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Research Article

Pathologists Recommend Repeat Pap Testing: In Clinical Practice What Do Gylecologist Do?

Patologlar Pap Testinin Tekrarlanmasını Öneriyor: Klinik Uygulamada Jinekologlar Ne Yapıyor?

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Abstract
The aim of this study is to evaluate the frequency and results of retests in patients whose cervical cytology was reported as 'Negative for intraepithelial lesion or malignancy', but for whom retest was recommended due to borderline adequacy, borderline evaluability or mild atypia attributable to benign causes. All cervical cytology reports evaluated in the pathology laboratory of Şanlıurfa Training and Research Hospital between 01.06.2022-01.06.2023 were examined. Four thousand three hundred eighty-five of the 5025 cervical cytologies were reported as negative. Retests were recommended for 820. Inflammation was detected in 96.22% of cases, parabasal cell predominance in 11.58%, hypocellularity in 6.58%, fibrin in 5.73%, bacterial vaginosis in 2.80%, Candida in 2.56%. Human papillomavirus (HPV) negativity was detected in 38.30% of patients, HPV positivity in 1.80%. Previous normal cervical cytology was present in 17.20% of patients, previous abnormal cervical cytology in 2.20%, previous normal cervical biopsy in 0.90%, previous abnormal cervical biopsy in 2.00%. Only 7.20% of patients underwent repeat testing within the following one year, and abnormalities were reported in only 6.78% of these retests. The retest rate was significantly higher in patients with previous normal cervical cytology and previous abnormal cervical biopsy. No significant relationship was found between the frequency of repeat testing and age, residence, the clinic where the patients were examined, presence and results of HPV test, presence and results of follow up biopsy. Both the frequency of repeat testing and the frequency of abnormality detected in the retest are low. However, since it is a minimal invasive procedure, we believe that repeat cytology when recommended, is an appropriate approach to identify this small number of abnormal patients.
Keywords: Cervix, Cervical Smear, Papanicolaou Smear, Pap Smear, Pap Test.

Özet
Bu çalışmanın amacı, servikal sitolojileri 'İntraepitelyal lezyon veya malignite açısından negatif' olarak raporlanan, ancak sınırda yeterlilik, sınırda değerlendirilebilirlik veya iyi huylu nedenlere atfedilebilen hafif atipi nedenleriyle test tekrarı önerilen hastalarda tekrar test sıklığını ve tekrar test sonuçlarını değerlendirmektir. 01.06.2022-01.06.2023 tarihleri arasında Şanlıurfa Eğitim ve Araştırma Hastanesi patoloji laboratuvarında değerlendirilen tüm servikal sitoloji raporları incelenmiştir. İncelenmiş olan 5025 servikal sitolojinin 4385'i negatif olarak raporlandırılmıştır. Bunların 820'sine test tekrarı önerilmiştir. Bu hastaların %96.22'sinde inflamasyon, %11.58'inde parabazal hücre hakimiyeti, %6.58'inde hiposellülarite, %5.73'ünde fibrin, %2.80'inde bakteriyel vajinozis, %2.56'sında Candida tespit edilmiştir. Hastaların %38.30'unda Human papillomavirus (HPV) negatifliği, %1.80'inde HPV pozitifliği, %17.20'sinde eski normal servikal sitoloji, %2.20'sinde eski anormal servikal sitoloji, %0.90'ında eski normal servikal biyopsi, %2.00'sinde eski anormal servikal biyopsi belirlenmiştir. Takip eden bir yıl içinde hastaların sadece %7.20'sine test tekrarı yapılmıştır. Tekrar testlerinin sadece %6.78'inde anormallik bildirilmiştir. Test tekrarı oranı, eski normal servikal sitolojisi ve eski anormal servikal biyopsisi olan hastalarda önemli ölçüde daha yüksektir. Test tekrarı sıklığı ile yaş, adres, hastaların muayene edildiği klinik, HPV testi varlığı ve sonucu, takip biyopsisi varlığı ve sonucu arasında anlamlı bir ilişki bulunamıştır. Hem tekrar test sıklığı hem de tekrar testte anormallik tespit edilme sıklığı düşüktür. Bununla birlikte, minimal invaziv bir işlem olduğu için, önerildiğinde sitolojinin tekrarlanmasının bu az sayıdaki anormal hastayı yakalamak için uygun bir yaklaşım olduğuna inanıyoruz.
Anahtar Kelimeler: Serviks, Servikal Smear, Papanicolaou Smear, Pap Smear, Pap Test.

Introduction

Cervical cancer is the fourth most common cancer among women worldwide, according to the World Health Organization (WHO) 2022 data (1). Persistent Human papillomavirus (HPV) infection is the most significant risk factor identified (2).

The Papanicolaou (Pap) test, developed by George Papanicolaou in 1943, is a cytological examination used to examine cervical cells. It quickly became a standard screening test for cervical cancer (3). Current cervical cancer screening guidelines recommend that women aged 21-65 be screened every three years with cervical cytology (CC) alone, or women aged 30-65 be screened every five years with both CC and HPV cotest (4).

In 1991, the Bethesda system was developed to improve communication between pathologists, who examine and report CC, and gynecologists, who perform the test and manage patient follow up or treatment based on the test results. Under this system, CC deemed satisfactory for evaluation is classified into two main categories: 'Negative for intraepithelial lesion or malignancy (NILM)' and 'Epithelial cell abnormality' (ECA). When ECA is detected, the type of abnormal epithelial cells is specified, and the severity of the abnormality is graded (3).

The Bethesda system recommends that liquid-based preparations contain at least 5000 squamous cells to be considered satisfactory. If the number of squamous cells is between 5000 and 20,000, this is classified as low cellularity, and this condition should be noted in the report. Even if a preparation is cellularly satisfactory, it should be deemed unsatisfactory if more than 75% of the squamous cells are obscured by factors such as blood, inflammation or bacterial vaginosis. If obscuring factors cover 50-75% of the cells, this condition should also be noted in the report (5).

Cellular changes resulting from metaplasia, atrophy, inflammation, foreign microorganisms, pregnancy, hormone therapy, intrauterine devices or radiation are reported under the NILM category. These factors can lead to changes such as nuclear enlargement, increased nucleus/cytoplasm ratio, hyperchromasia, membrane irregularities and cytoplasmic clearing. Particularly, changes related to pregnancy, the postpartum period or radiation can be misinterpreted as severe cellular atypia and reported as ECA (6).

The most commonly reported ECA in CC is 'Atypical squamous cells of undetermined significance (ASC-US)' (7). In this category, cellular changes are present but they are insufficient to classify as a 'Low grade squamous intraepithelial lesion (LSIL)' (8). It is crucial to provide comprehensive patient information to the pathologist, as they may interpret mild cellular changes as NILM if these changes can be attributed to a specific cause. Thus, detailed patient information from the gynecologist can help prevent unnecessary ASC-US diagnoses.

When CC is reported as 'Unsatisfactory for evaluation (UE),' repeat cervical cytology (RCC) should be performed between two and four months. If two consecutive CC are unsatisfactory, a colposcopy is recommended (5). For patients with NILM, if HPV test has not been performed, CC is recommended after three years. If HPV negativity is confirmed, CC is recommended after five years (4). Patients with ASC-US require a more complex management approach. If HPV test was not performed, RCC should be performed in one year. If HPV negativity is known, RCC should be performed in three years. If the RCC result is ASC-US again, colposcopy is suggested. Patients with a positive HPV test should be referred directly for colposcopy. For patients with normal cervical biopsy (CB) results, HPV test and CC should be repeated after one year. If CB taken during colposcopy identifies a cervical abnormality, follow up and treatment should be based on this abnormality (7).

This study aims to evaluate RCC frequency and RCC results in patients whose CC were reported as NILM but had a recommendation about RCC due to borderline cellular adequacy, borderline evaluability or mild cellular atypia attributable to a benign cause.

Material and Method

All CC (Liquid-based preparation, ThinPrep) reports evaluated in the pathology department of Şanlıurfa Training and Research Hospital between 01.06.2022-01.06.2023 were determined by reviewing the pathology report archive. Our study included the patients who were both reported as NILM and advised RCC.

The reasons for being recommended RCC, clinic performing the test, age and address (city/district) of the patients were obtained from the pathology reports. The hospital system was reviewed for HPV test (Abbott RealTime High Risk HPV test, detectable genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), CC and CB, that were performed in the previous five years. It was reviewed also for CC and CB that were

performed within the following one year. Address groups were created based on the districts of Şanlıurfa. Three most developed central districts (Karaköprü, Haliliye, Eyyübiye) were grouped separately, and all other districts were grouped as 'Small Districts' (9). If any ECA was reported in previous or follow up CCs and CBs, the group was classified as 'Abnormal,' regardless of the type of abnormalities. If multiple previous CCs and CBs were available, the presence of at least one abnormality was sufficient to classify the group as 'Abnormal'. If multiple previous HPV tests were available, the presence of at least one positivity was sufficient to classify the group as 'Positive', regardless of the HPV type.

The IBM Statistical Package for the Social Sciences (SPSS) version 26 for Windows was used for most statistical calculations. Descriptive statistics were calculated, including minimum, maximum, average, counts and percentages. Relationships between categorical variables were analyzed using the Chi-square test or Fisher's exact test where appropriate. In cases where contingency tables exceeded 2×2 dimensions and expected cell counts were low, the Fisher-Freeman-Halton test was applied using R software version 4.5.1. A p-value of <0.05 was considered statistically significant.

Ethical approval for the study was obtained from the Ethics Committee of Harran University Faculty of Medicine (Approval Number: HRÜ/24.11.07, Date: 05.08.2024).

Results

A total of 5025 CCs was evaluated within the specified period. 242 (4.82%) of them were UE, 4385 (87.26%) were NILM, 351 (6.98%) were ASC-US, 13 (0.26%) were 'Atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H)', 1 (0.02%) were 'Atypical glandular cells', 30 (0.60%) were LSIL, 3 (0.06%) were 'High grade squamous intraepithelial lesion (HSIL)'. 820 (18.70%) of the CCs reported as NILM were recommended to RCC for various reasons.

A total of eight hundred twenty patients whose CC was reported as NILM and recommended RCC were included in our study. Patients ranged in age from 18 to 84 years, with a mean age of 40.87 years. 60.36% (n= 495) of the patients were aged 30-47 years, 23.05% (n= 189) were 48-65 years, 14.27% (n= 117) were 18-29 years, and 2.32% (n= 19) were 66-84 years. 45.00% of the patients (n= 369) resided in Eyyübiye, the third most socio-economically developed central district of Şanlıurfa, where the hospital is located. 29.60% (n= 243) resided in Haliliye, the second most developed central district, located near the hospital. 13.00% (n= 107) resided in less developed small districts. 6.50% (n= 53) resided in Karaköprü, the most developed central district, which is far from the hospital (9). Address information was unavailable for 5.90% of the patients (n= 48). 96.80% of the patients (n= 794) were examined in the gynecology clinics and 2.00% (n= 16) in the gynecologic oncology clinics. Clinic information was unavailable for 1.20% of the patients (n= 10). The reason for recommending RCC was inflammation in 96.22% (n= 789) of patients, parabasal cell predominance in 11.58% (n= 95), hypocellularity in 6.58% (n= 54), blood/fibrin in 5.73% (n= 47), flora compatible with bacterial vaginosis in 2.80% (n= 23) and fungal spores and hyphae compatible with Candida in 2.56% (n= 21). Some CC reports included more than one reason. HPV testing was performed in 40.10% (n= 329) of the patients and 4.56% (n= 15) of them were positive. 19.40% (n= 159) of the patients had previous CC and abnormalities were reported in 11.32% (n= 18) of them. 2.80% (n= 23) of the patients had previous CB and abnormalities were reported in 69.56% (n= 16). Although RCC was recommended for all patients, RCC was performed in only 7.20% (n= 59) of the patients and abnormalities were reported in only 6.78% (n= 4). 1.20% (n= 10) of the patients had follow up CB and abnormalities were reported in 50.00% (n= 5) (Table 1).

No statistically significant relationship was found between patient age, place of residence, clinic where the patient was examined, HPV test status, follow up CB status, and RCC ($p > 0.05$). The frequency of RCC was found to be significantly higher in patients who had a previous CC with a normal result or a previous CB with an abnormal result ($p= 0.007$, $p= 0.027$) (Table 2).

No statistically significant relationship was found between the RCC result, follow up CB result, and HPV test result ($p > 0.05$) (Table 3).

Discussion

According to the cervical cancer screening program organized by the Ministry of Health in Türkiye, women aged 30-65 are screened free of charge every five years with CC and HPV test (10). As a result of this program, the incidence of cervical cancer among Turkish women has dropped to twelfth position according to the WHO

(11). In our study, the majority of patients (83.41%) were within this screening age range. Şanlıurfa is the province with the second lowest average maternal age at first birth in Türkiye (12). It is thought that the fact that 14.27% of our patients were in the 18-29 age group may be related to the need for early gynecological examinations among women in this region.

Most of our patients (45.00%) resided in the central district Eyyübiye, where the hospital is located. CCs were predominantly performed in gynecology clinics (96.80%).

Table 1. Descriptive statistics

Parameters			Frequency (n)	%
Age	Minimum 18			
	Maximum 84			
	Average 40.87			
	Between 18-29		117	14.27
	Between 30-47		495	60.36
	Between 48-65		189	23.05
	Between 66-84		19	2.32
Address	Karaköprü		53	6.50
	Haliliye		243	29.60
	Eyyübiye		369	45.00
	Small districts		107	13.00
	Unspecified		48	5.90
Clinician	Gynecology		794	96.80
	Gynecologic Oncology		16	2.00
	Unspecified		10	1.20
CC findings	Inflammation		789	96.22
	Parabasal cell dominance		95	11.58
	Hypocellularity		54	6.58
	Blood/Fibrin		47	5.73
	Flora compatible with bacterial vaginosis		23	2.80
	Fungal spores and hyphae compatible with candida		21	2.56
HPV test	Not found		491	59.90
	Found	(-)	314	38.30
		(+)	15	1.80
Previous CC	Not found		661	80.60
	Found	Normal	141	17.20
		Abnormal	18	2.20
Previous CB	Not found		797	97.20
	Found	Normal	7	0.90
		Abnormal	16	2.00
RCC	Not found		761	92.80
	Found	Normal	55	6.70
		Abnormal	4	0.50
Follow up CB	Not found		810	98.80
	Found	Normal	5	0.60
		Abnormal	5	0.60

CC: Cervical cytology, HPV: Human papillomavirus, CB: Cervical biopsy, RCC: Repear cervical cytology.

Table 2. The relationship between parameters with repeat cervical cytology

Parameters			RCC		
			Found n (%)	Not found n (%)	P value
Age	Between 18-29		7 (5.98) (11.86)	110 (94.02) (14.45)	0.534
	Between 30-47		36 (7.27) (61.02)	459 (92.73) (60.32)	
	Between 48-65		16 (8.47) (27.12)	173 (91.53) (22.73)	
	Between 66-84		0 (0.00) (0.00)	19 (100.0) (2.50)	
Address	Karaköprü		6 (11.32) (11.11)	47 (88.68) (6.54)	0.228
	Haliliye		11 (4.53) (20.37)	232 (95.47) (32.32)	
	Eyyübiye		28 (7.59) (51.85)	341 (92.41) (47.49)	
	Small districts		9 (8.41) (16.67)	98 (91.59) (13.65)	
Clinician	Gynecology		55 (6.93) (96.49)	739 (93.07) (98.14)	0.312
	Gynecologic Oncology		2 (12.50) (3.51)	14 (87.50) (1.86)	
HPV test	Not found		36 (7.33) (61.02)	455 (92.67) (59.79)	0.612
	Found	(-)	21 (6.69) (35.59)	293 (93.31) (38.50)	
		(+)	2 (13.33) (3.39)	13 (86.67) (1.71)	
Previous CC	Not found		39 (5.90) (66.10)	622 (94.10) (81.74)	**0.007**
	Found	Normal	19 (13.48) (32.20)	122 (86.52) (16.03)	
		Abnormal	1 (5.56) (1.70)	17 (94.44) (2.23)	
Previous CB	Not found		54 (6.78) (91.53)	743 (93.22) (97.63)	**0.027**
	Found	Normal	1 (14.29) (1.69)	6 (85.71) (0.79)	
		Abnormal	4 (25.00) (6.78)	12 (75.00) (1.58)	
Follow up CB	Not found		57 (7.04) (96.61)	753 (92.96) (98.95)	0.158
	Found		2 (20.00) (3.39)	8 (80.00) (1.05)	

CC: Cervical cytology, HPV: Human papillomavirus, CB: Cervical biopsy, RCC: Repear cervical cytology

Table 3. The relationships between results of RCC and CB performed within the following 1 year with HPV test results.

Parameters		HPV test		
		(-) n (%)	(+) n (%)	P value
RCC results	Normal	17 (89.47) (80.95)	2 (10.53) (100.0)	>0.999
	Abnormal	4 (100.0) (19.05)	0 (0.00) (0.00)	
Follow up CB result	Normal	2 (100.0) (66.67)	0 (0.00) (0.00)	0.400
	Abnormal	1 (33.33) (33.33)	2 (66.67) (100.0)	

HPV: Human papillomavirus, RCC: Repear cervical cytology, CB: Cervical biopsy

Inflammation was the most common reason (96.22%) for recommending a RCC to our cases. Inflammation can obscure squamous cells, reducing their visibility and causing cellular changes that may lead to suspicion of atypia (13). A study examining 47,541 conventional smears found that 5.84% of these were reported as UE. The most common reason was inflammation with a rate of 71.3%, which is consistent with our finding (14). However, in our cases, due to the lower severity of the inflammation, diagnoses were given as NILM rather UE, noting that the conditions were suboptimal. In some cases, the diagnosis was recorded as NILM rather than ASC-US, interpreting slight cellular changes as secondary to inflammation. In both scenarios, RCC was recommended after treating the inflammation.

Estrogen and progesterone are the two main hormones affecting squamous cells. Under the influence of these hormones, squamous cells may exhibit superficial, intermediate, or parabasal morphology (15). Parabasal cell dominance was observed in 11.58% of our cases. Factors such as menopause, bilateral oophorectomy, pregnancy, the postpartum period, and hormone therapy can contribute to parabasal cell dominance in CC (15,16). Especially if the patient's age does not align with menopause and if the factors causing parabasal cell dominance are not reported by the clinician, nuclear changes in parabasal cells, due to inflammation or drying artifact, may be mistaken for ECA (13,17). A study found that HSIL or higher lesions were not detected in the CB of 37.5% of postmenopausal women whose CCs were reported as HSIL (18). Another study revealed that 19 out of 128 women diagnosed with squamous cell carcinoma based on CC results had false positivity upon hysterectomy or conization, with 12 of these women being postmenopausal (19). Koilocyte like changes or cells showing atypical parakeratosis were observed in 10.5% of women over 55 diagnosed with ASC-US. Additionally, 60.9% of these women had HPV negativity (20). When there is parabasal cell dominance, RCC may be recommended after estrogen treatment to exclude suspicious changes before CB (13,17).

A study reported that the most common cause of inadequacy in conventional smear was hypocellularity, occurring in 66.6% of cases (21). Similarly, another study identified low cellularity as the most common cause of inadequacy in liquid based cytologies, accounting for 95.68% of the cases (22). Given that the sensitivity of CC for detecting abnormalities decreases with a lower number of represented squamous cells, RCC was recommended for 6.58% of our patients due to hypocellularity.

In a study, the cause was bleeding in 11.04% of insufficient CCs (14). Since blood covers the epithelial cells and prevents examination, it is recommended that CC should be performed on the twelfth day away from menstrual bleeding in patients if possible (23). At the same time, vaginal bleeding is one of the early symptoms of cervical cancer (24). Therefore, bleeding tests should be examined carefully and blood should be noted in the report. In 5.73% of our patients, although no obvious cellular abnormality was observed, RCC was recommended because the squamous cells were covered with fibrin.

In 2.80% of our patients, there were coccobacilli consistent with bacterial vaginosis above the epithelial cells. In bacterial vaginosis, the squamous cells may be less examinable and cellular changes resulting from the toxicity of the amines the bacteria secrete may be interpreted in favor of atypia (13).

Fungal spores or hyphae compatible with Candida species were observed in 2.56% of our patients. Since the cellular changes they caused were found exaggerated and raised suspicion of atypia, RCC was recommended after treatment. In a study, the sensitivity of the CC test in detecting fungus was found to be 25.25% (25). Especially when fungi are not represented, the cellular changes it causes can easily mimic ECA (13).

1.80% of our patients had HPV test positivity, 2.20% had previous abnormal CC and 2.00% had previous abnormal CB. 92.80% of the patients did not have an RCC, although it was recommended in the pathology report. Only 6.78% of the patients who were performed an RCC were reported abnormality. Only 1.20% of the patients had a CB in a year. Half of them were abnormal.

In a study, cervical intraepithelial neoplasia 1 (CIN1) was reported in the CB of 14% of patients whose CC results were interpreted as benign cellular changes. All of these patients had a previous abnormal CC result (26). Interestingly, the RCC rate was found to be statistically significantly higher in our patients who had previous normal CC results ($p= 0.007$). This situation may be interpreted as conscious patients who cared for cancer screening and gave regular CC even if they have no complaints had a RCC. The presence of a previous abnormal CB result was found to have a statistically significant effect on RCC ($p= 0.027$). RCC rates were higher in

those who had a positive HPV test (p= 0.612). When there is abnormal previous CB result or HPV infection that is proven to be associated with precancerous and cancerous lesions of the cervix, it is understandable that the gynecologist insists on RCC or the patient is enthusiastic about RCC. Even though the RCC rate was found to be high in HPV positivity, it was found to be lower than our expectations. The fact that only two of the 15 patients with HPV positivity were repeated CC and another two of them were performed CB showed that the majority of patients did not go for further examination. Our study was conducted in the city of Şanlıurfa with the second lowest average education period according to 2022 data (27). Therefore, it was more likely that this situation was due to the patients not following and not taking serious the CCs report and gynecologist's recommendations rather than the gynecologists not following the patients well.

The highest rate of retest was found in patients residing in Karaköprü, the most developed district of Şanlıurfa. This may be related to the awareness level of the patients. But this relationship was not statically significant (p= 0.228). It was observed that the RCC rate of the few patients coming from gynecologic oncology clinics was higher than the patients coming from gynecology clinics. This may be related to the fact that oncologists take CC, a cancer screening test, more seriously because of their branche. But this relationship was not statically significant (p= 0.312). There was no statistically significant relationship between RCC with age or presence of follow up CB (p= 0.534, p= 0.158).

Additionaly, we looked at the relationship between HPV test results and RCC and following up CB results. Only 25 (7.59%) of the patients with HPV test underwent RCC. The RCC results of two patients with HPV positivity were normal and there was no significant relationship between HPV and RCC results (p= 1.000). Only 5 (1.51%) of the patients with HPV test underwent follow up CB. The CB results of two patients with HPV positivity were abnormal but this relationship was not statistically significant (p= 0.400).

Conclusion

When evaluating CC, pathologists often tend to base their diagnostic comments on the clearly evaluable epithelial cells instead of saying UE. In addition, they prefer to ignore mild cellular changes that they can attribute to a specific cause without saying ASC-US. As a result, they report the cytology as NILM, and recommend to be repeated CC, after appropriate interval, treatment or HPV test. In our study, we observed that the RCC rate was low, although it was recommended in the CC report. The retest rate was significantly higher in patients with previous normal cervical cytology and previous abnormal cervical biopsy. No significant relationship was found between the frequency of repeat testing and age, the place of residence, the clinic where the patients were examined, presence and results of HPV test, presence and results of follow up CB. Abnormality was reported in a very small proportion of patients undergoing RCC. Due to the low RCC rate in our study, we think that this approach of pathologist does not go beyond sharing the legal responsibility with the gynecologist or the patient. Although frequency and abnormality of RCC results frequency were low, in order not to avoid missing these small number of abnormal patients, we believe it is prudent to repeat CC especially in patients with previous abnormal CC, CB or HPV positivity.

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Conflict of interest statement

There is no conflict of interest.

Ethics Committee Approval

Ethical approval for the study was obtained from the Ethics Committee of Harran Faculty of Medicine (Approval Number: HRÜ/24.11.07, Date: 05.08.2024).

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