

Colposcopy, cervical cytology and human papillomavirus detection as screening tools for cervical cancer

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تنظير المهبل والفحص الخلوي لعنق الرحم واكتشاف فيروس الورم الحليمي البشري، كوسائل لتحرّي سرطان عنق الرحم

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خلاصة: أجري التنظير المهبل لمجموعة من سبعة وسبعين امرأة تمت إحالتهم إلى عيادة التنظير المهبل للفحص الروتيني أو لتحرّي أي منظر غير طبيعي في اختبار بابانيكولاو. وقد تم تشخيص المستحضرات ذات الفعالية السائلة والملونة بملون بابانيكولاو، وتصنيفها وفقاً لنظام بيتسدا. أما الثمالات المتبقية على أداة أخذ العينة فقد استعملت لاكتشاف دنا فيروس الورم الحليمي البشري الذي هو من أشد عوامل الاحتظار في إحداث الأورام. ومع أن معدل فشل التنظير المهبل كان أكبر من معدل فشل الفحص الخلوي، إلا أنه لم تقلت أية آفة عند استعمال الطريقتين معاً. وقد سجلت أنواع عالية الاحتظار في 24% من المريضات اللاتي لديهن خلايا حرشفية لا نموذجية، وفي 45% من لديهن آفات حرشفية داخل الظهارة من درجة منخفضة، وفي 79% من لديهن آفات حرشفية داخل الظهارة من درجة مرتفعة، الأمر الذي يشير إلى إمكانية تحسين كفاءة التحرّي بالفحص الخلوي وذلك بالاستعانة باكتشاف فيروس الورم الحليمي.

ABSTRACT A cohort of 77 women referred for routine screening or investigation of Pap test abnormality underwent colposcopic examination. Pap-stained liquid-based preparations were diagnosed and categorized according to the Bethesda system. Residual material on the sampling device was used to detect high-risk oncogenic human papillomavirus DNA. Although the colposcopic failure rate was higher than that of cytology, no lesion was missed when both methods were used together. High-risk types were recorded in 24% of patients with atypical squamous cells of undetermined significance, 45% with low-grade squamous intraepithelial lesions and 79% with high grade squamous intraepithelial lesions indicating that the efficacy of cytological screening can be improved by papillomavirus detection.

Colposcopie, cytologie cervicale et détection du papillomavirus humain comme instruments de dépistage du cancer du col utérin

RESUME Une cohorte de 77 femmes, adressées en orientation-recours pour dépistage systématique ou examen d'une anomalie du test de Papanicolaou, a subi un examen colposcopique. Des préparations en milieu liquide ayant subi une coloration de Papanicolaou ont fait l'objet d'un diagnostic et ont été classées selon le système de Bethesda. Les matières résiduelles sur l'échantillonneur ont été utilisées pour détecter l'ADN du papillomavirus humain à haut risque oncogène. Bien que le taux d'échec de la colposcopie soit plus élevé que celui de la cytologie, aucune lésion n'a été oubliée lorsque les deux méthodes ont été utilisées ensemble. Des types à haut risque ont été enregistrés chez 24 % des patientes ayant des cellules squameuses atypiques d'importance indéterminée, 45 % ayant des lésions intraépithéliales squameuses de bas grade et 79 % ayant des lésions intraépithéliales squameuses de haut grade, indiquant que l'efficacité du dépistage cytologique peut être améliorée par la détection du papillomavirus.

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Introduction

Cervical cancer is the second most common cancer affecting women worldwide, accounting for 437 000 new cases each year [1]. In developing countries, it is a leading cause of death among middle-aged women, where an estimated three-quarters of the global burden occurs [2]. Although the incidence rates of this cancer in Iraq are relatively low, as in most other Islamic countries, the majority of the cases usually present in advanced stages with poor prospects of cure. According to the latest Iraqi Cancer Registry [3], cervical cancer ranks 9th among the 10 most common female cancers (forming 3.6% of total female malignancies). As more than two-thirds of the patients had late diagnoses (i.e. stages IIb, III or IV), a feasible control strategy would be to encourage Iraqi women to seek early detection of cervical intraepithelial neoplasia (CIN) [4].

This preliminary study was initiated at the Department of Obstetrics and Gynaecology of the Geneva University Hospital (September–October, 1999), and is continuing at the Medical City Teaching Hospital in Baghdad. The main objective was to compare the performance of colposcopy with that of cervical cytology (considered the main screening tool for the detection of CIN). The correlation of the presence of human papillomavirus (HPV) with the aforementioned test outcomes was also investigated.

Patients and methods

At the Colposcopy Clinic and Department of Obstetrics and Gynaecology

During the period of the study, 77 women (aged 19–69 years) referred to the Colposcopy Clinic for routine screening or for in-

vestigation of a Pap test abnormality, were approached. Using ZEISS PLUS® colposcope, the vulva, vagina and cervix were magnified (mainly 10×) and inspected for abnormalities. Colposcopic examination was aided by 3% acetic acid followed by Schiller iodine test. For collection of cellular samples, the cervix was scraped with a cytobrush, which was rotated within the cervical canal. The brush was then placed into a vial of fixative, and the material thus obtained later used for both cytological diagnoses and HPV detection. Endocervical curettage was performed in cases with unsatisfactory colposcopic examination (i.e. when the transformation zone was not entirely inspected). In cases with colposcopically recorded abnormal findings, directed biopsies were taken from the most severely affected areas.

At the Cytopathology Department

The Thin Prep® Pap Test (TP), a thin-layer liquid-based cytologic preparation, was used (Thin Prep Processor, Cytoc Corporation, Boxborough, Massachusetts, United States of America) [5]. The Cytoc system passes the cell suspension through a polycarbonate filter until a specified density of cells has been deposited and then touches the filter to a slide. Only one specimen is processed at a time. Papanicolaou-stained slides were cytologically diagnosed and categorized according to the Bethesda system [6].

At the Virology Department

The residual material on the sampling device was used in the detection of high-risk oncogenic type of HPV DNA using the Hybrid Capture II® test (Digene Corporation, Silver Spring, Maryland, United States of America) [7]. The test was applied on 131 specimens with different cytologic diagnoses. Samples containing the target DNA

Table 1 Colposcopy-cytology correlations

Cytological diagnosis	Colposcopic findings							Total
	Unsatisfactory			Normal			Total	
	Leukoplakia	SCP ^a /CINI	CIN I/CIN II ^b	Condylomata	Abnormal	CIN II ^c /CIN III		
Normal limits			1	2			1	14
Koilocytes/HPV				2	6			8
Hyperkeratosis				2	3			5
ASCUS	3			3	1	2		9
Koilocytes/LSIL				3	8	3		17
LSIL (CIN I)				1		7	1	9
HSIL (CIN II and III) ^c	2				4	9	9	15
Total	6			21	11	17	10	77

^aSubclinical papillomavirus (SCP) infection with or without low-grade squamous intraepithelial lesions (LSIL); colposcopy score = 1-2.

^bCervical intraepithelial neoplasia (CIN) grades 1 and 2; colposcopy score = 3-5.

^cHigh-grade squamous intraepithelial lesions (HSIL); colposcopy score = 6-8.

HPV = human papillomavirus.

ASCUS = atypical squamous cells of undetermined significance.

were hybridized with a specific HPV RNA probe cocktail. The resultant RNA-DNA hybrids were captured onto the surface of a microplate well coated with antibodies specific for the RNA-DNA hybrids. Immobilized hybrids were then reacted with specific alkaline phosphatase conjugated antibodies and detected with a chemiluminescent substrate.

Results and discussion

On recording the colposcopic findings (Table 1), a classification modified from the Colposcopic Index [8] and the European Proposal for Colposcopic Terminology [9] was adopted. Both cytology and colposcopy are primarily screening methods for detection of early cervical cancer. Although each of these methods is associated with a certain margin of error, the colposcopic failure rate remains higher than that of a good cytology test, simply because 10%-15% (13% in the present study) of atypical lesions are situated deep into the cervical canal out of reach of the colposcope [10]. Another 5% of error results from misinterpretation of the ectocervical changes (as in cases of immature metaplasia, acanthosis, subclinical papilloma infections). Thus, it has been reported that the accepted rate of colposcopic accuracy in the detection of cervical cancer precursors does not exceed 80% [10]. In the present study, the accuracy rates of colposcopy and cytology in diagnosing CIN II and CIN III [i.e.

high-grade squamous intraepithelial lesions (HSIL)], were 89.5% and 96.3%, respectively.

Errors when using the conventional Pap cytology are inevitable, despite the excellent performance of the smear in reducing morbidity and mortality from cervical cancer over the past 40 years [11,12]. The sources of error begin with cell sampling and relate to many aspects of specimen preservation, slide preparation and staining [13]. Such artefacts have been considerably reduced by the introduction of liquid-based cytology, a new method designed to convert a fluid suspension of cervical sample into a consistently staining homogenized thin layer of cells [5,14]. Cytologically, the terminology of the Bethesda system [6] was applied within this report. In our opinion, this system simplifies Pap smear cytologic reporting, making it more reproducible and thus refining the process of appropriate patient care by improving communication between cytologists and attending clinicians. Available data from liquid-based studies have shown overall increased detection of epithelial abnor-

malities with a considerable reduction in atypical squamous cells of undetermined significance (ASCUS) [14], so that by using a dual cytologist and computer image evaluation, a false-negative rate as low as 1.8% has been achieved [15].

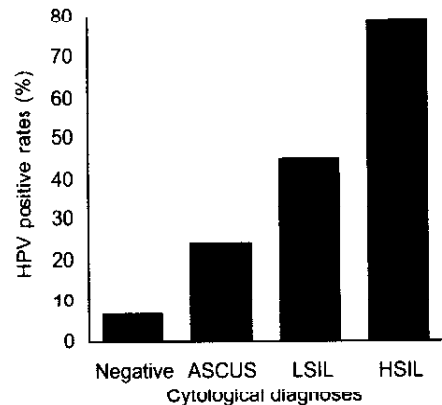
Since cytology is a screening test, abnormal findings should be confirmed histologically. In this preliminary report, the recorded sensitivity and specificity rates of cytology and colposcopy in the detection of HSIL were: 88.9% and 98.4% respectively for cytology; and 72.7% and 95.3% respectively for colposcopy (Table 2). Nevertheless, it was quite obvious that both cytology and colposcopy complemented each other, since no lesion was missed when both methods were used in concert.

Table 2 Analytical comparative evaluation of the accuracy of colposcopy versus cytology in the diagnosis of HSIL (i.e. CIN II and CIN III)

Statistical test	Colposcopy %	Cytology %
Sensitivity	72.7	88.9
Specificity	95.3	98.4
Positive-predictive value	84.2	94.1
Negative-predictive value	91.0	96.8
Efficiency	89.5	96.3

HSIL = high-grade squamous intraepithelial lesion (according to the Bethesda nomenclature).

CIN = cervical intraepithelial neoplasia.



ASCUS = atypical squamous cells of undetermined significance

LSIL = low-grade squamous intraepithelial lesion (CIN I)

HSIL = high-grade squamous intraepithelial lesion (CIN II and CIN III)

Figure 1 Rates of human papillomavirus (HPV) positivity, by Digene Hybrid Capture II® test, in various cytological diagnoses (n = 131 cases).

The diagnosis of condylomata has attracted a great deal of interest in recent years. The strong correlation between the presence of HPV and the severity of cervical disease has been restricted to the high-risk oncogenic types of HPV (mainly 16 and 18), which are considered to be the major recognized risk factors for the development of cervical carcinoma [16].

In the present work, the Digene Hybrid Capture II® test (Figure 1) demonstrated these high-risk types in 7% of women with normal cytology (1 of 14 cases), 24% of those with ASCUS (13 of 55 cases), 45% with low-grade squamous intraepithelial lesions (LSIL) (17 of 38 cases) and 79% with HSIL (19 of 24 cases). Even higher frequencies of the same oncogenic viruses within the cervixes of patients with LSIL and HSIL were recorded by Vassilakos [17] (60% and 88% respectively), indicating that the IIPV test could be used as a quality control measure to evaluate the diagnostic performance of cytology. That fact has been confirmed by a follow-up study of women with abnormal cytology, where clinical progression occurred only in those

who had persistent high-risk HPV infection, thus emphasizing that the efficacy of cytological screening can be improved by HPV detection [18].

Recently, a World Health Organization pilot study demonstration project to screen for cervical cancer precursors in Iraq has been initiated as a first step towards establishing an organized national cancer control programme. It is hoped that the study will provide precise information on the comparative performance of various methods used in early detection of cervical cancer. Detailed results of the aforementioned project will be presented in due course.

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