Vulvar intraepithelial neoplasia and vulvar squamous cell carcinoma: a clinicopathologic study of 18 cases

Ebru Demiralay^{1*}, Nihan Haberal Reyhan¹, Özlem Özen¹, Burcu Ksa², Ali Ayhan² and Beyhan Demirhan¹

Correspondence: ebrudemiralay@yahoo.co.uk

¹Department of Pathology, ²Department of Obstetrics and Gynecology, Faculty of Medicine, Baskent University, Ankara, Turkey.

Abstract

Background: The vulvar intraepithelial neoplasia is divided into two groups: usual type and differentiated type. The differentiated vulvar intraepithelial neoplasia which is frequently seen together with the invasive squamous cell carcinoma can be confused with some benign lesions. To analyse p16, p53, and Ki-67 expression characteristics of different histological types of invasive squamous cell carcinoma of the vulva and vulvar intraepithelial neoplasia is aimed in this study.

Methods: In this study, immunohistochemical analysis of 18 vulvectomy cases with p16, p53, and Ki-67 was performed.

Results: Of 18 patient who underwent vulvectomy, 9 had invasive squamous cell carcinoma and 9 vulvar intraepithelial neoplasia. 3 additional vulvar intraepithelial neoplasia lesions were found accompanying the invasive squamous cell carcinomas. Mean Ki-67 PI was 32,3% in usual type (human papilloma virus-related) vulvar intraepithelial neoplasia cases (n:9), and 26,4% in differentiated vulvar intraepithelial neoplasia cases (n:3)(p>0,05). Mean p16 staining degree was 2,6 for usual type vulvar intraepithelial neoplasias. No p53 expression was present in squamous cell hyperplasia or lichen sclerosis lesions.

Conclusions: Ki-67 PI does not have significant value in recognizing vulvar intraepithelial neoplasia usual type and differentiated vulvar intraepithelial neoplasia. p53 positivity can be of value in distinguishing especially differentiated type vulvar intraepithelial neoplasia from benign lesions.

Keywords: vulva, vulvar intraepithelial neoplasia, invasive squamous cell carcinoma

Background

Based on histological observations, approximately 90 % of all primary malign carcinomas of the vulvar tumors are invasive squamous cell carcinoma (ISCC) [1,2]. According to the currently popular classification system, vulvar invasive squamous cell carcinoma (ISCCV) is divided into 3 main histological groups; basaloid, warty and keratinizing ('conventional' or 'typical' keratinizing) types [1,3,4]. Conventional type is the most common histological type of ISCCVs. Basaloid and warty types of ISCCV are thought to originate from basaloid and warty type vulvar intraepithelial neoplasia (VIN). This type of VINs has strongly been associated with human papilloma virus (HPV) [4]. VIN terminology was modified in 2004 by The International Society for the Study of Vulvovaginal Disease (ISSVD), and was mainly divided into two groups: VIN usual type and VIN differentiated (simplex) type. These terms account for VIN 2 and VIN 3 in the older terminology, and the term VIN 1 has not been used anymore [5,6-8].

ISCCVs are grouped into two general types according to their different clinicopathologic features. The first group is the type seen in young age groups (35-65 years old) and is associated with HPV, and the second group is the type seen mostly in older women (55-85 years old), is unrelated to HPV, and has different etiological factors [1,2,9].

The type related to HPV accounts for 1/3 of all ISCCVs. The group named as classic VIN, whether warty or basaloid is characterized by accompanying highly differentiated VIN and high Ki-67 proliferation index (Ki-67 PI). In addition, there is increase in the risk of squamous neoplasia, particularly of cervix and other parts of lower genital tract. The patients in this group appear to have a better prognosis than the group of patients with ISCC unrelated to HPV [1,2,9].

The second group (unrelated to HPV) accounts for 2/3 of all ISCCs. The patients in this group typically develop a conventional (keratinizing) type ISCC associated with lichen sclerosis (LS) and squamous hyperplasia (SCH), and recent

studies have shown the relationship with differentiated (simplex) VIN. Moreover, mutation in p53 tumor suppressing gene has often been observed [1,2,10,11].

The differentiated VIN often observed together with the second group accounts for 2- 10% of all VINs [12]. These lesions have often been left unnoticed because histological criteria for differentiated VIN have not been clearly described and because there have been only a few cases in literature.

The aim of this study was to study 8 ISCC, one verrucous carcinoma and 9 VIN cases diagnosed at Baskent University Pathology Department between the years 2007-2009, and to analyze the immunohistochemical features of p16, p53 and Ki-67 expression and to compare the histopathologic features of the cases with accompanying other vulvar lesions.

Methods

In this study, all available preparations and paraffin blocks belonging to radical and skinning vulvectomy materials of 18 cases were retrieved from the surgical pathology files of Baskent University Ankara Hospital. All 18 cases of ISSCV and VIN had been operated from January 2007 through June 2009 at Baskent University Ankara Hospital, by the Gynecological Oncology team. Institutional Board Ethical approval for the study was obtained.

Hematoxylin and eosin (H&E) stained sections for all 18 cases are present. Vulvar squamous cell carcinomas have been grouped into 3 histopathologic types as basaloid, warty and keratinizing, and VINs were evaluated according to 2004 ISSVD terminology (6). The latter were classified as differentiated VIN and classical-usual VIN (which were previously classified as VIN II and VIN III).

Pathology reports were reviewed to obtain the ages of the patients and the localization of tumors. In addition, for the invasive carcinomas, the widest horizontal diameter of the tumor, tumor thickness, the depth of invasion, lymphovascular invasion, lymph node involvement, accompanying cervical or vaginal lesions and vulvar benign lesions were re-evaluated.

Immunohistochemical stains for the paraffin block sections were prepared using p16 (clon E6H4, mouse monoclonal, CINtec), Ki-67 (MIB-1 clone, rabbit polyclonal, Neomarkers) and p53 (DO-7 clone, mouse monoclonal, Neomarkers) antibodies using peroxidase-antiperoxidase method. Strong nuclear and cytoplasmic staining was evaluated positive reaction for p16 expression. p16 reaction pattern was scored semiquantitatively as follows; negative (-) when less than 1% of cells were positive, 1+: positive reaction in 1-10% cells, 2+: positive reaction in 11-50% cells, 3+: when more than 50% of cells were positive. Immunostaining for p53, which was always only nuclear, was graded using the same scoring system. The total of 1000 cells in the areas where Ki-67 positive cells peaked, was measured as proliferation index (PI).

Results

Histologic features

Table 1. Clinicopathological features

Case number	Age	Previous lesion			Non-neoplastic lesion		Additional lesions	
		VIN3	ISCC	Operation procedure	SCH	LS	Va-gen	Cer-vix
1	80	W	-	SV	-	-	-	-
2	26	W	-	SV	+	-	-	-
3	29	W	-	SV	+	-	-	-
4	40	W	-	SV	-	-	-	-
5	50	B	-	SV	-	-	VAIN	LSIL
6	36	W	-	SV	-	-	-	HSIL
7	29	W	-	SV	-	-	-	-
8	56	D	-	SV	+	+	-	-
9	52	W	-	SV	+	-	-	-
10	80	W	W	DWE	-	-	-	-
11	73	-	K	HV	-	-	-	-
12	58	-	K	V	+	+	-	-
13	61	-	K	HV	-	-	-	-
14	56	D	MI	RV	-	-	-	-
15	64	-	K	V	+	-	-	-
16	66	D	K	HV	-	-	-	-
17	52	-	K	V	-	-	-	-
18	84	-	V	RV	+	-	-	-

VIN-Vulvar intraepithelial neoplasia, ISCC-Invasive squamous cell carcinoma, W-Warty type, B-Basaloid type, D-Differentiated type, K-Keratinizing type, MI-Microinvasive, VC-Verrucous carcinoma, SV-Skinning vulvectomy, DWE-Deep wide excision, HV-Hemivulvectomy, V-Vulvectomy, RV-Radical vulvectomy, SCH-squamous hyperplasia, LS-lichen sclerosis, VAIN-Vaginal intraepithelial neoplasia, LSIL-Low grade squamous intraepithelial lesion, HSIL-High grade squamous intraepithelial lesion.

Of 18 patients who underwent radical and skinning vulvectomy, 9 had ISSCV (6 keratinized type, 1 warty type, 1 microinvasive, 1 other verrucous carcinoma) and of the other 9 patients with VIN. **Table 1** shows the clinicopathologic features of the patients.

Association with other conditions

Together with VIN lesions observed also in invasive carcinoma cases, 3 of the total 12 VIN lesions were classified as differentiated, 1 as basaloid, and 8 as warty types.

SCH areas were also present in 4 patients with VIN and 3 patients with ISSCV. LS was present in 1 case with keratinizing type invasive carcinoma and 1 case with differentiated VIN.

Immunohistochemical features

Ki-67 PI: In a total of 9 HPV related VIN cases (8 warty, 1 basaloid), the mean Ki-67 PI was 32,3%, whereas this value was 26,4% in 3 differentiated VIN cases.

In keratinizing type invasive carcinoma, the mean Ki-67 proliferation index was 16,75%, and those of one warty and one microinvasive carcinoma case were 20% and 10,8%, respectively.

Figure 1a. Strong p16 expression was detected in carcinoma of keratinizing type without the accompanying of HPV-related VIN, **1b.** and high p53 expression was observed in the same case, immunoperoxidase x100.

Figure 2. p53 expression was observed in differentiated VIN area in carcinoma of keratinizing type, immunoperoxidase x400.

Table 2. p16 expression pattern, clinical features and follow-up information invasive carcinoma patients.

	1+	2+	3+	4+
Total	2	1	2	3
Age	54	64	62	71,3
Ki-67 PI				
≤%30	2	1	2	3
>%30	-	-	-	-
Local recurrency	0	0	0	0
Metastatic disease				
<i>Lymph node</i>	0	1	1	1
<i>Distant</i>	0	0	1	0
Stage				
1A	-	-	-	1
1B	2	-	1	1
2	-	-	-	-
3	-	1	1	1
4	-	-	-	-

Table 3: p53 expression pattern, clinical features and follow-up information invasive carcinoma patients.

	1+	2+	3+	4+
Total	1	1	2	4
Age	56	80	56,5	65,25
Ki-67 PI				
≤%30	1	1	2	4
>%30	-	-	-	-
Local recurrency	0	0	0	0
Metastatic disease				
<i>Lymph node</i>	0	0	1	2
<i>Distant</i>	0	0	0	1
Clinical Stage				
1A	-	1	-	-
1B	1	-	1	2
2	-	-	-	-
3	-	-	1	2
4	-	-	-	-

Ki-67 proliferation index had a mean 8,07% in SCH accompanying the lesions. Ki-67 proliferation was found to be 8% and 7% in two cases with LS.

P16: p16 staining degree had a mean of 2,6 for warty and basaloid type VINs. No p16 expression was detected in differentiated type VINs. Strong p16 expression was detected in 3 carcinomas of keratinizing type (**Figure 1a**). No p16 immunoreactivity were present in benign vulvar lesions.

P53: P53 expression were observed as 4+ in 4 cases with ISCCV, as 3+ in cases, 2+ in cases, 1+ in cases (**Figure 1b**). **Tables 2** and **3** summarize the p16 and p53 expression patterns together with clinical and observational information. When evaluated semiquantatively, p53 expression in usual type VINs had a mean of 1,2 and it was 1,6 in differentiated VINs

Figure 3a. No p53 expression was present in squamous hiperplasia, immunoperoxidase x100, **3b.** No p53 expression was present in another case with squamous hiperplasia, immunoperoxidase x200.

(**Figure 2**). No p53 immunoreactivity were present in benign vulvar lesions (**Figure 3a,3b**).

Discussion

VIN classification has been reviewed after the determination of HPV role in VINs. Haefner *et al.*, observed HPV DNA in 78% of 58 classic VIN lesions and 14% of differentiated VINs [13]. Contrary to the studies performed by Haefner *et al.*, many studies using molecular techniques have shown no HPV DNA in vulvar invasive carcinomas [4,14]. While warty and basaloid VINs are easily defined because of histopathologic features and immunohistochemical p16 positivity, it is difficult to diagnose differentiated VIN because it can be confused with some benign lesions such as SCH [5,12]. Therefore, the number of cases with warty and basaloid VIN diagnosis is more than the ones with differentiated type VIN. For this reason, to decrease the mortality rate and its frequency, immunohistochemical

staining of Ki-67 and p53 has gained importance to recognize preinvasive vulvar lesions earlier. While Ki-67 PI is determined in full thickness of epithelium and diffusely in VIN cases of warty and basaloid characteristics, it has no diagnostic value in differentiated VIN cases. However, it has been stated that increased p53 expression can be used to discriminate the differentiated VIN from other benign lesions [1,3,12,15]. Our study supports some studies showing the presence of p53 expression in differentiated VIN and keratinizing type ISCCs accompanied by differentiated VIN. Similar to the cases in literature, no p16 expression was detected in these cases [2,14,16]. On the other hand, in 3 cases with keratinizing type carcinoma showing strong p16 immunoreactivity and not accompanied by usual type VIN, diffuse p53 expression was found. According to these results, p53 expression can be seen in both usual and differentiated VIN and invasive carcinoma cases, and because there was no evidence of p53 expression in SCH or LS lesions observed in our cases, p53 staining can be of reliable utility in distinguishing benign lesions from the malign ones. It has been observed that Ki-67 PI can be found in almost similar ratios in lesions associated with HPV and irrelevant with HPV. However, it has been noted that in strong p53 positivity, Ki-67 PI is also strongly positive.

In contrast to many studies stating that keratinizing type invasive carcinomas have no association with HPV, in the present study, strong p16 positivity was detected in 3 ISCCs which were morphologically keratinizing type [3,4]. Al-Ghamdi *et al.*, similar to our results, have found in their series of 21 cases that 18 of the invasive carcinomas associated with HPV were of keratinizing type in young patients [1]. The reason for this may be that there is a significant degree of subjectivity in subclassifying invasive vulvar carcinomas and that most of the cases show evidence of keratinization.

Verrucous carcinoma is a discrete variant of ISCC and there is no clear evidence of association with HPV. However, in their series of 10 cases with verrucous carcinoma, Gaulco *et al.*, found that all the cases were found to be HPV negative in in-situ DNA hybridization analysis performed by HPV 6/11, 16/18, 31/35/51 probes [17]. In addition, Santos *et al.*, supported the study of Gaulco *et al.*, by their studies stating that p53 was negative in all the verrucous carcinoma cases [11,17]. In our study, in contrast to the studies mentioned above, p53 showed low grade positivity (1+), but in accordance with the cases in literature, Ki-67 proliferation index was rather low (6%) and p16 was negative.

Conclusions

ISCCVs are divided into two groups; one associated with HPV and the other not associated with HPV, and it is difficult to subclassify invasive carcinomas into warty, basaloid or keratinizing types depending on morphological features because some of the cases showing strong p16 expression also show evidence of strong keratinization. Although Ki-67 proliferation index does not have significant value in

recognizing VIN, p53 positivity can be of value in distinguishing especially differentiated type VIN from benign lesions.

Competing interests

The authors declare that they have no competing interests.

Publication history

Senior Editor: Jiaoti Huang, David Geffen

School of Medicine at UCLA, USA.

EIC: G.J. Peters, VU University Medical Center, Netherlands.

Received: 03-Aug-2012 Revised: 16-Aug-2012

Accepted: 17-Aug-2012 Published: 27-Aug-2012

References

- Al-Ghamdi A, Freedman D, Miller D, Poh C, Rosin M, Zhang L, *et al.*: **Vulvar squamous cell carcinoma in young women: a clinicopathologic study of 21 cases.** *Gynecol Oncol* 2002, **84**(1):94-101. | [Article](#) | [PubMed](#)
- Santos M, Landolfi S, Olivella A, Lloveras B, Klaustermeier J, Suarez H, *et al.*: **p16 overexpression identifies HPV-positive vulvar squamous cell carcinomas.** *Am J Surg Pathol* 2006, **30**(11):1347-56. | [Article](#) | [PubMed](#)
- Kurman RJ, Toki T, Schiffman MH: **Basaloid and warty carcinomas of the vulva. Distinctive types of squamous cell carcinoma frequently associated with human papillomaviruses.** *Am J Surg Pathol* 1993, **17**(2):133-45. | [PubMed](#)
- Chiesa-Vottero A, Dvoretzky PM, Hart WR: **Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with analysis of adjacent vulvar intraepithelial neoplasia types and lichen sclerosis.** *Am J Surg Pathol* 2006, **30**(3):310-8. | [Article](#) | [PubMed](#)
- Preti M, Van Seters M, Sideri M, Van Beurden M: **Squamous vulvar intraepithelial neoplasia.** *Clin Obstet Gynecol* 2005, **48**(4):845-61. | [Article](#) | [PubMed](#)
- Heller DS: **Report of a new ISSVD classification of VIN.** *J Low Genit Tract Dis* 2007, **11**(1):46-7. | [Article](#) | [PubMed](#)
- Santegoets LA, Seters M, Helmerhorst TJ, Heijmans-Antonissen C, Hanifi-Moghaddam P, Ewing PC, *et al.*: **HPV related VIN: highly proliferative and diminished responsiveness to extracellular signals.** *Int J Cancer* 2007, **121**(4):759-66. | [Article](#) | [PubMed](#)
- Scurry J, Campion M, Scurry B, Kim SN, Hacker N: **Pathologic audit of 164 consecutive cases of vulvar intraepithelial neoplasia.** *Int J Gynecol Pathol* 2006, **25**(2):176-81. | [Article](#) | [PubMed](#)
- van der Avoort IA, van der Laak JA, Paffen A, Grefte JM, Massuger LF, de Wilde PC, *et al.*: **MIB1 expression in basal cell layer: a diagnostic tool to identify premalignancies of the vulva.** *Mod Pathol* 2007, **20**(7):770-8. | [Article](#) | [PubMed](#)
- Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, *et al.*: **Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee.** *J Reprod Med* 2005, **50**(11):807-10. | [PubMed](#)
- Santos M, Montagut C, Mellado B, Garcia A, Ramon y Cajal S, Cardesa A, *et al.*: **Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva.** *Int J Gynecol Pathol* 2004, **23**(3):206-14. | [Article](#) | [PubMed](#)
- Hart WR: **Simplex (differentiated) VIN: an underappreciated threat.** *Ob/Gyn Apr.* 1, 2003; 48: 59-83.
- Haefner HK, Tate JE, McLachlin CM, Crum CP: **Vulvar intraepithelial neoplasia: age, morphological phenotype, papillomavirus DNA, and coexisting invasive carcinoma.** *Hum Pathol* 1995, **26**(2):147-54. | [Article](#) | [PubMed](#)
- Ordi J, Alejo M, Fuste V, Lloveras B, Del Pino M, Alonso I, *et al.*: **HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN.** *Am J Surg Pathol* 2009, **33**(11):1659-65. | [Article](#) | [PubMed](#)
- Yang B, Hart WR: **Vulvar Intraepithelial Neoplasia of the Simplex (Differentiated) Type: A Clinicopathologic Study Including Analysis of HPV and p53 Expression.** *The American Journal of Surgical Pathology* 2000, **24**(3). | [Article](#)
- van de Nieuwenhof HP, van Kempen LC, de Hullu JA, Bekkers RL, Bulten J, Melchers WJ, *et al.*: **The etiologic role of HPV in vulvar squamous cell carcinoma fine tuned.** *Cancer Epidemiol Biomarkers Prev* 2009, **18**(7):2061-7. | [Article](#) | [PubMed](#)
- Gualco M, Bonin S, Foglia G, Fulcheri E, Odicino F, Prefumo F, *et al.*: **Morphologic and biologic studies on ten cases of verrucous carcinoma of the vulva supporting the theory of a discrete clinico-pathologic entity.** *Int J Gynecol Cancer* 2003, **13**(3):317-24. | [Article](#) | [PubMed](#)

Citation:

Demiralay E, Reyhan NH, özen ö, Ksa B, Ayhan A and Demirhan B: **Vulvar intraepithelial neoplasia and vulvar squamous cell carcinoma: a clinicopathologic study of 18 cases.** *Journal of Cancer Therapeutics and Research* 2012, **1**:24.
http://dx.doi.org/10.7243/2049-7962-1-24