Comparison of human papillomavirus testing and cervical cytology with colposcopic examination and biopsy in cervical cancer screening in a cohort of patients with Sjogren's syndrome

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Summary

Objective: The purpose of this study was to evaluate women with Sjögren Syndrome by using cervical cytology, colposcopic examination and HPV-DNA testing and to compare these findings with those obtained from the control group.

Method: A total of 100 women, who were referred to Ege University, School of Medicine, Department of Obstetrics and Gynecology for cervical cytological screening between September 2004 and March 2005 and 33 of whom had Sjögren syndrome were included in this study. The patients were informed and subjected to cervical cytology, colposcopic examination and HPV-DNA testing. Colposcopic biopsy and endocervical canal curettage were carried out in cases of suspicious colposcopic examination and cytological findings. The findings obtained from 33 women with Sjögren syndrome and 67 subjects in the control group were compared.

Results: Normal cervical cytology was detected in five women (5.7%), while suspicious cervical cytology was reported in 62 women (92.5%) in the control group. The prevalence of normal cytology in patients with Sjögren syndrome was 93.9% (n = 31), where 6.1% (n = 2) of the women had suspicious cervical cytology findings. HPV-DNA findings were negative in 66 women (98.5%) in the control group, where the test result of one women (1.5%) was positive. HPV-DNA findings of patients with Sjögren syndrome were positive in one women (3%) and negative in 32 (97%). Colposcopic findings were normal in 63 women (94%) in the control group, where abnormal colposcopic findings were observed in four women (6%). Normal colposcopic findings were observed in 32 women (97%) with Sjögren syndrome, while pathological findings were recorded in one woman (3%). Suspicious cervical cytology, positive findings at colposcopic examination and biopsy and positive HPV-DNA tests were observed together in only one 40-year-old woman who was diagnosed with Sjögren syndrome for a period of four years. Prevalence of dyspareunia and vaginal dryness (atrophic vaginitis) symptoms were observed in Sjögren syndrome and control groups as 36.3% and 22.3%, respectively.

Conclusion: No significant differences were observed between Sjögren syndrome and the control group who were evaluated by using cervical cytology, colposcopic examination and HPV-DNA tests. A higher prevalence of dyspareunia and vaginal dryness were observed in patients with Sjögren syndrome, yet this difference was not considered as significant with respect to either colposcopic or histopathological findings.

Key words: HPV; Cervical cytology; Colposcopy; Sjögren's syndrome.

Introduction

Sjögren Syndrome is a chronic progressive autoimmune disorder, characterized by exocrine glandular manifestations. Ninety percent of the patients are female, and the disorder is most frequently seen in the fourth and fifth decades. Primary Sjögren syndrome has been reported in 5% of the population over age 60. Lymphoproliferative disorders consequently develop in a considerable number of patients [1].

Definitive etiology of the disorder has not been clariefied. Viruses of retrovirus and Herpes simplex groups have occasionally been taken under consideration. Prevalence of Sjögren syndrome has been reported to be 30 times higher in HIV-positive subjects when compared with those in the control group [1]. T-lymphocyte activation, increased antibody production and decreased immune tolerance are among the characteristic properties of this disorder. Exocrine glands are infiltrated by lymphocytes. Progressive clinical components are classified as glandular and extraglandular. Major glandular symptoms consist of xerosthomia due to parotid glandular involvement and xeropthalmia due to lacrimal glandular involvement. Other exocrine glands are less frequently involved.

Findings related to genital involvement are external genital dryness and dyspareunia. Frequent gynecological complaints, such as vaginal dryness and dyspareunia, have occasionally been considered as research subjects. Prevalence of vaginal dryness in subjects with Sjögren syndrome was found to be significantly higher when compared with the normal population [2]. Therefore, it is recommended that young patients with dyspareunia com-

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plaints be taken under close investigation for Sjögren syndrome [3].

Human papillomavirus infection is effective in development of pre-invasive cervical cancer [4]. Ninety percent of cervical intraepithelial neoplasms develop due to HPV infection [5]. HPV DNA is diagnosed in many female subjects with cervical neoplasms [5, 6]. The risk of cervical neoplasm is increased ten times with a diagnosis of HPV DNA [5]. Prevalence of invasive cervical cancer has significantly decreased with cervical cytology screening.

With the viral etiology and known relationship with malignancy of the disorder, the probability of involvement of cervical exocrine glands has drawn the attention of gynecologists. The importance of cervical cytology and colposcopy in early diagnosis of cervix cancer with its well established viral etiology (HPV) [4-10], have also led to the idea of using HPV-DNA, cervical cytology and colposcopy in evaluation of subjects with Sjögren syndrome.

HPV-DNA screening, cervical cytology, colposcopic examination and biopsy were applied to a group of subjects diagnosed with Sjögren syndrome in accordance with international criteria, and the results were compared with the control group in this study.

Materials and Method

Subjects. A total of 100 subjects, including 33 women with Sjögren syndrome, referred to the Department of Obstetrics and Gynecology, Ege University School of Medicine for cervical cytological screening between September 2004 and March 2005 were included in the study. Patients were evaluated with respect to age, number of sexual partners, smoking habits, use of intrauterine devices and the duration, and use of oral contraceptives; and there were no significant differences between the two groups.

Specimens. Women were advised to avoid using vaginal lubricants for one week and to practice sexual abstinence for 48 hours until cervical cytology specimens were obtained. A dry speculum was placed before taking specimens. The procedure was postponed in case of menstruation or uterine bleeding. Cervical specimens were taken with a brush from the intracervical canal and with an Ayre spatula from the exocervical transformation area. Cytological evaluation was carried out at the Department of Pathology, Ege University, School of Medicine. The results were classified according to the Bethesda system.

HPV DNA Determination. Determination of HPV DNA was carried out by using the Hybrid capture technique (Digene Hybrid Capture System, Digene, London, UK). HPV subtypes of 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52 and 56 can be determined with this qualitative technique. Additionally, low-risk types (type 6, 11, 42, 43 and 44) and medium/high-risk types (type 16, 18, 31, 33, 35, 45, 51, 52 and 56) could be distinguished. Cervical cytology specimens were transported by using the 'Digene Specimen Transport Medium' (Digene, London, UK) kit.

Target DNA (HPV DNA) is hybridized by using a mixture containing specific HPV RNA probes. Resulting RNA:DNA hybrids are transferred into a tube whose inner surface is covered with an antibody specific to RNA:DNA hybrids. Stabilized hybrids react with alkaline phosphatase-conjugated antibodies specific to RNA:DNA hybrids and are determined by using a chemical-luminescent substrate.

Colposcopy. Colposcopic examination was carried out on women with abnormal cervical cytology findings. After elimination of cervical secretions in the dorsal lithotomic position, the cervix was macroscopically evaluated and followed by application of an acetic acid solution. The transformation area was inspected with a colposcope. Specimens were collected from suspicious areas by using a Kevorkian punch biopsy tool. Curettage of the cervical canal was performed.

Statistics. SPSS software was used for statistical evaluation. The Student's t-test was used as the distribution of comparison values among groups were normal. The chi-square test was used for the comparison of percentage values. Values were expressed as mean ± standard deviation; $p < 0.05$ was considered as statistically significant.

Results

Demographic values of subjects are presented in Table 1. Mean ages for control and study groups were 47.8 and 47.4, respectively. Sixteen women (48.4%) in the study group were in the postmenopausal period, while 17 women (51.6%) were in the premenopausal period.

Table 1. — *Demographic values of study and control groups.*

	Group	No.	Mean value	Standard deviation	p value
Age (years)	Control group	67	47.8	9.1	0.86
	Sjögren group	33	47.4	10.3	
No. of parturitions	Control group	67	2.4	1.3	0.12
	Sjögren group	33	1.9	1.4	
No. of abortions	Control group	67	1.5	1.5	0.59
	Sjögren group	33	1.3	1.4	
Marital age (years)	Control group	67	20.9	3.8	0.24
	Sjögren group	33	21.9	3.9	
Menopausal age (years)	Control group	67	1.4	0.4	0.36
	Sjögren group	33	1.5152	0.5	

Normal cervical cytological findings were determined in 62 women (92.5%) in the control group, while suspicious cervical cytological findings were observed in five women (7.5%). Normal cervical cytological findings were observed in 31 women (93.9%) in the study group, while suspicious cervical cytological findings were evident in two (6.1%). The results of the HPV-DNA scanning test were negative in 66 women (98.5%) and positive in one in the control group. The HPV-DNA screening test results in women with Sjögren syndrome were found to be negative in 32 (97%) and positive in one (3%). Women with suspicious cervical cytology results are presented in Table 2.

Colposcopic findings in the control group were normal in 63 women (94%), while abnormal colposcopic findings were observed in four women (6%). Colposcopic findings in women with Sjögren syndrome were normal in 32 (97%), while abnormal colposcopic findings were

Table 2. — *Women with suspicious cervical cytology results.*

Cases	Colposcopic examination	Histopathology	HPV-DNA
Control 1	White epithelium at 12 and 9 o'clock	CIN I at 12 o'clock	Negative
Control 2	Ordinary	Coilocytotic changes	Negative
Control 3	Ordinary	ASCUS	Negative
Control 4	White epithelium at 6 and 12 o'clock	CIN I at 6 and 12 o'clock	Negative
Control 5	White epithelium at 12 and 9 o'clock	CIN I at 12 o'clock	Negative
Study 1 (Sjögren)	White epithelium at 12 o'clock	Coilocytosis on cervix surface epithelium	Positive
Study 2 (Sjögren)	Ordinary	Coilocytosis on cervix surface epithelium	Negative

Table 3. — *Women with abnormal colposcopic examination findings.*

Cases	Colposcopic examination	Histopathology	HPV-DNA
Control 1	White epithelium at 12 and 9 o'clock	CIN I at 12 o'clock Cervix surface epithelium at 9 o'clock	Negative
Control 2	White epithelium at 11 o'clock	Benign	Negative
Control 3	White epithelium at 6 and 12 o'clock	CIN I at 6 and 12 o'clock	Negative
Control 4	White epithelium at 9 and 12 o'clock	CIN I at 12 o'clock	Negative
Study 1 (Sjögren)	White epithelium at 12 o'clock	Coilocytosis on cervix surface epithelium	Positive

evident in one (3%). Clinical results of women with abnormal colposcopic findings are presented in Table 3.

Patients with CIN I were treated with cryotherapy and annual follow-up with cervical cytology was proposed. Suspicious cervical cytology, positive colposcopic examination and biopsy findings and positive HPV-DNA tests were observed together in only one 40-year-old woman in the study group, who was diagnosed with Sjögren syndrome for a period of four years.

Discussion

HPV plays an important role in cervical cancer etiology, and development of cervical dysplasia increases particularly in immunologically suppressed subjects [11].

In a study by Rebello *et al.* [12], 333 subjects with cervical cytology findings at critical levels or slightly dyskaryotic were inspected for high-risk HPV types by acquiring additional specimens for the Digene Hybrid Capture test with a cervical brush. Women with lesions were treated with wide loop excision of the transformation zone. Prevalence of positive HPV findings in 166 women under the age of 30 were higher when compared to 167 women over age 30 (79% and 45%, respectively, $p = 0.001$) and high-grade cervical lesions were observed more frequently in women under 30 than those in women over 30 (43% and 45%, respectively, $p < 0.01$).

HPV testing was considered to be beneficial in evaluating patients with suspicious cervical cytology findings at critical levels. Due to the high cost, colposcopy cannot be directly applied to all women in developed countries. HPV testing has gained importance in classification of cervical cytology findings at critical levels. Colposcopic examination and biopsy should be recommended particularly in women over 30 years of age with positive results for high-risk HPV type [13].

In a study by Woodman *et al.* [14], the natural pattern of HPV infections and their relationship in the development of cervical intraepithelial neoplasms were investigated. In a total of 1,075 females with normal cytological findings and negative HPV, cumulative risk for development of HPV infection in three years' time was 44% (n = 473) and the most frequently observed was HPV type 16. In the same study, it was also observed that the risk of cervical intraepithelial neoplasm was the highest in the group infected with HPV type 16, and this risk was at its highest level during the first 6-12 month period following determination of HPV type 16. The results of HPV tests in five women with high-grade intraepithelial neoplasms were negative. In 69 women in this study, Pap cervical cytology results were negative, while positive results were obtained in three. Human papillomavirus testing was found to be negative in women with positive Pap cervical cytology findings. No significant differences with respect to risk factors were observed between women with negative and positive human papillomavirus screening test results.

In a study by Levert *et al.* [15], a total of 3,778 women received Pap cervical cytology, HPV-DNA tests (Hybrid Capture DNA Assay II) in search of high-grade cervical intraepithelial lesions. Colposcopic examination and biopsy were performed on women with abnormal cervical cytology. Pap tests were repeated after six months in women with normal Pap cervical cytology findings, yet positive HPV-DNA test results. Positive HPV-DNA test results were obtained in all high-grade intraepithelial lesions, 76% of lower grade lesions, 57% of women with atypical squamous cells of undetermined significance (ASCUS) and 10% of normal Pap smear cases. Positive results for HPV-DNA subtypes with a high-risk factor were observed in all cases where high-grade intraepithelial lesions were identified during colposcopy and follow-up. However, it was seen that the results of the first Pap smear test were reported as high-grade lesions in 76% of these cases. Among the group of women in which Pap smear test results were reported as lower grade lesions or atypical squamous cells of undetermined significance, high-grade lesions were observed only in women with positive high-risk HPV-DNA results.

In a study by Nasiell *et al.* [16], a total of 555 women with mild cervical dysplasia were observed through follow-up controls without any treatments. Rate of regression was reported as 62%, where rate of persistence was 22% and progression was 16% among the women observed. Invasive cervical cancer (Stage IB) was diagnosed in two cases among 89 subjects with progressing lesions. It was observed through analysis of the results, that probability of transformation of mild dysplasia into severe dysplasia/carcinoma in situ/invasive carcinoma during follow-up periods of two and 12 years were 7% and 23%, respectively. When the results were reevaluated

in accordance with the Bethesda system, it was observed that the rate of regression was 62% and rate of persistence was 10%, while transformation into high-grade intraepithelial lesions was found in less than 1% of the cases.

Vaginal dryness is a common symptom in subjects with Sjögren syndrome. In a study by Mulherin *et al.* [3], cases with chronic dyspareunia complaints associated with musculoskeletal, oral and ocular symptoms and rheumatological symptoms, such as Raynaud phenomenon were evaluated with respect to Sjögren diagnostic criteria and laboratory findings. It was determined that the mean duration for development of dyspareunia in subjects diagnosed with Sjögren syndrome was seven years, while mean duration for the oral symptoms to begin was reported as one and a half years. Therefore, it was recommended by the researchers that young patients describing dyspareunia should be taken under close follow-up controls.

On the other hand, 51 women with Sjögren syndrome were compared with 54 women in the control group with respect to their gynecologic-obstetric history and gynecological inspection findings in a study by Skopouli *et al.* [17], and no significant differences in menstrual history and vaginal dryness were found. It was stated that vaginal dryness showed a correlation with age and menopausal state with respect to cervical mucus secretion, but no significant relationship with Sjögren syndrome was determined.

In a study by Capriello *et al.* [18], a total of 26 women with Sjögren syndrome at a mean age of 46 were evaluated by using gynecological inspection, colposcopic examination and cervical biopsy. Vaginal dryness was noted in all of the subjects. However, a significant relationship with Sjögren syndrome was not observed. Dystrophic changes secondary to atrophy were found in 50% of the women during colposcopic examination, and the most frequently observed histopathological finding was chronic cervicitis.

Marchesoni *et al.* [19] compared 36 women who had primary Sjögren syndrome with 43 women in the control group, with respect to commonly observed complaints in cases with Sjögren syndrome, such as dyspareunia and vaginal dryness. Prevalence of vaginal dryness and dyspareunia in Sjögren syndrome subjects was 55% and 61%, respectively; while prevalence of vaginal dryness and dyspareunia in the control group was 33% and 39%, respectively. Nevertheless, no significant differences were observed between the gynecological evaluations of premenopausal and postmenopausal women in either group. Additionally, no correlations were observed between oral and ocular symptoms and findings and vaginal complaints in women with Sjögren syndrome.

In the present study, no significant differences were observed between 33 women with Sjögren syndrome and 67 women in the control group during the evaluations made by using cervical cytology, colposcopic examination and HPV-DNA tests. Dyspareunia and vaginal dryness complaints were more frequently observed in the group of women with Sjögren syndrome. The prevalence of dyspareunia and vaginal dryness was found as 36.3% (12 cases) in women with Sjögren syndrome and 22.3% (15 cases) in the control group, yet this difference did not present any colposcopic or histopathological significance.

Sjögren syndrome, which has been reported to act as a predisposing factor for lymphoproliferative disorders and in whose etiology induction of T-lymphocytes has been questioned, does not incur any risks with respect to cervical malignancies (considering the rates of precancerous lesions) according to the results of this study. Likewise, cervical cytology findings did not exhibit any significant differences in the control group.

It has been well established that human papillomavirus penetrates the basal layers in squamocolumnar junctional cells with the help of microtraumas. Increased rate of microtraumas and consequently the higher probability of HPV infection due to impaired cervical gland function and eliminated preventive barrier effect of the mucus did not receive any support from the results of this study.

Colposcopic examination was performed on all women under the same conditions, and the inconvenience of cases with complaints of vaginal dryness did not constitute any significant problems for a proper examination. Results obtained were similar in both groups.

Within the limitations of this study, it was concluded that routine gynecological follow-up controls are sufficient for women with Sjögren syndrome.

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