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SQUAMOUS CELL CARCINOMA OF THE VULVA AND ADJACENT LESIONS TREATED AT NAGOYA UNIVERSITY HOSPITAL FROM 1965 TO 1997

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ABSTRACT

Japan has a lower incidence of vulvar squamous cell carcinoma (VSCC) than Western nations. To pinpoint the reasons for this, we reviewed biopsy samples from all cases treated at Nagoya University Hospital over the past 33 years in order to investigate the background lesions for VSCC. Two of 36 VSCC patients had adjacent or coexisting lichen sclerosus (LS), 5 had squamous cell hyperplasia (SCH), and 16 had vulvar intraepithelial neoplasia (VIN). There were 8 cases in which these lesions were thought to be the origin of the VSCC, 1 in which keratinizing squamous cell carcinoma (KSC) was seen in LS, 1 in which verrucous SCH was the origin, and 6 in which 4 basaloid carcinoma and 2 warty carcinoma developed from basaloid VIN and warty VIN, respectively. Although 8 other cases of keratinizing or non-keratinizing squamous cell carcinomas (NSC) coexisted with VIN NOS (not otherwise specified), differentiated VIN or basaloid VIN, we could not be histologically certain of the origin. Among 22 VSCC patients tested for HPV DNA, only an 84-year-old woman presenting a histological feature of KSC tested positive by in situ hybridization (ISH). It was considered that LS and SCH had little and VIN considerable capacity to cause the malignancy of VSCC. We surmise that in Japan the majority of squamous cell carcinoma is unrelated to HPV. One reason for the low incidence of VSCC is largely due to race; the homogeneous, monoethnic Japanese population, as well as the few cases of HPV-related VSCC.

Key Words: Squamous cell carcinoma of the vulva, lichen sclerosus, vulvar intraepithelial neoplasia-HPV

INTRODUCTION

On a population basis, the incidence of cancers of the female genitalia differs considerably by region and race.[1)2)] The same is also true when viewed on a hospital basis. The incidence of ovarian cancer in Japan is lower than in Western countries, while the incidence of squamous cell carcinoma of the uterine cervix is higher. Population-based statistics show that malignant tumors of the oviducts, vagina, and vulva, which make up the "Other female genitalia" category, occur at a lower incidence in Japan than in Western countries.[1)] Vulvar cancer accounts for a high proportion of cancers in the "Other female genitalia" category both in Japan and

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Western countries, so it may be assumed that there are fewer malignant tumors of the vulva in Japan than in the West. What are the reasons for this lower incidence? To date, a variety of precursor lesions have been investigated for vulvar squamous cell carcinoma (VSCC), which accounts for the majority of malignant tumors of the vulva. Lichen sclerosus (LS), squamous cell hyperplasia (SCH), and vulvar intraepithelial neoplasia (VIN) have been particularly well investigated.[3-7] However, LS and SCH are still not generally accepted as precursor lesions.[8-11] There are also reports indicating that atypical epithelium, which often accompanies LS and SCH, can be a precancerous lesion.[3)5)12)] Reports differ as to the percentage of VIN which progresses to VSCC. It has also been reported in recent years that VIN is increasing together with the sudden rise in human papillomavirus (HPV) infection.[13)] However, the increase in VIN does not necessarily parallel the increase in VSCC,[14)15)] and some investigators have voiced doubts as to whether VSCC originates as VIN.[2)16)] It should be noted that many problems remain to be resolved concerning the precursor lesions of VSCC. In Japan, the low incidence of this disease means that there is also a lack of data, making investigation of the lesions that develop into vulvar cancer very difficult.

In the present retrospective study, we review cases of vulvar lesions treated at Nagoya University Hospital over 33 years in order to help clarify the types of lesions that give rise to squamous cell carcinoma of the vulva in Japan.

MATERIALS AND METHODS

In the present study, we reviewed requests for pathology tests that are on file in the Department of Laboratory Medicine, Nagoya University Hospital, for the 33 years from 1965 through 1997. Among these cases, we also microscopivally reviewed under microscope biopsies taken from the vulva as well as glass slide specimens from surgery cases. The vulva is bounded anteriorly by the mons pubis, posteriorly by the anus, and laterally by the folds of the inguinal and gluteal regions. In the center it extends to the hymen, and it also includes the urethral orifice which is contiguous with the vestibule.

There were 1 or 2 vulva specimens from each biopsy, and 2 to 18 specimens (average 9) from the surgery cases. Almost all were stained with hematoxylin-eosin. Tissue classifications were made using the female genital tumor classifications of the ISSVD (1990)[17)] and WHO (1994).[18)] The AFIP-vulva[19)] was referred to for VIN subclassifications. The word “adjacent” is used only when there are 2 or more lesions in the same visual field in a specimen on a glass slide being magnified for microscopic observation at a power of 4 (4 mm diameter on the specimen). Thus, it was possible to observe “adjacent lesions” even in a small biopsy sample. Biopsy specimens other than those containing VSCC were treated as “adjacent tissue” in materials from multiple biopsies. Adjacent lesions were observed in 28 cases in total: all 12 of the surgery cases, 8 of 8 multiple biopsy cases, 3 of 3 excisional biopsy cases, and 5 of 14 incisional biopsy cases.

For in situ hybridization (ISH),[20)] ordinary biotin probes (Cat# Y1411, Y1412, Y1413, DAKO Kyoto JAPAN) and In-situ Hybridization Detection kit for DNA/RNA probes (Cat# K0600, DAKO Kyoto JAPAN) were used to search for HPV and identify its type (6/11, 16/18, 31/33) in 34 cases of condyloma acuminatum, 12 cases of VIN, and 22 cases of VSCC.

RESULTS

The total number of biopsies for vulvar lesions performed at the Nagoya University Hospital

during the 33 years in question accounted for 0.26% of the total number of all biopsies, about 260,000, performed in that same period. There were 511 patients (Table 1), of whom 93 (18%) had malignant tumors. Sixty-three had epithelial malignant tumors (excluding VIN, including Paget's disease), 2 had soft tissue tumors, 2 had other tumors, and 26 had metastatic lesions (Table 1).

Of the 146 patients with epithelial tumors and related lesions, tumors of the squamous epithelium accounted for 78% (114 cases) and glandular-type for 22% (33 cases). Among tumors of the squamous epithelium, VIN was only seen in 13 cases (11.4%) (VIN was present together with squamous cell carcinoma in 16 other cases, and with Paget's disease in 1), and squamous cell carcinoma was present in 36 cases (31.6%) (Table 2). Of the 33 cases of glandular tumors, Paget's disease accounted for 24 (72.7%).

Table 1 Vulvar lesions treated at Nagoya University Hospital from 1965-1997

	No. of cases	%	No. of malig.	% of malig.
Epithelial tumors and related lesions	146[22]	27.2	63	66.3
Soft tissue tumors	20 [1]	3.9	2 *	4.2
Miscellaneous tumors	8	1.5	2**	2.1
Secondary tumors	26	4.7	26	27.4
Tumor-like lesions and nonneoplastic disorders	333[23]	62.7		
Total	533[23]	100	93	100

Twenty-three of the 511 patients appear in the tabulation twice, thus accounting for a total of 533.
** Both embryonal rhabdomyosarcoma. **both malignant melanoma*

Table 2 Squamous lesions

	No. of cases
Epithelial papillomas and polyps	22
Condyloma acuminatum	41
Seborrheic keratosis	1
Squamous intraepithelial lesions (VIN)	
Mild dysplasia (VIN1)	1
Moderate dysplasia (VIN2)	1
Severe dysplasia (VIN3)	7
Carcinoma in situ (VIN3)	4
Squamous cell carcinoma*	
Keratinizing	24
Nonkeratinizing	3
Basaloid	6
Warty	3
Total	114

**Sixteen of 36 squamous carcinomas coexist with VINs.*

In Table 3, 36 VSCC patients are arranged by histologic subtype and order of age, 27 of whom had the usual type of squamous cell carcinoma (keratinizing or nonkeratinizing). The ages of the 36 squamous cell carcinoma patients ranged from 35 to 87 years (mean age 64.8 years), and 73% were 60 years of age or older. The most common complaint during the initial hospital visit was that the patient had felt a lump (22 cases), followed by pain, bleeding, and ulcer. The primary site of the VSCC was in or included the labia in 68% of the patients (23 cases), followed by 27% (10 cases) in which the primary site included the vaginal vestibule. There were 2 patients with multiple lesions appearing in 2 or more locations (Case 1 and 7),

Table 3 Thirty-six patients with vulvar squamous cell carcinoma. Age, histological subtypes, site of squamous cell carcinoma, adjacent tissue, adjacent lesions, results of tests for HPV DNA (ISH) and other associated cancers or diseases are shown

No. of cases	Age (yrs)	Histologic subtype	Site of vulvar SCC	Adjacent tissue detectable	Adjacent lesions			HPV (ISH)	Other assoc. cancer or diseases
1	38	KSC	min-maj, anus	+				ND	
2	43	KSC	vest-perin	+	LP	SCH	D-VIN		
3	48	KSC	vest	+				-	
4	49	KSC	vag-vest	+				-	
5	51	KSC	min	-				-	
6	57	KSC	vag-vest	+	LP			-	
7	59	KSC	min, maj	+	LP&LS	SCH	D-VIN	ND	RA
8	60	KSC	min-maj	+			VIN	ND	
9	61	KSC	min	+	LP&LS			-	
10	61	KSC	min-maj	-				ND	renal fail. D
11	63	KSC	min	+	LP	SCH	VIN	ND	hypertension, DOD
12	64	KSC	maj	+				-	SCC cervix
13	65	KSC	maj	-				-	
14	67	KSC	maj	+				ND	
15	68	KSC	maj	-				-	DOD
16	69	KSC	maj	-				-	
17	70	NSC	maj	-				-	bladder TCC
18	71	KSC	vest-ureth	+			B-VIN	ND	bladder TCC
19	71	KSC	perin-vest	+			D-VIN	ND	
20	72	NSC	vag-min-maj	+	LP		VIN	ND	
21	72	KSC	min	+			D-VIN	-	
22	72	KSC	maj	+	LP	SCH			
23	73	KSC	maj	+				ND	
24	75	NSC	vest	-				-	hypertension
25	76	KSC	min	-				ND	nephritis
26	80	KSC	vag-min-maj	+		SCH	D-VIN	-	hypertension
27	84	KSC	min	+			B-VIN	+	
28	63	BC	vag-perin	+				ND	
29	71	BC	maj-perin	+			B-VIN	ND	
30	73	BC	maj-min	+			B-VIN	-	Tbc of lung
31	73	BC	ureth-vest	+			B-VIN	-	
32	76	BC	vest-perin	+				-	hypertension
33	87	BC	min-maj	+			B-VIN	-	
34	35	WC	vag-vest-labia	+			W-VIN	-	CIS cervix
35	50	WC	perin	+				-	
36	53	WC	vest	+			W-VIN	-	

KSC: keratinizing type, NSC: non-keratinizing type, BC: basaloid type, VC: verrucous type, WC: warty type, vag: vagina, vest: vestibule, min: labia minor, maj: labia major, perin: perineum, ureth: urethra, LP: leukoplakia, LS: lichen sclerosus, SCH: squamous cell hyperplasia, VIN: vulvar intraepithelial neoplasia not oterwise specified, D-, B-, W-VIN: differentiaed type, basaloid, warty VIN. HPV: human papilloma virus, ISH: in situ hybridization, ND: not detected, RA: rheumatoid arthritis, D: died, DOD: died of the disease, TCC: transitional cell carcinoma, Tbc: tuberculosis

but in both cases the lesions were on the same side, and it cannot be ruled out that, histologically, one was a metastasis of the other.

Of the 36 squamous cell carcinoma patients, the adjacent tissue could be evaluated in 28, among leukoplakia was seen clinically adjacent to or coexisting with the squamous cell carcinoma in 7 and LS was confirmed histologically in 2 (8%), one of which was accompanied by atypical epithelium. In one of these cases (Case 7), typical LS (Fig. 1) was distributed symmetrically, mainly in the labium majus, on both sides, and a relatively small VSCC nidus was observed in one portion of the LS in the labium majus on one side. The LS surrounding the VSCC was differentiated VIN, and findings indicated that it was gradually being displaced by VSCC (Fig. 2). Histologically, the process of developing into VSCC could be followed. This was thought to be a case in which LS passed through a phase of differentiated VIN to become VSCC.

In the other patient, LS was coexistent with but separate from VSCC, so it could not be determined that LS was the source that developed into VSCC. The histologic picture of the 5 other patients diagnosed clinically as having leukoplakia included lichen planus in 2, fibrosis of the dermis in 1, and hyperplasia accompanied by hyperkeratosis in 2, none of which corresponded to the histological picture of LS in the WHO.

Among the 36 cases of VSCC, hyperplasia was also present in 5. Of great interest was one KSC patient (Case 2) who was observed over 17 years for verrucous SCH (Fig. 3), during which time differentiated VIN with acantholysis (Fig. 4) and actinic-like differentiated VIN appeared in one part of the SCH that extended over a broad area. This was followed by KSC (Fig. 5). Surgery was performed 19 years after the initial examination. This was a case in which SCH might be the source of KSC. In 14 cases, dermatosis other than LS and SCH was

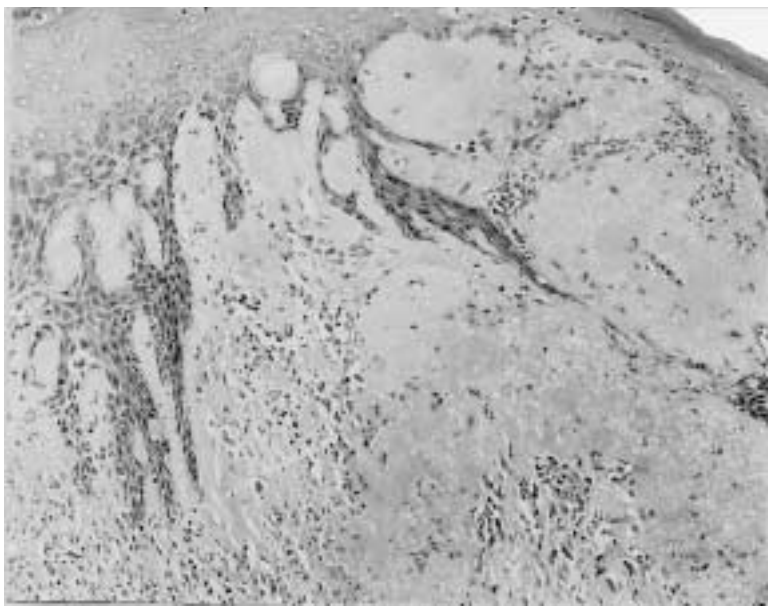

Fig. 1 Case 7. Keratinizing squamous cell carcinoma. Typical lichen sclerosus with squamous cell hyperplasia is found.

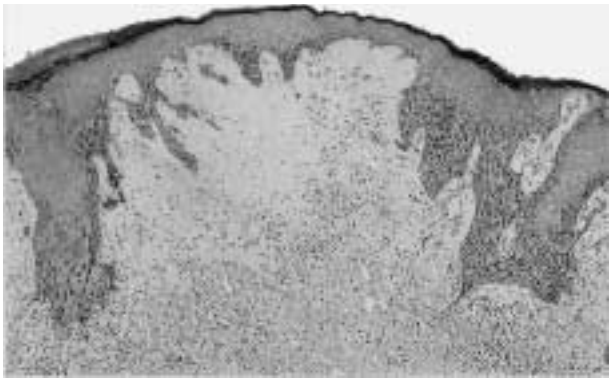

Fig. 2 Case 7. Keratinizing squamous cell carcinoma. Well-differentiated squamous cell carcinoma arising in the area of lichen sclerosus. In the superficial dermis, traces of lichen sclerosus are seen.

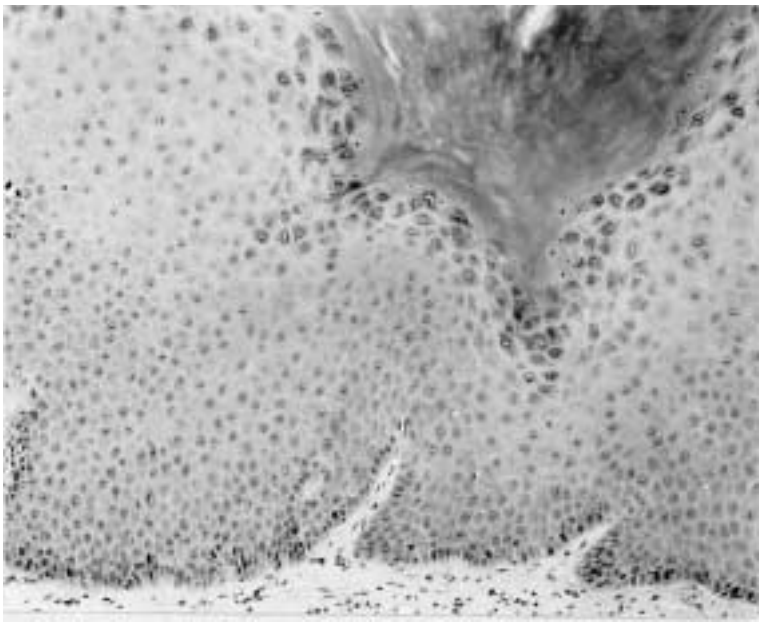

Fig. 3 Case 2. Keratinizing squamous cell carcinoma. Verrucous squamous cell hyperplasia was followed for 17 years.

adjacent to VSCC. Lichen sclerosus chronicus was present in 2 cases, and most of the others were of non-specific dermatitis.

Sixteen of the 36 cases of VSCC also had VIN. Among the 28 cases in which the adjacent tissue could be observed, VSCC alone was noted in 6 cases (excluding dermatitis). As Table 3 demonstrates, KSC or NSC was associated to some degree with differentiated type VIN, basaloid carcinoma closely with basaloid VIN (Fig. 6), and warty carcinoma with warty VIN

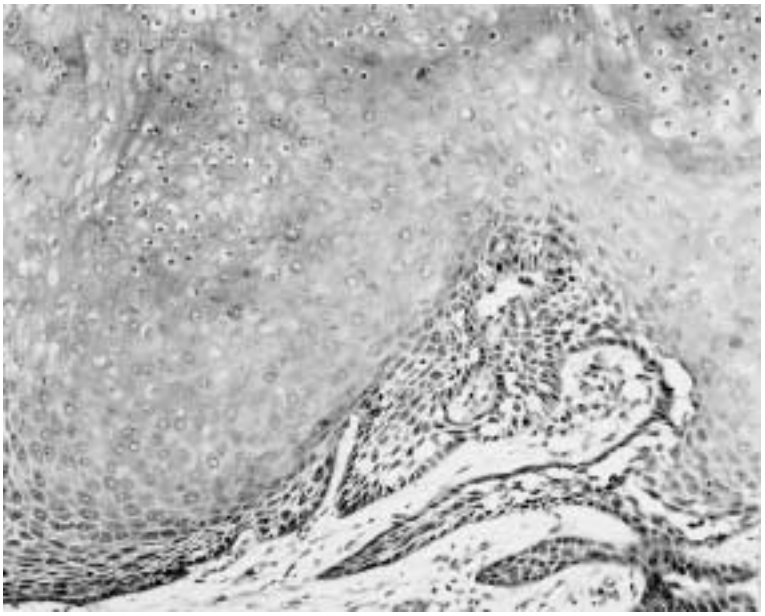

Fig. 4 Case 2. Keratinizing squamous cell carcinoma. Differentiated VIN with acantholysis is seen in the basal and parabasal layers of the epithelium.

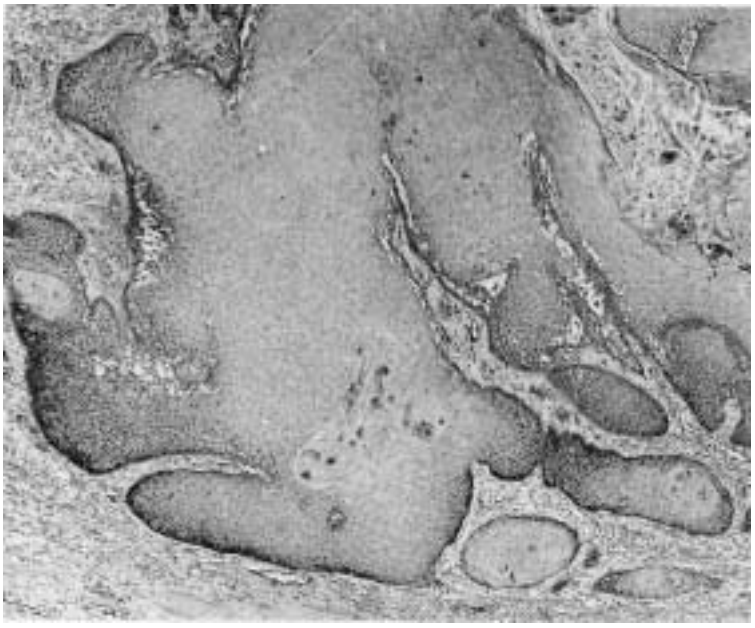

Fig. 5 Case 2. Keratinizing squamous cell carcinoma diagnosed 19 years after the first visit.

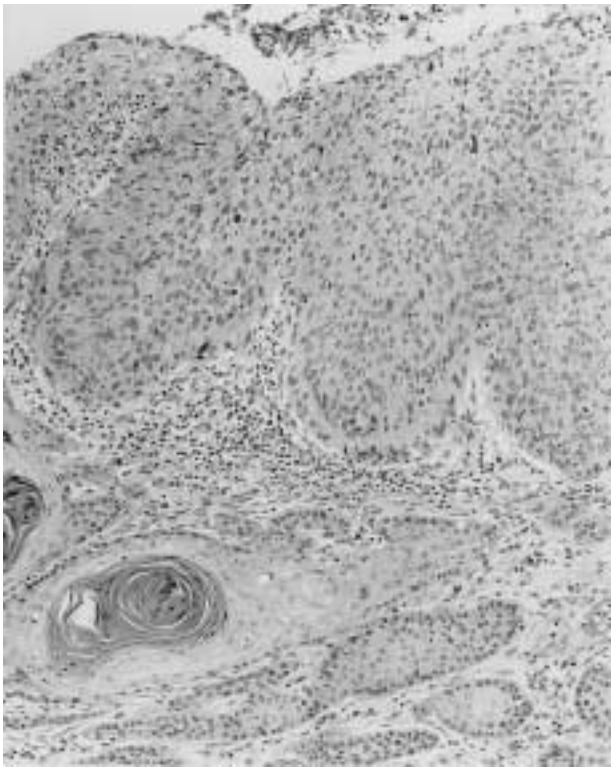

Fig. 6 Case 34. Keratinizing squamous cell carcinoma lies below an area of vulvar basaloid VIN.

Table 4 HPV detected by in situ hybridization, and HPV types

	ISH	HPV types detected		
	positive	6/11	31/33	16/18
Condyloma acuminatum (34)	14	14	0	0
Vulvar intraepithelial neoplasia (12)	4	1	0	3
Squamous cell carcinoma (22)	1	0	0	1

ISH: in situ hybridization, HPV: human papilloma virus, Parentheses: Number of cases detected.

(Fig. 7). The average age of warty carcinoma was younger (46 years old) in comparison with those of the usual type (KSC or NSC) (64.4 years old) and basaloid carcinoma (73.8 years old).

VSCC, condyloma, and VIN were tested for HPV DNA by ISH, and in those cases that were positive the type of HPV was investigated (Tables 3, 4). Only 1 of 22 patients with squamous cell carcinoma was positive by ISH.

As far as could be determined in our investigation, no coexisting condyloma acuminatum was found either at the same or different times. There was 1 case each of metachronous coexistence of uterine cervical carcinoma in situ (Case 34), uterine carcinoma of the cervix (Case

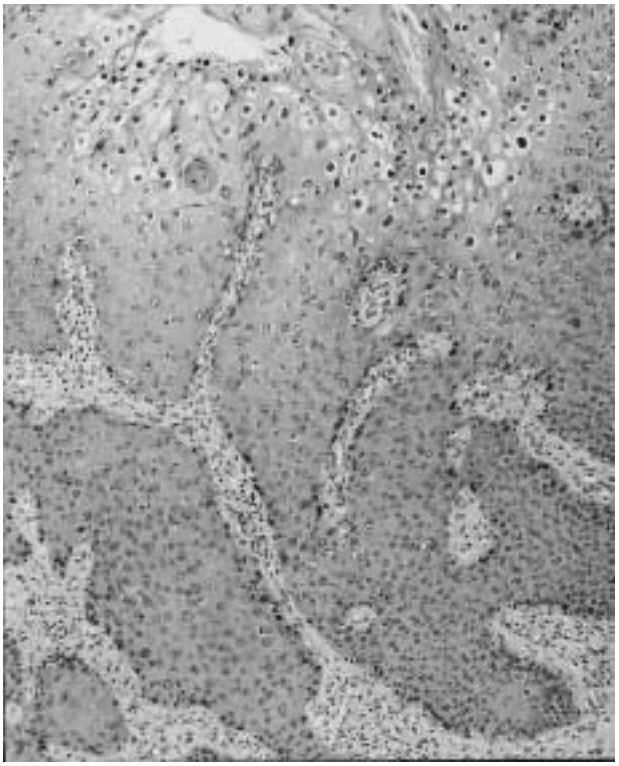

Fig. 7 Case 34. Warty carcinoma lies below an area of vulvar warty VIN.

12), and bladder transitional cell carcinoma (Case 17 and 18). Two patients (Cases 11 and 15) died of the tumor 4 months after the initial examination. One other patient (Case 10) died 5 years later of kidney failure.

DISCUSSION

It may be surmised from a review of population-based statistics[1] on malignant tumors that there are fewer cases of squamous cell carcinoma of the vulva in Japan than in the Western world. Based on hospital-based statistics as well, VSCC at our hospital accounted for 1.7% of all female genital tumors, lower than the reported 5–12% in Europe and the U.S.A.[22-24] or the 3% in South America.[25] Overall, VSCC in Japan, as is true of skin cancers in general, is absolutely lower in incidence than among Caucasians in the West, and may also be assumed to have a relatively lower incidence among malignant tumors of the female genitalia. VSCC commonly occurs in women over the age of 60 years, and a slight increase has been reported[23] with the aging of the population in recent years. However, no confirmation of this has been made in Japan.

To understand the reasons for the lower incidence of VSCC in Japan than in Europe and the U.S.A., it is first necessary to gain a clear picture of all vulvar lesions, and then to investigate the concurrent lesions among VSCC patients.

Epithelial lesions coexisting with VSCC are usually classified as either nonneoplastic epithelial disorders or VIN. Among the nonneoplastic epithelial disorders LS, SCH, and LS/SCH (formerly referred to as "vulvar dystrophies") have been the subjects of much research.[4) 7) 26) 27)] The reported frequency with which VSCC is accompanied by LS varies widely, from 6 to 61%, a difference thought to stem from differences in the accuracy of the investigator making the diagnosis and the diagnostic criteria adopted.[28)] Buscema et al.[8)] state that more than 50% of VSCC patients have accompanying dystrophy, which acts as a long-term ongoing stimulus triggering the onset of VSCC. Moreover, there are many reports stating that atypical epithelium itself, which is recognized to coexist with LS, is a major precursor lesion.[3-7) 10) 13)] McAdam,[3)] Lavery[10)] and others, have reported that vulvar dystrophy is usually not a precancerous lesion, but that it initially carries a risk of developing into VSCC when accompanied by atypical epithelium. However, Hart et al.[9)] indicated that among 107 patients with lichen sclerosus et atrophicus (LS&A), there were 5 cases of VSCC and 1 case with coexisting carcinoma in situ, but that lichen sclerosus et atrophicus was not observed clinically before biopsy in any of the cases. Hart et al. concluded that LS&A has extremely little or absolutely no capacity to cause the malignancy of VSCC. In the present study, one (Case 7) of the 28 VSCCs was thought to be a case in which LS passed through a phase of differentiated VIN to become VSCC. In the other patient, LS was coexistent with but separate from VSCC, so it could not be determined whether LS was the source that developed into VSCC.

Five (18%) of the same 28 patients had accompanying SCH, but only one (Case 2) had adjacent VSCC. This was a case in which SCH could be the source of KSC. Kim[29)] conducted a molecular biological investigation of healthy skin adjacent to HPV-unrelated VSCC, and SCH. Even when the cancer was monoclonal, the surrounding healthy skin and SCH were polyclonal, and SCH was a reaction to VSCC. However, the picture that we decided to be SCH among our patients included SCH that was a reaction, and SCH that was the basis for developing into VSCC.

Meyrick et al.[30)] observed 350 LS patients for an average of 10.5 years of whom 17 (5%, 17/350) developed squamous cell carcinoma. During a follow-up of 158 LS patients, Langley[31)] reported 1 case (0.6%) of VSCC, and Carli,[32)] after following 211 LS patients for an average of 20 months, reported 2 cases (1%) of VSCC. Judging from data in the literature and our findings on LS and SCH, the percentage of LS and SCH that develops into VSCC is between 0.6 and 5%, and probably closer to 0.6%.

A question requiring further investigation is whether intraepithelial neoplasias of the vulva are precursor lesions that develop into VSCC at about the same rate with which intraepithelial neoplastic precursor lesions appearing in the uterine cervix develop into cervical cancer.

There were 16 patients in the present study with coexisting VIN and VSCC. It has been pointed out that KSC showed a tendency to coexist with differentiated VIN, basaloid carcinoma with basaloid VIN, and warty carcinoma with warty VIN. Assuming the presence of a koilocytotic change in epithelial cell HPV infection, three cases of warty carcinoma could develop from warty VIN under the influence of HPV. Basaloid carcinoma is often associated with basaloid VIN, and they transform each other as is always the case in uterine cervical cancer with cervical intraepithelial neoplasia. At least basaloid carcinoma and warty carcinoma were considered to have VIN as their precursor lesion. Among the cases of keratinizing or nonkeratinizing squamous cell carcinoma, there were some patients in whom the carcinoma was considered to develop from differentiated VIN or basaloid VIN. In this retrospective study a considerable number of VSCC cases could have VIN as their precursor.

Crum reported that 5 of 41 cases of VIN (10.2%) developed into VSCC.[33)] In prospective studies of the natural history of VIN, Jones and Mclean[34)] reported [1994][35)] that 87.5% of un-

treated cases and 3.5% of treated cases developed into cancer, with an average of 6.5 years for VIN to develop into VSCC. There are several other reports of severe dysplasia or carcinoma in situ (CIS) progressing to cancer.[36] In contrast, Hording et al.[36] reported that only 4% of VIN 3 became cancerous. Buscema et al.[8] indicated that CIS in young people rarely developed into squamous cell carcinoma. There are also reports of the spontaneous remission of CIS.[33,37] In the U.S.A. as well, no great changes in the incidence of VSCC have been reported, even during the same period in which there was a dramatic rise in the incidence rate of VIN.[2,16] Thus, no increase in VSCC corresponding to the sudden rise in VIN in recent years has been reported. Borrowing from Trimble et al.,[38] we looked at the incidence of VSCC in 2 different periods, 1965-1980 and 1981-1997 (the number of biopsies in the latter period was 1.3 times that in the former), and found the same trend. The natural history of VIN must therefore be considered only partially elucidated.

Recent advances in virology and molecular biology have brought new developments in the pathogenesis of VSCC. However, although the relationship between cervical cancer and HPV infection has been fairly well elucidated, the role of HPV infection in the onset of VSCC is still insufficiently understood.[11] Of the 22 VSCC patients tested for HPV DNA in the present study, only 1 (4.5%) was positive (Tables 3, 4). We assume that the majority of patients at Nagoya University had HPV-unrelated VSCC. HPV DNA detection rates reported for VSCC vary considerably by institution, ranging from 19 to 90%.[28,39,40] Among cancers of the female genitalia, those of the cervix, in which HPV is recognized as having a high level of involvement, are far more prevalent in Japan than in the West; nevertheless, the incidence of VSCC is lower. This likely means that HPV infection is not involved in the onset of VSCC to the extent that it is involved in cervical cancers.

One of the reasons for the lower incidence of VSCC in Japan than in the West may therefore be the fewer cases of HPV-related VSCC among younger women.

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