Progression of 189 Women Diagnosed with Uterine Cervical Dysplasia Based on Abnormal Results in Mass Screening

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During the five-year period from January 1997 to December 2001, cytological abnormalities in the uterine cervix were confirmed in 189 women (class IIIa: 172, class IIIb: 9, class IV: 7, and class V: 1) who underwent cytology screening of the uterine cervix at the Tokai University Health Evaluation and Promotion Center. Biopsy samples from the uterine cervix showed that the 172 women categorized into class IIIa based on cytology included 28 with no atypical lesions, 53 with mild dysplasia, 24 with moderate dysplasia, 3 with severe dysplasia; and the 9 women in class IIIb included 2 with mild dysplasia, 5 with moderate dysplasia, 1 with carcinoma in situ, and 1 with invasive carcinoma. The conformity rates between the cytology data and the biopsy samples were 71.3% and 11.1% in class IIIa and class IIIb, respectively. A three-year follow-up survey of the class IIIa and class IIIb subjects confirmed progression (PRO) in 8 (4.7%), continuous (CON) symptoms in 48 (27.9%), and regression (REG) in 116 (67.4%) in class IIIa, and PRO, CON and REG in 3 (33.3%), 4 (44.4%), and 2 (22.2%), respectively, in class IIIb; the percentage of subjects in the CON + REG group was significantly higher than in the PRO group ($p = 0.0052$). Twelve subjects underwent resection because uterine carcinoma was suspected in the punch biopsy; these subjects have remained under observation and have now made a complete recovery. Our results suggest that patients with uterine abnormal cells should undergo regular cytology and colposcopy for detection of high-risk patients and to allow treatment at an early stage.

Key words: cervical cancer screening, dysplasia, uterine cancer

INTRODUCTION

Cytological analysis of the uterine cervix is a useful auxiliary diagnostic method for detection of cervical cancer. In basic cytology of the uterine cervix, subjects are categorized into class IIIa, class IIIb, class IV and class V based on the Papanicolaou classification; these classes reflect patients with mild/moderate dysplasia of the uterine cervical epithelium with development of atypical cells in the superficial and intermediate cells; with severe dysplasia of the uterine cervical epithelium; with carcinoma in situ; and with microinvasive carcinoma, respectively. For this categorization, atypical nuclei in lateral basal cells must be identified, and data regarding the number and amount of atypical cells are required to make a diagnosis. In addition, a relatively high correlation is observed between the results of biopsy and cytology using this classification, and it is thought that more than 70% of biopsy results can be predicted correctly based on the results of screening using cytology.

Tokai University Health Evaluation and Promotion Center started a comprehensive health screening in January 1976 and cervical cancer screening is included among our screening items [1]. Most of the examinees are employees of nearby companies and approximately 80% are aged 30 to 60 years old. For 5 years from January 1997 to December 2001, 33,372 women underwent cervical cancer screening at the Center and 189 (0.57%) were diagnosed with abnormal cells in the cervix (class IIIa or higher) by cervical cytology and followed up for 3 years. Our results allow identification of important aspects of cases showing progression, and additionally we provide a discussion of the controversy surrounding human papilloma virus (HPV) infection as a possible cause of uterine cervical neoplasm [2-5].

SUBJECTS AND METHODS

For 5 years from January 1997 to December 2001, 33,372 women (age ≤29 yrs: 168 (0.5%), 30-39 yrs: 2,340 (7.0%), 40-49 yrs: 11,069 (33.2%), 50-59 yrs: 13,333 (40.0%), 60-69 yrs: 5,286 (15.8%) and ≥70 yrs: 1,158 (3.5%)) underwent cervical cancer screening (primary examination) at the Tokai University Health Evaluation and Promotion Center (Figure 1) and 189 (0.57%) were diagnosed with abnormal cells in the cervix (class IIIa or higher) by cervical cytology (class IIIa: 172, class IIIb: 9, class IV: 7, and class V: 1). Cytology, colposcopy and a secondary examination using punch biopsy were performed for these subjects at the Department of Obstetrics and Gynecology at our hospital; these procedures were performed to examine the outcome for subjects in classes IIIa and IIIb over

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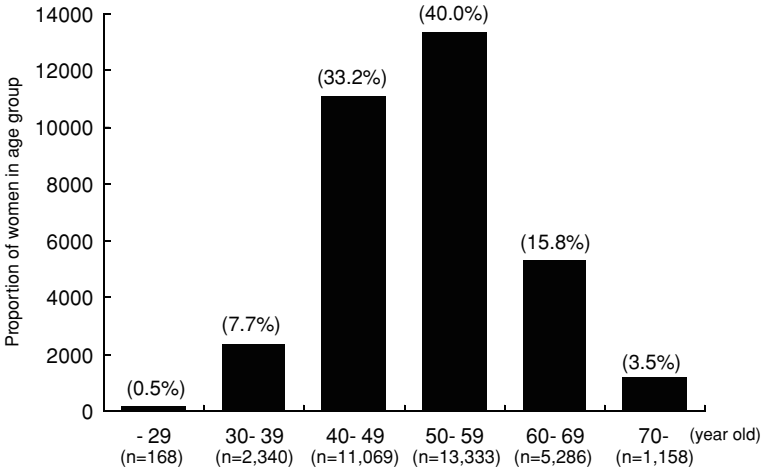

Fig. 1 33,372 women received mass screening by uterine cervical cytology during the five-year period from January 1997 to December 2001.

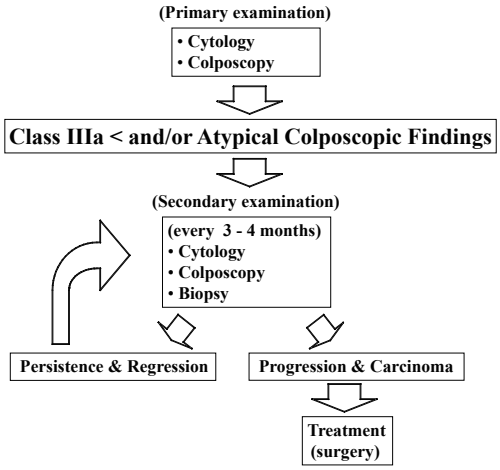

Fig. 2 Strategy for uterine cervical cancer screening.

Table 1 The relationship between cytology data and punch biopsy of the uterine cervix.

Cytology			Punch biopsy					
Class	No.	ACF	no dysplasia	dysplasia			CIS	INV
				mild	moderate	severe		
IIIa	172	*108	28	53	24	3	0	0
IIIb	9	9	0	2	5	0	1	1
IV	7	7	0	0	0	4	1	2
V	1	1	0	0	0	0	1	0

ACF: atypical colposcopic findings.
172: *108 patients with ACF (64 patients without ACF)
CIS: carcinoma in situ. INV: invasive carcinoma.

three years and to determine the conformity between cytology and punch biopsy results.

Exfoliative cell samples were collected using cotton swabs, and a Papanicolaou stain was performed after fixation with 95% ethanol. A diagnosis was made by cytology screeners and pathologists, and the subjects were categorized into six groups based on the following classification: class I, no abnormal findings; class II, abnormal findings but benign changes; class III, mild to moderate dysplasia (IIIa) and severe dysplasia (IIIb); class IV, carcinoma in situ; class V, invasive carcinoma. For subjects in class IIIa and class IIIb, follow-up examinations were performed at the Department of Obstetrics and Gynecology every 3-4 months. Statistical analysis was performed using a Chi-square test with a significance level of $p < 0.05$. The goals of the study were explained to the subjects and informed consent was obtained.

RESULTS

Atypical neoplastic changes in the uterine cervix over a 5-year period

During the five-year period from January 1997 to December 2001, cervical cancer screening revealed abnormalities of class IIIa or higher in 189 women, including 172 categorized as class IIIa, 9 as class IIIb, 7 as class IV, and 1 as class V. For these subjects, a secondary examination was performed at the Department of Obstetrics and Gynecology at our hospital (Table 1, Fig. 2).

Cytology, colposcopy and punch biopsy

For the 189 subjects in whom an abnormality was found in the initial cytology test, a re-examination was performed, and for those in whom the abnormality was confirmed by colposcopy, a punch biopsy was conducted. The punch biopsy was performed for 108 of the 172 class IIIa subjects because of atypical colposcopic findings. The results of the biopsy showed no abnormalities in 28 subjects (25.9%), mild dysplasia in 53 (49.1%), moderate dysplasia in 24 (12.5%), and severe dysplasia in 3 (2.8%). Atypical colposcopic findings were confirmed in all 16 class IIIb and class IV subjects, and the results of punch biopsy showed mild or moderate dysplasia in 7 subjects (43.8%), severe dysplasia in 4 (25%), carcinoma in situ (CIS) in 2 (12.5%), and invasive carcinoma (INV) in 3 (18.8%). CIS was confirmed by biopsy in the one class V subject (Table 1).

Progression of class IIIa and IIIb subjects over three years

Three-year follow-up was performed for 181 class IIIa and class IIIb subjects in whom an abnormality was found by cytology. The 172 subjects in class IIIa included 8 (4.7%) who showed progression (PRO) of symptoms, 48 (27.9%) who showed continuance (CON), and 116 (67.4%) who showed regression (REG). Of the 9 class IIIb subjects, 3 (33.3%), 4 (44.4%) and 2 (22.2%) were classified as PRO, CON and REG, respectively (Table 2). The number of class IIIa and class IIIb subjects classified as CON + REG was significantly higher

Table 2 Results for class IIIa and IIIb subjects over three years.

class	patients	progression (%)	continuance (%)	remission (%)
IIIa	172	8 (4.7)	48 (27.9)	116 (67.4)
IIIb	9	3 (33.3)	4 (44.4)	2 (22.2)

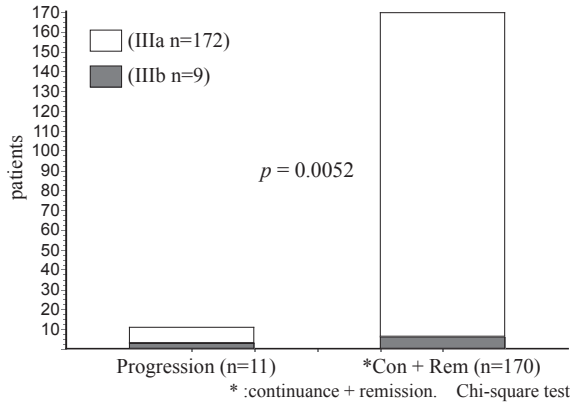

Fig. 3 The relationship between progression and continuance & remission in class IIIa and IIIb subjects.

than the number classified as PRO (170 vs. 11, $p = 0.0052$; Fig. 3).

Patients with progressive atypical neoplasias or carcinomas in the uterine cervix

In the three-year follow-up period, 12 subjects (class IIIa: 4, class IIIb: 3, class IV: 4, and class V: 1) underwent surgery due to possible progression (PRO) or carcinoma. Of the 8 PRO subjects in class IIIa, 4 were transferred to other hospitals. Of the remaining 4 subjects, 2 underwent surgery due to severe dysplasia and 1 due to squamous cell carcinoma, which were confirmed in a punch biopsy performed 12 months after commencement of the study. A postoperative diagnosis of moderate dysplasia was made for all three subjects. In addition, a punch biopsy performed 24 months after commencement of the study showed CIS in one subject and atypical hyperplasia in the endometrium in another subject, and therefore these 2 subjects underwent surgery. Postoperative diagnosis showed no clear malignant findings in the former subject, and endometrioid adenocarcinoma (G1) in the latter subject. The 3 class IIIb subjects underwent surgery due to confirmation of carcinoma in a punch biopsy performed during the study, and surgery was also performed for the 5 subjects in class IV and class V because carcinoma was confirmed by punch biopsy. All the subjects have shown complete recovery postoperatively (Table 3, Fig. 4).

DISCUSSION

A three-year follow-up study was performed for 189 subjects who were categorized into class IIIa or higher in cervical cancer screening performed during a five-year period from January 1997 to December 2001. The study was used to examine changes in symptoms based on punch biopsy for subjects in whom an abnormality was found in the initial cytology test. Colposcopy and punch biopsy were performed in the secondary examination at the Department of Gynecology of our hospital. To assess the conformity between the cytology and punch biopsy data, punch biopsy was performed for 108 of the 172 subjects in class IIIa following confirmation of atypical colposcopic findings in these subjects. Agreement between the cytology and biopsy data was confirmed in 77 subjects (mild dysplasia: 53, moderate dysplasia: 24) (71.3%), and 3 subjects (2.8%) showed severe dysplasia in the biopsy, which showed greater progression than indicated in the cytology test. In addition, for the 16 subjects categorized as class IIIb and class IV based on cytology, consistency with the punch biopsy was found in 6 subjects (37.5%) and 3 subjects (18.8%) showed invasive carcinoma in the biopsy that was more pro-

Table 3 Characteristics of twelve patients who underwent surgery due to atypical neoplastic changes in the uterine cervix.

Case	Age	Class	Observation	Punch biopsy (Pre operation)	Operation	Post operative diagnosis
1.	45	IIIa	12	Severe dysplasia	ATH	Moderate dysplasia
2.	58	IIIa	12	Squamous cell carcinoma	ATH	Moderate dysplasia
3.	67	IIIa	24	Carcinoma in situ	EXT	No malignant cells
4.	50	IIIa	24	Atypical hyperplasia in EM cell	EXT	Endometrioid adenocarcinoma (G1)
5.	43	IIIb	12	Squamous cell carcinoma	Se-Radical	Carcinoma in situ
6.	56	IIIb	36	Carcinoma in situ	Se-Radical	Carcinoma in situ
7.	49	IIIb	2	Adenocarcinoma in situ	Radical	Adenocarcinoma in situ
8.	57	IV	-	Carcinoma in situ	Se-Radical	Carcinoma in situ
9.	57	IV	-	Squamous cell carcinoma	Se-Radical	Microinvasive squamous cell carcinoma
10.	38	IV	-	Carcinoma in situ	Conization	Carcinoma in situ
11.	51	IV	-	Carcinoma in situ	Se-Radical	Carcinoma in situ
12.	50	V	-	Carcinoma in situ	Se-Radical	Microinvasive squamous cell carcinoma

Observation: months. EM: endometrium. Progression: period of months. ATH: abdominal total hysterectomy.
EXT: extended total hysterectomy. Se-Radical: semi-radical hysterectomy. Radical: radical hysterectomy.
All 12 patients are surviving and disease-free.

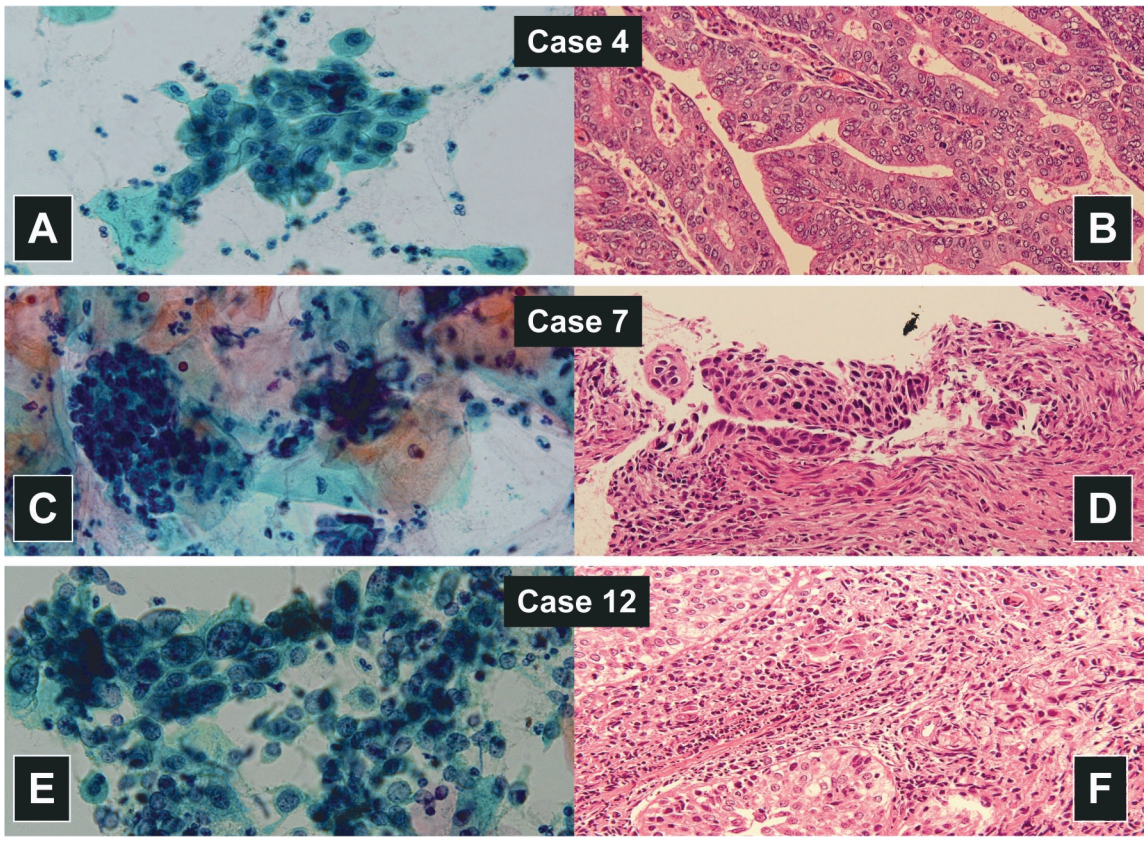

Fig. 4 Cervical cytology of mass screening and post operative histology. Case 4: **A**, class IIIa, sheet of atypical glandular cells with slightly increased chromatin. **B**. endometrioid adenocarcinoma with glandular and cribriform, grade 1. Case 7: **C**, class IIIb, cluster of cells with a high N/C ratio and hyperchromatic nuclei. **D**, small intraluminal protrusions. Case 12: **E** class V, irregular squamous cell carcinoma with a high N/C ratio. **F**, invasive keratinizing- squamous cell carcinoma.
(**A**, **C** and **E**, Papanicolaou stain × 40. **B**, **D** and **F**, Hematoxylin and eosin stain × 20)

gressed than indicated by cytology. The one class V subject showed CIS in the biopsy.

In our hospital, all cell samples are collected using cotton swabs. Using this method, the amount of cells collected is small and many of the samples are surface cells, because firm pressure cannot be applied during sample collection and it is difficult to collect cell samples from deep layers. This is a particular problem when collecting samples of invasive cancer, since the surface layer has many necrotic cells and it is difficult to obtain cancer cells from deeper layers. Punch biopsy is performed after confirmation of cervical findings by colposcopy, but even if atypical colposcopic findings are observed over a wide area the biopsy is performed at 2-3 sites at most; therefore, the tissue collected may not be representative of the complete area, and this may partially explain the inconsistency between the cytology and biopsy data.

Uterine cervical cancer can be histologically categorized into squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma is thought to begin with an increase of reserve cells and develop to invasive carcinoma via squamous metaplasia, mild, moderate and high-grade dysplasia, and development of carcinoma in situ. However, not all of these precancerous lesions develop into cancers: most mild or moderate dysplasias show no changes or resolve within two years, and only approximately 20% of high-grade dysplasias develops into cancer [6]. During our three-year follow-up survey, progression was observed in 8 of 172 subjects in class IIIa (4.7%), and 3 of 9 subjects in class IIIb (33.3%). According to prospective studies on cervical cancer screening performed by Flannelly *et al.* and Soutter *et al.*, progression may be observed in 40% of cases of mild and moderate dysplasia [7, 8]. However, there have been many contradictory reports in Japan showing that many cases of mild dysplasia recover naturally during their clinical course, and the outcome of such mild or moderate dysplasia therefore remains unclear. It has also been reported that an atypical cervical epithelium and development of cervical cancer are closely associated with human papilloma virus (HPV) infection; in particular, HPV16 and 18 are considered to be high-risk HPVs that may lead to atypical changes in the cervical epithelium and result in cervical cancer [9-11].

At present, approximately 80 types of HPV have been identified, and about 4-20% of healthy women are thought to be HPV-positive. Several reports show that middle to high risk types of HPV, such as types 16, 18, 33, 35 and 52, may cause development of high-grade dysplasia of the epithelium within a few years, and therefore close follow-up observation is required for HPV-positive patients with dysplasia of the epithelium and HPV of these types [12]. Cervical cancer screening is performed every year in Japan and Germany, once

every 1-3 years in the USA and Canada, and once every 3-5 years in some EC countries. However, it may be reasonable to lengthen the interval between screening examinations for middle to high risk type subjects who are HPV DNA-negative [13], thus achieving a large decrease in the cost of mass screening. Discussions on the development and clinical use of a vaccine for HPV that is related to the onset of uterine cervical cancer are also currently ongoing [14].

A study of histological differences in HPV infection and development of cervical cancer was conducted in the Netherlands and the results of cervical cytology were analyzed retrospectively using the Mantel-Haenszel common odds ratio (OR_{MH}) with a 95% confidence interval. Fourteen high-risk HPVs (HPV16, 18, 31, 33, 35, 39, 45, 51, 51, 56, 58, 58, 66 and 68) were examined in 1,467 women with normal cytology (mean age: 37 years old; range: 17-63 years old) and 17.6% were diagnosed with multiple HPV infection; furthermore, HPV infection was found in 21.3% of 61 women with adenocarcinoma in situ (ACIS) (mean: 37.1 years old; range: 23-55 years old), 7.1% of 70 women with adenocarcinoma (AdCx) (mean: 44.7 years old; range: 28-79 years old), and 10.8% of 83 women with squamous cell carcinoma (SCC) (mean: 49 years old; range: 27-88 years old). In particular, HPV18 infection was shown to be a significant risk factor for ACIS and AdCx development (OR_{MH}: 15.0; 95% CI: 8.6-26.1 and OR_{MH}: 21.8; 95% CI: 11.9-39.8, respectively). Similarly, HPV16 infection (exclusive of HPV18 infection) was also found to be a risk factor for ACIS and AdCx development (OR_{MH}: 6.6; 95% CI: 2.8-16.0 and OR_{MH}: 9.4; 95% CI: 2.8-31.2, respectively). In addition, OR_{MH} of the SCC patients was 4.3 (95% CI: 1.6-11.6), and therefore HPV16 infection was considered to be a risk factor for both adenocarcinoma and SCC development [15]. Based on the above results, 11 advanced patients of 181 with smears of class IIIa and IIIb who were followed up for 3 years (Table 2) were probably infected by high-risk HPV. In our hospital, an HPV-DNA test is still not performed routinely in screening, but we conclude that this test should be introduced into cervical cancer screening after the problem of cost is solved. The patients (Case Nos.: 5 to 12) who had advanced dysplasia and underwent surgery will definitely be examined for HPV infection after informed consent is obtained.

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