 (20.3)		
	Ear	9 (6.1)	6 (6.7)	3 (5.1)		
	Forehead	12 (8.1)	6 (6.7)	6 (10.2)		
	Cheek	38 (25.7)	28 (31.4)	10 (16.9)		
	Scalp	18 (12.2)	12 (13.4)	6 (10.2)		
	Chin	1 (0.7)	1 (1.1)	0		
	Upper lip	1 (0.7)	1 (1.1)	0		
	Neck	8 (5.4)	6 (6.7)	2 (3.4)		
	Back	14 (9.5)	8 (9.0)	6 (10.2)		
	Chest	9 (6.1)	3 (3.3)	6 (10.2)		
	Abdomen	2 (1.4)	0	2 (3.4)		
	Arms	8 (5.4)	5 (5.6)	3 (5.1)		
	Legs	5 (3.4)	2 (2.2)	3 (5.1)		

SD: standard deviation.
*Total % can be larger than 100% because some patients had a cancer on 2 locations.

Table 2. Immunohistochemical expression of MMP-1 and COX-2 in squamous cell (n=89) and basal cell skin carcinoma (n=59)

Immunohistochemical expression*		Squamous cell carcinoma (n=89)	Basal cell carcinoma (n=59)	Statistics	*P*-value
MMP-1		0 (0-20)	30 (0-100)	Z=3.612	<0.001
	0	60 (67.4)	23 (39.0)		
	1	2 (2.3)	0	χ2=15.064	0.002
	2	15 (16.9)	16 (27.1)		
	3	12 (13.5)	20 (33.9)		
COX-2		50 (0-100)	100 (20-100)	Z=3.369	<0.001
	0	23 (26.1)	13 (22.0)		
	1	4 (4.6)	0	χ2=14.430	0.002
	2	18 (20.5)	2 (3.4)		
	3	43 (48.9)	44 (74.6)		

*Immunohistochemical expression is presented as median and interquartile range and as semiquantitative categories: 0 - negative expression, 1 - mild positive expression (<10% of tumor cells), 2 - moderate positive expression (10-50% of tumor cells), 3 - strong positive expression (>50% of tumor cells).

histological subtype. In this way, it was determined whether there was a connection between the analyzed antibodies and within the cancer subgroups or only at the level of the histological type of the tumor.

Immunohistochemical response for both observed proteins (MMP-1 and COX-2) manifested as positivity in the cytoplasm of tumor cells of varying intensity, from a moderately positive reaction to MMP-1 (Figure 1) to a strong positive reaction to COX-2 (Figure 2).

Data analysis

Statistical analyses were performed using Statistica, version 12 (StatSoft Inc., Tulsa, OK) and MedCalc, version 17.8 (MedCalc Software bvba, Ostend, Belgium). Categorical variables are presented as frequency and proportion (%), and continuous variables as mean and standard deviation (SD) or as median and interquartile range (IQR) depending on the type of distribution. Normality of distribution was assessed with the Kolmogorov-Smirnov test. Comparison of categorical variables between groups was assessed using the χ2 test, and comparison of continuous variables was done using the Student's t-test or Mann-Whitney U-test depending on the type of distribution. Association between immunohistochemical expression of MMP-1 and COX-2 and level of differentiation of squamous cell carcinoma and infiltrative subtypes of basal cell carcinoma was assessed using univariate logistic regression. Multivariate logistic regression was used to analyze independent associations of sex, age, location of skin carcinoma, expression of MMP-1 and COX-2 with the type of skin carcinoma, level of differentiation of

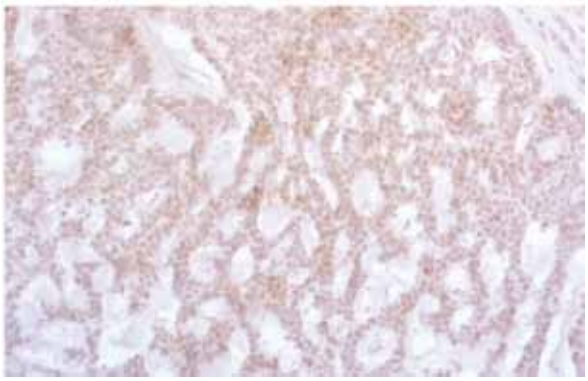

Figure 1. Moderately positive reaction to MMP-1 in the cytoplasm of adenoid basal cell carcinoma cells (IMH ×20).

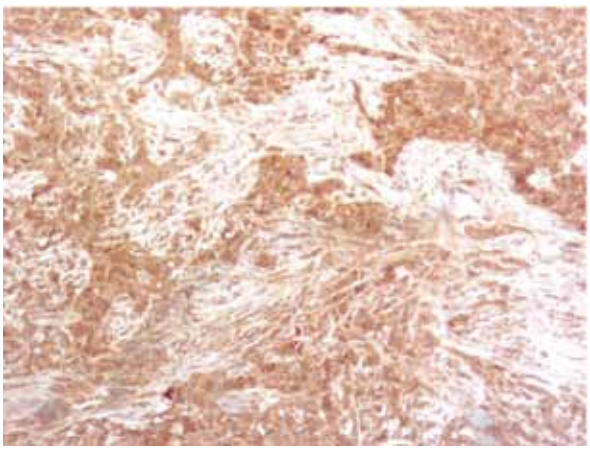

Figure 2. Strong positive reaction to COX-2 in the cytoplasm of squamous cell carcinoma cells (IMH ×20).

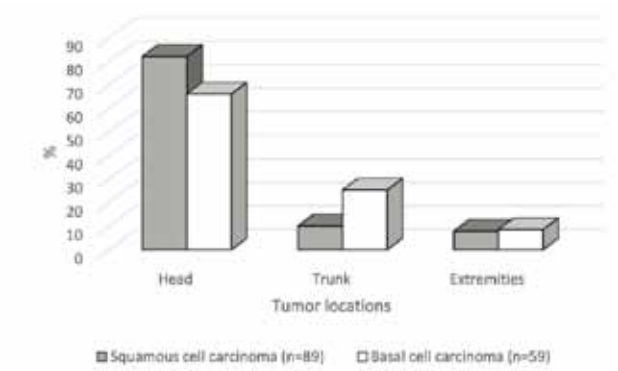

Figure 3. Skin cancer locations according to type (squamous cell or basal cell skin carcinoma) (N=148).

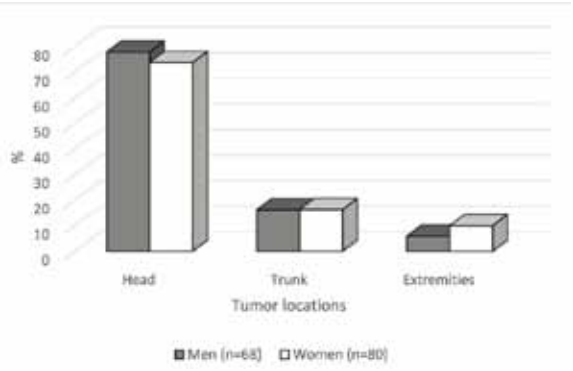

Figure 4. Skin cancer locations according to sex (N=148).

squamous cell carcinoma, and infiltrative subtypes of basal cell carcinoma. Results of logistic regression analyses are presented as odds ratios (ORs) with 95% confidence intervals (CIs) and as area under the curve (AUC) with 95% CIs. Receiver operating characteristic (ROC) curve analysis was used to assess significant associations of immunohistochemical expression of MMP-1 with the type of skin carcinoma and infiltrative subtypes of BCC, with results presented as a ROC curve, associated criterion, sensitivity, and specificity. All tests were computed as two-sided with the level of significance set at 95% (P<0.05).

RESULTS

This analysis included histopathology samples from 148 patients who went through a surgical procedure because of a primary skin cancer (SCC and BCC) in which immunohistochemical expression of MMP-1 and COX-2 was assessed. Peritumor skin was analyzed as a control, but the results were negative for both MMP-1 and COX-2 expression for all the samples, so these results will not be presented any further.

Baseline characteristics of patients and skin cancer locations

Baseline characteristics are presented in Table 1. Analyzed samples originate from 68 (46.0%) men and 80 women (54.0%) of an average age of 73.0 (11.3 SD) years, of which 89 were diagnosed as squamous cell and 59 as basal cell skin carcinoma (Table 1). No significant difference was found for sex distribution between types of carcinoma (P=0.478), but patients with SCC were significantly older (76.8 vs. 67.3 years, P<0.001) (Table 1). Significant difference was found regarding the tumor location between types of carcinoma (P=0.042), with both having the head as the most frequent location (75.7%), while BCC was more frequent on the trunk (25.4% vs. 10.1%) Table 1 and Figure 3) with no difference in locations regarding sex (Figure 4). The most frequent location of SCC was in the cheeks area (31.4%), followed by the nose (15.7%) and scalp areas (13.4%). BCC was most frequent in the area of the nose (20.3%), followed by the cheeks (16.9%) and forehead, scalp, back, and chest areas (all location with 10.2%) (Table 1).

Immunohistochemical expression of MMP-1 and COX-2 according to the type of tumor

Immunohistochemical expression of MMP-1 and COX-2 according to the type of carcinoma is presented in Table 2 and Figure 5. Significantly greater immunohistochemical expression of both MMP-1 and COX-2 was found in BCC (P<0.001 for both) (Table 2). Negative expression of MMP-1 was significantly more frequent in SCC (67.4% vs. 39.0% of samples), while

Table 3. Multivariate logistic regression analysis for the type of tumor (squamous cell vs. basal cell skin carcinoma)

Variable	Coefficient	SE	Wald	OR	95% CI	*P*-value
COX-2 (-)	0.190	0.452	0.177	1.210	0.498-2.935	0.674
MMP-1 (+)	-1.089	0.438	6.176	0.336	0.142-0.794	**0.013**
Tumor location	0.134	0.752	0.032	1.144	0.262-4.991	0.858
Sex (men)	0.496	0.393	1.596	1.643	0.761-3.548	0.206
Age (years)	0.093	0.021	18.794	1.097	1.052-1.144	**<0.001**

χ2=37.227, df=5, P<0.001 – for the model. SE – standard error, OR – odds ratio, CI – confidence interval.

Table 4. Immunohistochemical expression of MMP-1 and COX-2 in SCC according to the levels of differentiation (N=88)

		Immunohistochemical expression of MMP-1			
Level of differentiation		0	1	2	3
Grade I	(n=30)	20 (66.7)	1 (3.3)	3 (10.0)	6 (20.0)
Grade II	(n=31)	20 (64.5)	0	7 (22.6)	4 (12.9)
Grade III	(n=27)	19 (70.4)	1 (3.7)	5 (18.5)	2 (7.4)
		Negative		Positive	
Well / moderately differentiated	(n=61)	41 (67.2)		20 (32.8)	
Poorly differentiated	(n=27)	20 (74.1)		7 (25.9)	

		Immunohistochemical expression of COX-2			
		0	1	2	3
Grade I	(n=30)	11 (36.7)	0	7 (23.3)	12 (40.0)
Grade II	(n=31)	7 (22.6)	1 (3.2)	9 (29.0)	14 (45.2)
Grade III	(n=27)	4 (15.4)	3 (11.5)	3 (11.5)	17 (65.4)
		Negative		Positive	
Well/moderately	(n=61)	19 (31.2)		42 (68.8)	
Poorly	(n=27)	7 (25.9)		20 (74.1)	

Immunohistochemical expression: 0 - negative expression, 1 - mild positive expression (<10% of tumor cells), 2 - moderate positive expression (10-50% of tumor cells), 3 - strong positive expression (>50% of tumor cells). Grade IV is not included in this table due to the small number of cases (1 patient).

strong positive expression was significantly more frequent in BCC (33.9% vs. 13.5% of samples, *P*=0.002) (Table 2). Negative expression for COX-2 was comparably frequent in both SCC and BCC, but strong positive expression was significantly more frequent in BCC (74.6% vs. 48.9%, *P*=0.002) (Table 2 and Figure 5).

Significant association between the type of carcinoma (SCC vs. BCC) and immunohistochemical expression for MMP-1 was found (OR 0.30, 95% CI 0.15-0.62, *P*=0.001, univariate logistic regression analysis); positive expression of MMP-1 reduced the odds for SCC by 70%. Significant association between the type of carcinoma (SCC vs. BCC) and immunohistochemical expression for COX-2 was also found (OR 2.12, 95% CI 1.02-4.43, *P*=0.045, univariate logistic regression analysis); positive expression of COX-2 increased the odds for BCC by 112%. Multivariate analysis for the type of carcinoma (SCC vs. BCC) revealed that it was significantly associated with the age of patient (odds for SCC increases with each year of age by 9.7%, *P*<0.001) and with positive expression of MMP-1 (positive expression of MMP-1 reduced the odds for SCC by 66.4%, *P*=0.013) ($\chi 2$=37.227, df=5, *P*<0.001 – for the model) (Table 3).

ROC curve analysis (Figure 6) for the diagnostic threshold of immunohistochemical expression of MMP-1 to discriminate the types of carcinoma (SCC vs. BCC) found a value of ≤20% (AUC 0.676, 95% CI 0.594-0.751, z=4.182, *P*<0.001) with a sensitivity of 80.70% and specificity of 52.54%.

Immunohistochemical expression of MMP-1 and COX-2 in SCC according to the levels of differentiation

Immunohistochemical expression of MMP-1 and COX-2 in SCC according to the levels of differentiation is presented Table 4 and Figure 7. We found compara-

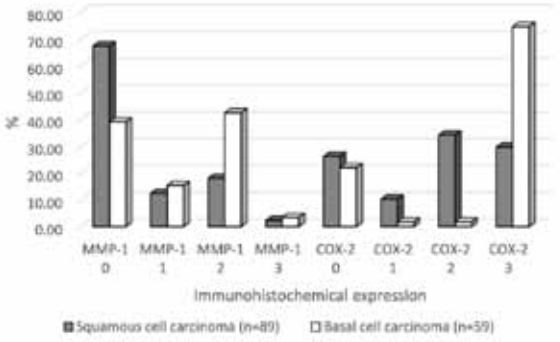

Figure 5. Immunohistochemical expression of MMP-1 and COX-2 in squamous cell (n=89) and basal cell skin carcinoma (n=59).

Table 5. Multivariate logistic regression analysis for the level of differentiation of squamous cell skin carcinoma (well / moderately vs. poorly differentiated, n=89)

Variable	Coefficient	SE	Wald	OR	95% CI	*P*-value
COX-2 (-)	-0.195	0.528	0.137	0.823	0.293-2.314	0.712
MMP-1 (+)	-0.711	0.678	1.100	0.491	0.130-1.854	0.294
Tumor location	-0.998	1.157	0.744	0.369	0.038-3.561	0.388
Sex (men)	0.977	0.514	3.615	2.656	0.970-7.269	0.057
Age (years)	0.0116	0.026	0.199	1.012	0.962-1.064	0.655

$\chi2$=5.794, df=5, *P*=0.327 – for the model. SE – standard error, OR – odds ratio, CI – confidence interval.

tive expression of MMP-1 and COX-2 in SCC according to the levels of differentiation (MMP-1, $\chi2$=0.414, p=0.520; COX-2, $\chi2$=0.245, *P*=0.621) (Table 4). On the other hand, the proportion of positively expressed samples for MMP-1 in poorly differentiated SCC was low (25.9%) compared with a high proportion (74.1%) of positively expressed samples for COX-2.

We did not find a significant association of the level of differentiation of SCC with the immunohistochemical expression for MMP-1 (OR 0.58, 95% CI 0.17-1.97, *P*=0.387, univariate logistic regression analysis) or for COX-2 (OR 0.96, 95% CI 0.38-2.47, *P*=0.938, univariate logistic regression analysis). Multivariate analysis for the level of differentiation of SCC did not reveal a significant association with any of the predictors (immunohistochemical expression for MMP-1 and COX-2, tumor location, sex and age) ($\chi2$=5.794, df=5, *P*=0.327 – for the model) (Table 5).

Immunohistochemical expression of MMP-1 and COX-2 in basal cell carcinoma based on infiltrative subtypes

Immunohistochemical expression of MMP-1 and COX-2 in BCC based on infiltrative subtypes is presented in Table 6 and Figure 8. Contrary to SCC, in BCC we found a significantly greater proportion of expression of MMP-1 in non-ifiltrative subtypes (94.1% vs. 47.6%, $\chi2$=11.000, *P*<0.001) (Table 6). Additionally, a significantly greater proportion of expression of COX-2 in non-infiltrative BCC subtypes was found, although the difference in infiltrative subtype was not that large (94.1% vs. 71.1%, $\chi2$=4.365, *P*=0.037) (Table 6).

A significant association between BCC according to infiltrative subtypes and immunohistochemical expression for MMP-1 was found (OR 0.10, 95% CI 0.02-0.39, *P*=0.001, univariate logistic regression analysis);

Table 6. Immunohistochemical expression of MMP-1 and COX-2 in basal cell skin carcinoma based on infiltrative subtypes (non-infiltrative vs. infiltrative subtypes, n=59)

		Immunohistochemical expression of MMP-1			
Subtype		0	1	2	3
Non-infiltrative	(n=17)	1 (5.9)	0	4 (23.5)	12 (70.6)
Infiltrative	(n=42)	22 (52.4)	0	12 (28.6)	8 (19.1)
		Negative		Positive	
Non-infiltrative	(n=17)	1 (5.9)		16 (94.1)	
Infiltrative	(n=42)	22 (52.4)		20 (47.6)	

		Immunohistochemical expression of COX-2			
		0	1	2	3
Non-infiltrative	(n=17)	1 (5.9)	0	0	16 (94.1)
Infiltrative	(n=42)	12 (28.6)	0	2 (4.8)	28 (66.7)
		Negative		Positive	
Non-infiltrative	(n=17)	1 (5.9)		16 (94.1)	
Infiltrative	(n=42)	12 (28.6)		30 (71.4)	

Immunohistochemical expression: 0 - negative expression, 1 - mild positive expression (<10% of tumor cells), 2 - moderate positive expression (10-50% of tumor cells), 3 - strong positive expression (>50% of tumor cells); non-infiltrative - superficial subtype, nodular and fibroepithelioma of Pinkus; infiltrative – morpheaform, sclerosing, infiltrative, micronodular, and basosquamous subtypes.

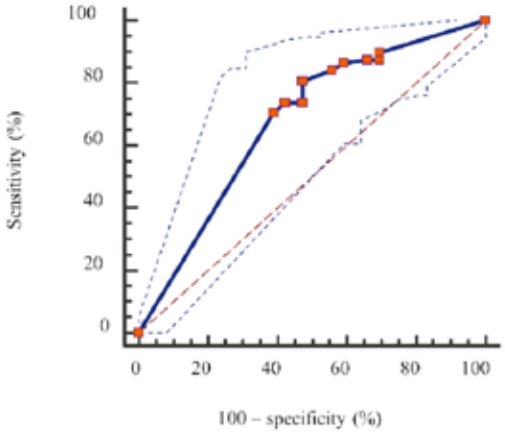

Figure 6. ROC curve analysis for immunohistochemical expression of MMP-1 (%) for the discrimination of type of skin cancer (squamous cell vs. basal cell carcinoma, N=148).

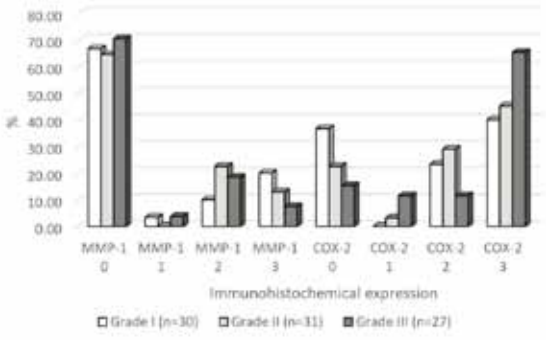

Figure 7. Immunohistochemical expression of MMP-1 and COX-2 in SCC according to differentiation levels (N=89).

positive expression for MMP-1 reduced the odds for the infiltrative subtypes by 90%. A marginally significant association between BCC according to infiltrative subtypes and immunohistochemical expression for COX-2 was also found (OR 7.17, 95% CI 0.86-59.97, *P*=0.069, univariate logistic regression analysis). Multivariate analysis for the infiltrative subtypes (non-infiltrative vs. infiltrative) of BCC revealed that it was significantly associated with the expression for MMP-1 (positive expression for MMP-1 reduced the odds for infiltrative subtypes of basal cell carcinoma by 87%, *P*=0.008) (χ2=15.590, df=5, p=0.008 – for the model) (Table 7).

ROC curve analysis (Figure 9) for the diagnostic threshold of immunohistochemical expression of MMP-1 to discriminate the BCC between non-infiltrative and infiltrative subtypes found a value of ≤15% (AUC 0.811, 95% CI 0.688-0.901, z=5.473, *P*<0.001) with a sensitivity of 64.3% and specificity of 94.1%.

DISCUSSION

Analysis of patients by sex and age did not show any statistically significant differences between tumor types (*P*=0.478), but patients receiving surgical procedures for SCC were statistically significantly older compared with patients receiving surgical procedures for BCC (76.8 versus 67.3, *P*<0.001) (Table 1), which is comparable to the literature data on the incidence of SCC and BCC (16,41,42).

Additionally, a statistically significant difference was found according to the type of cancer related to tumor localization (*P*=0.042). Both surgically removed tumors were most commonly located in the head area (75.7%), while BCC was more often (25.4%) localized in the trunk area, compared with 10.1% in SCC (Table 6). The most common localization of SCC was in the cheek area (31.4%), followed by localizations in the nose (15.7%) and scalp (13.4%). In contrast to SCC, the most common localization of BCC was the area of the nose (20.3%), cheeks (16.9%), and forehead, scalp, back, and chest (all localizations 10.2%) (Table 1).

In a retrospective analysis by Findik *et al.*, 400 cases of NMSC had a similar distribution of tumors by localization (42).

In our study, we found that the tumor type was statistically significantly associated with immunohistochemical expression for MMP-1, where the possibility of SCC with MMP-1 positivity was reduced by 66.4%. Following the obtained results, we also determined that the limit value of immunohistochemical

Table 7. Multivariate logistic regression analysis for the basal cell skin carcinoma based on infiltrative subtypes (non-infiltrative vs. infiltrative subtypes, n=59)

Variable	Coefficient	SE	Wald	OR	95% CI	*P*-value
COX-2 (-)	0.962	1.234	0.608	2.617	0.233-29.399	0.436
MMP-1 (+)	-2.040	0.773	6.969	0.130	0.029-0.591	**0.008**
Tumor location	-0.827	1.094	0.572	0.437	0.051-3.729	0.449
Sex (men)	-0.545	0.687	0.629	0.580	0.151-2.229	0.428
Age (years)	0.012	0.0303	0.145	1.012	0.953-1.074	0.704

χ2=15.590, df=5, *P*=0.008 – for the model. SE – standard error, OR – odds ratio, CI – confidence interval.

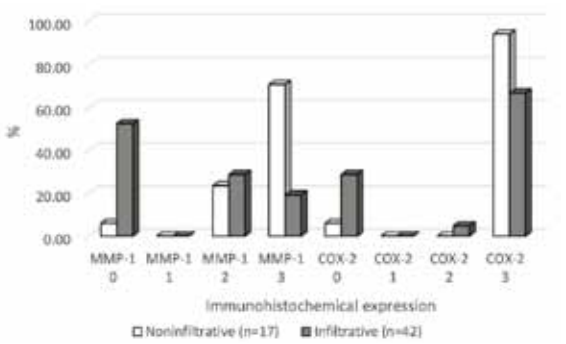

Figure 8. Immunohistochemical expression of MMP-1 and COX-2 in basal cell skin carcinoma based on infiltrative subtypes (non-infiltrative vs. infiltrative subtypes, n=59).

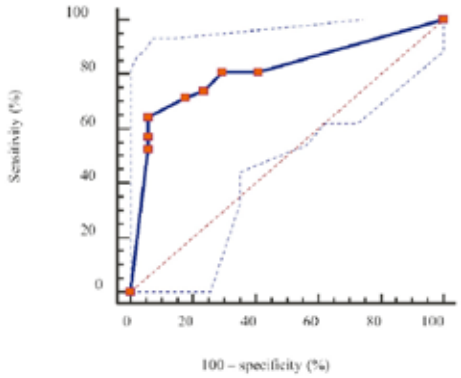

Figure 9. ROC curve analysis for the diagnostic threshold of immunohistochemical expression of MMP-1 (%) to discriminate the basal cell carcinoma between non-infiltrative and infiltrative subtypes (n=59).

expression for MMP-1 to differentiate tumor type was ≤20%, which would mean that if the immunohistochemical expression of MMP-1 in the pathohistological preparation is ≥20%, it is more likely to be BCC with a diagnostic capability of 3.76% and a diagnostic accuracy of 67.35%. This study is the only one that investigated the cut-off data, so this cannot be compared with other studies.

The use of these two markers could play a new role in the early diagnosis and differential diagnosis of these two malignancies.

In the past twenty years of research, immunohistochemical studies of COX-2 expression have not yet fully elucidated the role or pattern of expression in normal skin as well as in skin epithelial tumors.

Leong *et al.* found strong expression of COX-2 in cases of SCC, while BCC cells showed poor staining (43). The authors also found COX-2 expression of normal skin but limited to keratinocytes of the granular and spinous layers (43). Vogt *et al.* did not detect COX-2 protein in any of the 11 cases of BCC, but observed moderate and / or strong protein expression in nine of the 17 cases of SCC and poor staining in two of the six keratoacanthomas (44). The staining pattern observed for normal skin cells was similar to that described by Leong *et al.* (43,44). In a study by Putti *et al.*, COX-2 expression was detected in 13 of 17 cases of SCC and 8 of 24 cases of CA (45). Kim *et al.* were able to detect COX-2 expression in only 5 of 10 SCCs, 4 of 10 cases of BD, 5 of 10 AKs, and 2 of 10 cases of porokeratosis (46). At the same time, the authors detected COX-2 in 8 of 10 BCCs (46). Some of the COX-2 positive lesions showed strong staining: BD – 50%, AK – 20%, and BCC – 12.5% of positive cases (46).

From the above studies, it can be seen that several groups described similar or slightly higher expression of COX-2 in cases of SCC compared with precancerous lesions such as AK, KA, and BD (46-50).

The results of our study show that there was a comparable expression of MMP-1 and COX-2 in SCC according to the degree of differentiation, where the share of positively expressed samples for MMP-1 in poorly differentiated cases of SCC was low (25.9%), in contrast with the expression of COX-2, where the share of positively expressed samples was almost ¾ (74.1%).

We did not find a statistically significant association of the degree of SCC differentiation with immunohistochemical expression for MMP-1 or with immunohistochemical expression for COX-2. Since the role of stroma cells and their positivity to MMP-1 is more important than the response on tumor cells, which was not studied in this paper and from, it logically follows that tumor cells gave less positivity. Multivariate logistic regression for the degree of SCC differentiation did not establish an association with any of the factors (immunohistochemical expression for MMP-1 and COX-2, tumor localization, and sex and age of patients).

In our study, the immunohistochemical expression of MMP-1 was negative in 67.2% of cases of well- and moderately differentiated SCCs, while it was 74.1% in poorly differentiated cancers.

The results of our research are partially in line with the literature.

Tsukifuji *et al.* investigated the expression of MMP-1, MMP-2, and MMP-3 in cases of SCC and actinic keratosis (51). They found that MMP-1 mRNA was more present in stromal cells than in tumor cells (51). Son *et al.* found that MMP-1 expression in the stromal cell SCC was significantly correlated with the depth of invasion in univariate analysis (P=0.010), but not in multivariate analysis. The expression of MMP-1 tumor cells gradually increased with increasing depth

of invasion, but the differences were not statistically significant (52).

These results are partially in line with the results obtained in our study, because we did not find a statistically significant difference in the expression of MMP-1 among the examined groups of SCC. The share of positives in poorly differentiated SCCs was 25.9%, and 32.8%in poorly and moderately differentiated SCCs. It should certainly be noted that the number of patients in the present study, which is higher than the above studies, should not be neglected, which may also be the reason for the different levels of expression compared with other studies. Additionally, in other studies there were no data on UV exposure and skin pigmentation in the patient samples studied.

In most previous studies, as well as in this one, the immunohistochemical expression of MMP-1 and COX-2 was calculated using a semiquantitative method. This method may have an element of subjectivity stemming from the researcher analyzing the immunohistochemical expression, which may affect the end result.

In contrast to SCC, there was a statistically higher proportion of MMP-1 expression in non-infiltrative BCC subtypes (94.1% vs. 47.6%). A statistically significantly higher incidence of COX-2 markers was also found in the non-infiltrative BCC subtype, although the difference was not as pronounced (94.1% for non-infiltrative and 71.1% for infiltrative subtypes). Such a result is generally inconsistent with earlier reports from other groups that described very low COX-2 expression in most BCCs (46,49), or COX-2 absence in most cases, or low expression in only a small proportion of examined lesions (43-45,47,50). Considering the good reproducibility of the results of our study for a large number of samples and the consistency of the results obtained for BCC, it can be objectively concluded that reports of indeterminate COX-2 expression in smaller or larger BCC fractions examined in previous studies are due to insufficient sensitivity of COX-2 detection and not protein deficiency in BCC cells.

All of these data suggest that COX-2 expression is not proportional to the extent of malignancy and that COX-2 is more pronounced in differentiated cells than in immature cells.

A statistically significant association between BCC infiltration and immunohistochemical expression for MMP-1 was found. This means that the positive expression of MMP-1 reduces the 90% likelihood of an infiltrative type of BCC, with an established limit value of ≤15%. Furthermore, if the immunohistochemical expression of MMP-1 in the BCC is ≥15%, there is a diagnostic likelihood of 28.8% that it is a non-infiltrative subtype, with a diagnostic accuracy of 69.49%.

This result of our research is contradictory to the findings of Vanjaka Rogošić *et al.* In their analysis of 64 BCC specimens, they found that MMP-1 presence in tumor cells was associated with a morpheaform and recurrent form of BCC, suggesting its influence on cancer cells (53).

Cells that produce MMP and its production mechanism have created controversy in the literature. Some studies have reported that tumor cells are major producers of MMPs. Several cancers, including colon cancer, gastric cancer, and breast cancer, show that MMP expression in the cytoplasm of tumor cells is determined by immunohistochemical staining, while other researchers have reported MMP mRNA expression in fibroblasts around colon and breast cancer and SCC of the head and neck, which was determined by the *in situ* hybridization method (54,55).

The aim of our study on the immunohistochemical expression of MMP-1 and COX-2 was primarily an attempt to find biomarkers that would play a role in the prevention but also in the prognosis of these common malignancies, i.e. the isolation of relapsed BCC cases and SCC cases with more aggressive disease. A potential contribution of this study to the progress of the scientific field is the realization that matrix metalloproteinases, especially MMP-1, have prognostic value in non-melanoma skin tumors. In other words, not only does it have prognostic value in distinguishing SCC from BCC, but it is particularly significant for distinguishing infiltrative from non-infiltrative BCC. Our research has indicated that MMP-1 is an important indicator of BCC biological behavior.

Although previous studies have shown that the severity of MMPs is associated with tumor invasiveness, the mechanism of MMP production in tumor tissue has not yet been elucidated.

CONCLUSION

The analyzed SCC samples in our sutdy were from a significantly older population than BCC samples, with a majority coming from different head locations. Immunohistochemical expression of both MMP-1 and COX-2 markers was significantly greater in BCC, but age, sex ,and tumor location were not associated with the level of expression. We did not find a significant predictor for SCC expression levels for either of two markers, while the expression of MMP- in BCC 1 was significantly inversely associated with the infiltrative type (moderate sensitivity and high specificity). Further studies with larger sample sizes and better

definition of underlying pathological processes and phenotyping are needed to more precisely determine the role these enzymes have in this diseases.

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