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THE SIGNIFICANCE OF FHIT AND Bcl-2 IN PATIENTS WITH ORAL LICHEN PLANUS IN COMPARISON TO THE HEALTHY ORAL MUCOSA AND ORAL SQUAMOUS CELL CANCER

IVANA PAVIĆ[1], DRINKO BALIČEVIĆ[1], BORNA VRDOLJAK[2], MAŠA FILIPOVIĆ[2], JOSIP ZEKIC[3] and VANJA VUČIĆEVIĆ BORAS[4]

[1]Department of Pathology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia;
[2] School of Medicine, University of Zagreb, Croatia;
[3]Children's Hospital, Zagreb, Croatia;
[4]Department of Oral Medicine, School of Dental Medicine, University of Zagreb, Croatia

Summary

Background: Oral lichen planus (OLP) is a precancerous lesion which might progress into oral squamous cell cancer (OSCC) in 0-1.2 % of the affected patients. Albeit there are many published studies upon this topic, there are no universally accepted clinical and histopathological criteria which would suggest which patients will develop OSCC. Therefore, the aim of this study was to compare epithelial and sub-epithelial FHIT and Bcl-2 expression between patients with OLP, OSCC and healthy oral mucosa.Materials and Methods: Fifty patients with OLP, 20 with OSCC who had histologically confirmed diagnoses and 20 healthy controls were included in this study. Immuno-histochemical analysis was performed on primary monoclonal antibodies Bcl-2 (Dako, Finland) and FHIT (Zymed Laboratories Inc., USA) were used. Statistical analysis included Kolmogorov-Smirnoff test, $\chi 2$ test and Spearman's correlation. All p values lower than $p<0.05$ were considered as significant. Results: Expression of FHIT in the OLP and OSCC epithelium is significantly decreased when compared to the healthy oral mucosa. However, no significant differences between FHIT expression between OLP and OSCC could be found. OLP and OSCC patients have significantly increased expression values of Bcl-2 in the epithelium when compared to the epithelium of healthy participants. Furthermore, Bcl-2 expression is significantly increased in patients with OLP when compared to the patients with OSCC. Sub-epithelial infiltrate in OLP and OSCC reveals significantly higher Bcl-2 expression when compared to the healthy controls. However, Bcl-2 expression in the inflammatory infiltrate is significantly higher in OSCC patients when compared to the OLP patients. Conclusion: Loss of FHIT expression in the epithelium is not sufficient for malignant transformation in OLP patients. It seems that other molecular changes are needed for OLP progression into OSCC. Bcl-2 expression in the inflammatory infiltrate is significantly higher in OSCC patients when compared to the OLP patients and healthy controls, however bcl-2 expression in the epithelium does not correlate with precancerous (OLP) and OSCC lesions.

KEY WORDS: *oral lichen planus, oral squamous cell cancer, FHIT, Bcl-2*

ZNAČAJ FHIT I Bcl-2 U BOLESNIKA S ORALNIM LIHEN PLANUSOM U USPOREDBI S ZDRAVOM ORALNOM SLUZNICOM I ORALNIM KARCINOMOM PLOČASTIH STANICASAŽETAK

Sažetak

Uvod: Oralni lihen planus (OLP) je prekancerozna lezija koji u 0-1,2 % oboljelih može progredirati u oralni karcinom pločastih stanica (OKPS). Premda su objavljeni brojni radovi na ovu temu, ne postoje opće prihvaćeni klinički i patohistološki kriteriji koji bi upućivali na to u kojih će se bolesnika razviti OKPS. Dakle, cilj je ovog istraživanja bio usporediti

ekspresiju FHIT i Bcl-2 u epitelu i subepitelu pacijenata s OLP, OKPS te osoba sa zdravom oralnom sluznicom. Materijal i metode: U istraživanje je bilo uključeno pedeset pacijenata s histološki potvrđenom dijagnozom OLP, 20 pacijenata s histološki potvrđenom dijagnozom OKPS te 20 zdravih pacijenata kao kontrolna skupina. Korištene su imunohistokemijske analize na primarnim monoklonim protutijelima Bcl (Dako, Finska) i FHIT (Zymed Laboratories Inc., SAD). U statističkoj analizi korišteni su Kolmogorov-Smirnovljev test, hi kvadrat test i Spearmanov koeficijent korelacije. P vrijednosti manje od 0.05 su smatrane značajnima. Rezultati: Ekspresija FHIT u epitelu zahvaćenom s OLP i OKPS je značajno smanjena u usporedbi sa zdravom oralnom sluznicom. Međutim, nije nađena značajna razlika u FHIT ekspresiji kod OLP i OKPS. Pacijenti oboljeli od OLP i OKPS imaju značajno pojačanu ekspresiju Bcl-2 u usporedbi s zdravim sudionicima. Nadalje, Bcl-2 ekspresija je značajno veća u pacijenata s OLP, nego u pacijenata s OKPS. Subepitelni infiltrat u pacijenata s OLP i OKPS pokazuje značajno povećanje Bcl-2 ekspresije u odnosu na zdrave osobe iz kontrolne skupine. Međutim, Bcl-2 ekspresija u upalnom infiltratu je značajno viša u OKPS pacijenata nego li u OLP pacijenata. Zaključak: Gubitak FHIT ekspresije u epitelu sam po sebi nije dovoljan za zloćudnu transformaciju kod pacijenata koji boluju od OLP. Čini se da su za progresiju OLP u OKPS potrebne i druge molekularne promjene. Ekspresija Bcl-2 u upalnom infiltrate je znakovito visa u bolesnika s OPKS u odnosu na bolesnicke s OLP i zdrave kontrole, ipak, ekspresija bcl-2 u epitelu ne korelira s prekanceroznim (OLP) odnosno lezijama OPKS.

KLJUČNE RIJEČI: *oralni lihen planus, FHIT, Bcl-2, oralni planocelularni karcinom*

INTRODUCTION

Oral lichen planus is a chronic autoimmune disease of unknown aetiology which may affect skin, oral, vaginal, and anal and oesophageal mucosa (1). According to the WHO it is precancerous oral lesion with different malignant potential ranging from 0-1.2%. It seems that OLP lesions have higher proliferative level of the basal cells, therefore also the ability to accumulate irreparable DNA damage which adds to the malignant transformation. Key role in the accumulation of DNA damage has Bcl-2 family as they regulate apoptosis. Damage of Bcl-2 genes lead to the development of various cancers such as melanoma, breast cancer, prostate cancer, chronic leukaemia and lung cancer (2). Studies have shown that Bcl-2 expression in oral dysplastic changes is more frequent when compared to the hyperplastic oral changes; therefore increased Bcl-2 expression might correlate with the dysplasia or invasion of the tissues. Recent studies reported that cell proliferation in OLP is higher when compared to the healthy oral mucosa, but lower when compared to the epithelial dysplasia and OSCC (3). Increased Bcl-2 expression is not only essential for oral carcinogenesis; it also has influence on the disease progression as it increases the life of neoplastic cells and fosters genetic mutations (4). Gene FHIT (Fragile Histidine Triad) is a most fragile place in the human genome. Much depletion in the precancerous lesions and malignant tumours which manifested as lost or reduced expression have been described (5, 6, 7). Numerous researchers confirm that FHIT gene is significantly inactivated in the precancerous lesions as well as in cancer. Loss of FHIT expression especially in OSCC suggest poor prognosis of these patients. It seems that loss of FHIT expression might be potentially useful marker for early carcinogenesis (7).ne kuzim komentar recenzenta ovdje je jasno navedeno sto su bcl-2 I FHIT

We assumed that the difference in the FHIT and Bcl-2 expression in patients with OLP might differentiate subgroup of OLP patients which are prone to the malignant transformation into OSCC. Therefore, those patients might have benefit from more intensive follow-up.The aim of this study was:

1. To compare FHIT expression in the oral epithelium between patients with OLP, OSCC and healthy oral mucosa.
2. To compare Bcl-2 expression in the oral epithelium between patients with OLP, OSCC and healthy oral mucosa.
3. To compare Bcl-2 expression in the subepithelial inflammatory infiltrate between patients with OLP, OSCC and healthy oral mucosa.

MATERIALS AND METHODS

This study which was a retrospective one based on the histopathological data and was approved by Ethical Committee of the Sestre mi-

Table 1.

DIFFERENCES IN THE FHIT EXPRESSION WITHIN EPITHELIUM BETWEEN PATIENTS WITH OLP, OSCC AND HEALTHY ORAL MUCOSA.

		Groups						Difference between OLP and OSCC.	Difference between OLP and healthy oral mucosa	Difference between OSCC and healthy oral mucosa
		OLP		OSCC		Healthy oral mucosa				
		N	%	N	%	N	%			
FHIT epithelium	<10%	30	60%	15	75%	6	30%	X^2=1.400 df=1 P=0.237	X^2=5.147 df=1 **P=0.023**	X^2=8.120 df=1 **P=0.004**
	>10%	20	40%	5	25%	14	70%			

Table 2.

DIFFERENCES IN THE FHIT EXPRESSION WITHIN INFLAMMATORY INFILTRATE BETWEEN PATIENTS WITH OLP, OSCC AND HEALTHY ORAL MUCOSA.

		Groups						Difference between OLP and OSCC.	Difference between OLP and healthy oral mucosa	Difference between OSCC and healthy oral mucosa
		OLP		OSCC		Healthy oral mucosa				
		N			N	%				
FHIT inflammation	<10%	17	34%	9	45%	20	100%	X^2=0.740 df=1 P=0.390	X^2=24.973 df=1 **P<0.001**	X^2=15.172 df=1 **P<0.001**
	>10%	33	66%	11	55%	0	0%			

losrdnice University Hospital Center. Fifty samples of the OLP patients mainly situated on buccal mucosa, 20 samples of patients with OSCC majority developed on basis in oral cavity and 20 healthy controls without oral diseases some localized on buccal some on base of oral cavity were included in the study. Material was fixed in the 10% buffered formalin which is later put in the paraffin blocks and cut (5μm), deparaffinised and stained with hemalaun eosin. For immune-histochemical analysis sections of the aforementioned paraffin blocks were used and primary monoclonal antibodies Bcl-2 (code number No. MO887, Dako, Finland) and FHIT (code number 71-9000, Zymed Laboratories Inc., USA) were used. Immuno-histochemical reaction to Bcl-2 was semi-quantitatively determined in four categories; based on the number of positive citoplasmatic or membrene cell expression. Thus grade 1 expression included those samples with less than 10% expression, grade 2 included samples showing 10-30% expression, grade 3 included samples exhibiting 31-60% and grade 4 included samples showing greater than 61% positive cells according to Ravi et al. (8). Immuno-histochemical reaction to FHIT was semi-quantitatively determined in two categories; as 'negative' or 'low expression' (no staining or immunoreactivity staining present in <10% of tumor cells) or 'positive' (immunoreacitvity ≥10% of tumor cells) as decribed by Kujani et al. (9). Stats Direct version 3.0.86. was used for data analysis. Statistical analysis included Kolmogorov-Smirnoff test in order to check data distribution, and mostly nonparametric tests were used except for the age of the participants. $\chi 2$ test was used in order to distinguish categorical values between tested groups (OLP, OSCC and healthy controls). Spearman's correlation was used in order to obtain possible significant correlation between clinical parameters of the disease and age, i.e. certain parameters in the inflammatory infiltrate. All p values lower than $p<0.05$ were considered as significant.

RESULTS

Table 1. reveals differences in the FHIT expression within epithelium between patients with OLP, OSCC and healthy oral mucosa. FHIT expression >10% was significantly more present in the epithelium of healthy oral mucosa when compared to the OLP and OSCC. No differences between OLP and OSCC regarding FHIT expression within epithelium were found.

Table 2 shows differences in the FHIT expression within inflammatory infiltrate between pa-

Table 3.
DIFFERENCE IN BCL-2 EXPRESSION IN THE EPITHELIUM BETWEEN PATIENTS WITH OLP, OSCC AND HEALTHY ORAL MUCOSA.

		Groups						Difference between OLP and OSCC.	Difference between OLP and healthy oral mucosa	Difference between OSCC and healthy oral mucosa
		OLP		OSCC		Healthy oral mucosa				
		N				N	%			
Bcl-2	no reaction <10%	4	8%	11	55%	20	100%	X^2=22.489 df=3 **P<0.001**	X^2=53.667 df=3 **P<0.001**	X^2=11.613 df=3 **P=0.009**
	10-30%	30	60%	4	20%	0	0%			
	31-60%	13	26%	2	10%	0	0%			
	>60%	3	6%	3	15%	0	0%			

Table 4.
DIFFERENCE IN BCL-2 EXPRESSION IN THE INFLAMMATORY INFILTRATE BETWEEN PATIENTS WITH OLP, OSCC AND HEALTHY ORAL MUCOSA.

		Groups						Difference between OLP and OSCC.	Difference between OLP and healthy oral mucosa	Difference between OSCC and healthy oral mucosa
		OLP		OSCC		Healthy oral mucosa				
		N				N	%			
Bcl-2	No reaction <10%	7	14%	2	10%	17	85%	X^2=8.631 df=3 **P=0.035**	X^2=32.474 df=3 **P<0.001**	X^2=25.342 df=3 **P<0.001**
	10-30%	27	54%	5	25%	3	15%			
	31-60%	15	30%	10	50%	0	0%			
	>60%	1	2%	3	15%	0	0%			

tients with OLP, OSCC and healthy oral mucosa. FHIT expression >10% was significantly more present in the inflammatory infiltrate of the healthy oral mucosa when compared to OLP and OSCC. Difference in FHIT expression of the inflammatory infiltrate between OLP and OSCC was not significant.

When compared to the healthy oral mucosa, bcl-2 expression is significantly higher in the epithelium of the OLP and OSCC patients. Significantly higher bcl-2 expression is found in patients with OLP when compared to the ones with OSCC.

When compared to the healthy oral mucosa, bcl-2 expression is significantly higher in the inflammatory infiltrate of the OLP and OSCC patients. Significantly higher bcl-2 expression is found in the inflammatory infiltrate of patients with OSCC when compared to the ones with OLP.

DISCUSSION

Our results are in concordance with the results of Fitzpatrick et al. (10) and Reibel (11) who reported that malignant transformation of OLP was 1.09%. Latest research have shown that elevated Bcl-2 expression in the epithelium and inflammatory infiltrate might have role in the malignant transformation as well as in the progression of the disease as reported by Sudha et al. (12). Pigatti et al. (13) reported that staining for Bcl-2 in inflammatory infiltrate in OLP was positive in 92.9% of sections. Shailaja et al. (14) stated that significant expression of Bcl-2 marker in patients with OLP and oral epithelial dysplasia was seen. In this study significant Bcl-2 expression in the OLP epithelium when compared to the OSCC tissues might depict OLP patients which are prone to OSCC. Most of the OLP patients were grouped within Bcl-2 expression from 10-30%, therefore those patients might have benefit from more intensive follow-up. As this was a retrospective study this fact could not be confirmed as prospective studies are needed. It seems that other mechanisms besides epithelial changes are needed for OLP progression into OSCC. Contrary to our results, Nafarzadeh et al. (15) Bcl-2 was negative for all OLP and normal mucosa samples, and weak positivity was observed in OSCC samples. Leyva-Huerta et al. (16) reported that Bcl-2 was negative for all OLP and OSCC cases, and mild positivity was observed in the normal tissue. Furthermore,

Hadzi-Mihailovic et al. (17) concluded that Bcl-2 may not serve as a prognostic biomarker in oral cancer development from OLP, but it could help in selecting patients with higher need of follow up to prevent malignancy. de Sousa et al. (18) found no significant differences between the expression of bcl-2 in oral lichen planus and oral squamous cell carcinoma.

Significant difference in FHIT expression between patients with OLP and healthy oral mucosa as well as no difference in comparison to the OSCC might lead to the conclusion that OLP lesions only in some cases behave as precancerous lesions. van Heerden et al. (19) reported that FHIT tumour suppressor protein is abundantly expressed in normal epithelial cells of human organs and that this expression is lost or reduced in the majority of cancers arising in these epithelial tissues which was seen in about 60% of OSCC who have lost FHIT expression. However, significant difference in FHIT expression in OLP patients when compared to the healthy oral mucosa suggests that certain changes have occurred in the OLP epithelium, albeit these changes are not sufficient in order to differentiate OLP patients who are prone to the OSCC development. Yin et al. (20) reported that the rate of the negative or low FHIT expression in OSCC was 17% which was significantly lower when compared to the healthy oral mucosa and oral precancerous lesions. In the other study, Yin et al. (21) stated that the positivity rate of FHIT in healthy oral mucosa was 100% and medium and high FHIT expression levels were detected in oral precancerous lesions but without significance when compared to the healthy oral mucosa. The rate of negative or low FHIT expression in the oral squamous cell carcinoma was 17% which was significantly different from the normal oral mucosa and oral precancerous lesions.

CONCLUSION

1. Expression of FHIT in the epithelium of OLP and OSCC is significantly decreased when compared to the epithelium of the healthy oral mucosa. However, no significant differences between FHIT expression between OLP and OSCC could be found.
2. OLP and OSCC patients have significantly increased expression values of Bcl-2 in the epithelium when compared to the epithelium of healthy participants. Furthermore, Bcl-2 expression is significantly increased in patients with OLP when compared to the patients with OSCC.
3. Sub-epithelial infiltrate in OLP and OSCC patients reveals significantly higher Bcl-2 expression when compared to the healthy controls. However, Bcl-2 expression in the inflammatory infiltrate is significantly higher in OSCC patients when compared to the OLP patients.
4. Extremely strong and significant positive Bcl-2 expression in the epithelium and sub-epithelial inflammatory infiltrate in OLP patients could be noticed.
5. Loss of FHIT expression in the epithelium is necessary, however not sufficient for malignant transformation in OLP patients. It seems that other molecular changes are needed for OLP lesions to develop in OSCC.

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Corresponding author: Vanja Vučićević Boras, Department of Oral Medicine, School of Dental Medicine, University of Zagreb, Gundulićeva 5, 10000 Zagreb, Croatia; e-mail: boras@sfzg.hr