

Subclinical human papillomavirus infection of the cervix

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ABSTRACT

Objectives: A prospective study to investigate a group of Iraqi women with proved genital vulval warts, to seek evidence of human papillomavirus infection in apparently normal looking cervixes and to investigate the natural history of infection.

Methods: From December 1997 to August 1998, 20 women with vulval warts were enrolled along with 20 aged-matched control cases without warts. Their ages ranged between 19-48 years with a mean of 30.4 years, (+/- standard deviation = 2.3) for patients and 18-48 years with a mean of 29.7 (+/- standard deviation = 2.7) for the control group. General and gynecological examinations were carried out. Cervical swabs for associated genital infection, papilloma smears, speculotomy and directed punch biopsies were carried out to detect subclinical human papillomavirus infections of the cervix and associated intraepithelial neoplasm.

Result: Cytology results showed that 11 (55%) of patients had evidence of cervical infection by human papillomavirus, 6 (30%) showed mild dysplastic changes,

3 (15%) showed moderate dysplastic changes, whilst 2 (10%) showed no dysplastic changes. Speculotomy and acetowhitening was positive in 11 (55%) and collated histological results showed evidence of human papillomavirus infection in 9 patients (45%). As for the control group one case (5%) had evidence of human papillomavirus infection.

Conclusions: Subclinical human papillomavirus infection is more common than was previously thought among Iraqi women. It may appear alone or in association with vulval or exophytic cervical warts, or both, and may be more common than the clinically obvious disease. Speculotomy as an adjunctive method to colposcopy was found to be a simple and an easy to perform technique. Its combination with cytology gave relatively good results when it was used as a triage instrument, and may have a more promising performance in the future.

Keywords: Human papillomavirus, cervix.

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In recent years interest in cervical infection by human papillomavirus (HPV), the agent responsible for genital warts, has been growing. Condylomata acuminata of the cervix is a familiar condition which is associated with characteristic cytological findings of koilocytosis, dyskeratosis and nuclear atypia. It is now apparent that these findings are present in many women who do not have exophytic condylomata. In these women, condyloma acuminatum of the vulva is associated with

inconspicuous cervical lesions which are only visible by magnification.¹⁻³ These lesions have been called flat condylomata, non condylomatous cervical warts, or subclinical HPV infection,⁴ terms which indicate their essentially non papilliferous nature. The term subclinical HPV infection has been ascribed to different issues by different authors. Some authors call subclinical HPV infection those lesions that are not visible on routine inspection but become visible by colposcopy after acetic acid, and, which on

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histology contain typical HPV induced changes. There are others who used the term as identical to flat warts. In a 3rd group however, the term subclinical HPV infection in both sexes has been applied for lesions, which become visible only by colposcopy (peniscopy) after acetic acid application, and, which on light microscopy shows normal or minor epithelial changes not consistent with flat condylomas.⁵

Methods. The research and ethical committee approved this study, and patients consent was taken.

Patients. Twenty married female ladies with clinically evident external genital warts were selected from the outpatient clinics of the Department of Dermatology and Venereology and the Department of Gynecology, Baghdad Teaching Hospital, University of Baghdad during the period from December 1997 to August 1998. Twenty aged match ladies were recruited as a control group. From each patient a detailed medical history and physical examination was performed. Vaginal swabs and cervical smears were taken for direct examination and for culture and sensitivity tests. All patients had screening serological tests for syphilis venereal disease research laboratory (VDRL) and *Trponema pallidum* hemagglutination antibody test (TPHA). Pediculosis pubis, molluscum contagiosum, and scabies were diagnosed by there clinical appearances.

Cytological methods. Cervical and vaginal smears were taken from each patient using the Ayer's wooden spatulas, to collect cells from the squamocolumnar junction of the cervix and posterior fornix of the vagina. The samples were immediately spread on glass slides, fixed with 95% ethyl alcohol, stained with papanicolaou stains, subsequently examined microscopically, and evaluated for the presence of the 2 main important cells, the koilocytes, and dyskeratocytes.

Acetowhitening and speculscopy. The patients were examined in the lithotomy position as to allow easy exposure of the cervix using a bivalve speculum. Speculoscopy as an alternative and additive screening technique to colposcopy based on morphologic and physical principles has been used. Speculite is a chemiluminescent illumination source that is attached to the upper dilator blade of the vaginal speculum during a pap smear gynecological examination, intended as a general screening aid in the visualization of the acetowhite areas by a low power (x4-6) magnification, of vaginal and cervical mucosa that has been previously washed with acetic acid, 5% solution. A small plastic capsule containing a mixture of non-toxic chemicals serves as a light source (speculight B.W, Trylon corp. Torrance C.A.). After activation and attaching it to the upper blade of the vaginal speculum, a diffuse cold light is produced which has 3 maximum at 430, 540 and 580nm wavelengths giving a blue white effect. The capsule remains active for 15-20 minutes. The cervix was

liberally washed with 5% acetic acid using a large cotton swab or 4x4 gauze squares held in place for at least 60 seconds. With an approved low power (x4-6) hand held optical magnification source, visual examination was performed to detect the presence of acetowhite areas such as areas with bright whitening and sharply defined borders. Three criteria for non-condylomatous HPV infection of the cervix (flat and subclinical) were evaluated, these included apparent thickness, degree of acetowhitening and surface contour. Directed punch biopsies from the acetowhite areas of the cervix were obtained.

Histopathological studies. Two biopsy specimens for histopathological study were obtained from every patient, an excision biopsy from one of the clinically evident genital warts and a directed punch biopsy from the acetowhite areas of the cervix. Each specimen was formalin fixed processed, stained with hematoxylin and eosin stains and examined under light microscopy.

Results. Twenty women with vulval warts underwent evaluation during the course of the study. Cytological findings consistent with the presence of HPV infection in the cervix were positive in 11 cases (55%), such as the presence of both koilocytes and dyskeratocytes associated with mild to moderate dysplasia. In 2 cases (10%) the possibility of HPV infection could not be excluded. There were rounded cells, with vacuolated cytoplasm but not typical raisin nucleus. Five cases showed only polymorphs, lymphocytes, mature epithelial cells with and without metaplastic cells, changes of acute or chronic cervicitis. In 2 cases, the cervical

Table 1 - Results of cervical cytology in women with vulval warts.

Findings/type of cells	n	%
Koilocytes, dyskeratocytes and mild dysplastic changes (CINI)	6	30
Koilocytes, dyskeratocytes moderate dyplastic changes (CINII)	3	15
Only koilocytes + dyskeratosis - no dysplasia	2	10
Intermediate cells, rounded cells, vacuolated cytoplasm, no typical koilocytosis	2	10
Lymphocytes, polymorphs, mature epithial and metaplastic cells - acute or chronic cervicitis	5	25
Hypocellular smears	2	10
Total	20	100
CINI= cervical intraepithelial neoplasia grade I, CINII=cervical intraepithelial neoplasia grade II		

Table 2 - Correlation between acetowhite areas and histological sections with typical koilocytic changes.

Acetowhite positive areas	n %		Histological sections with typical koilocytic changes	
	n	%	n	%
Bright white with sharply defined margin and irregular border	12	60	9	75
Faint white with diffuse ill defined fuzzy border	3	15	0	0
Negative acetowhitening	5	25	0	0
Total	20	100	9	75

smears were hypo-cellular and inadequate for assessment. Of the 11 positive smears for HPV infection 6 cases (30%) had mild dysplasia (CINI), 3 cases (15%) moderate dysplasia (CINII) and 2 cases (10%) with no dysplasia (Table 1). Acetowhitening of the cervix after application of 5% acetic acid and screening by speculoscopy for definite bright white areas and sharply defined borders was positive in 12 cases (60%), mostly involving the anterior lip at 12, one and 7 o'clock positions. Diffuse faint acetowhite areas with ill defined margins were found in 3 cases (15%) and 5 cases 25% were acetowhite negative (Table 2).

Histological findings. In 6 cases (30%) the histological features were consistent with subclinical HPV infection of the cervix: acanthotic epithelium, accentuated rete ridges, elongated dermal papillae and a superficial layer with many koilocytic and dyskeratotic cells (giving a feature of a lace curtain appearance), in addition to mild-dysplastic changes (CINI). Two cases (10%) showed only mild acanthosis, superficial dyskeratosis and some intermediate cells with slightly vacuolated cytoplasm, few koilocytic cells and moderate dysplasia (CINII). Only one case (5%) showed features consistent with HPV infection only with no dysplasia. Eleven cases (55%) showed only features of acute or chronic inflammation. Of the control group, one case (5%) showed cytological and histological findings consistent with HPV infection but with no associated dysplasia.

Discussion. Human papillomavirus infection is very common among sexually active adolescent and young adults. Women in this study ranged in age between 19-48 years with a mean of 30.45 years. This range was slightly higher than other reported European studies. Most reported a range between 12-

18 years with a mean of 16.8 years¹ and 16-25 years.⁶⁻⁷ This is probably due to early sexual practicing habits, and multiple partners leading to a higher chance of harboring the virus. Many women with vulval warts in this study had an associated lower genital tract infection. The association of micro-organisms was similar to that found in other studies.⁸ Condylomata acuminata of the cervix (exophytic condyloma) is a familiar condition found in approximately 6-8% of women with anogenital warts.^{6,9} It is associated with microscopical findings of koilocytosis which is the hallmark of HPV infection, dyskeratosis, and nuclear atypia. It is now apparent that these findings are present in many women who do not have exophytic condylomata.⁸ Clearly there was a substantial amount of cervical abnormality among women with vulval warts whom we studied. Cytological evidence of HPV infection and cellular abnormalities consistent with cervical intraepithelial neoplasia CINI and CINII was found in 55% of the cases. Different results were obtained from other studies. Some gave evidence of 50%⁸ and other reported an evidence of HPV infection in 44%.⁹ Subsequent speculoscopy results combined with histological confirmation of HPV infection and CIN was present in 45% in this study. The discrepancy between our cytological and histological results could be explained by that some of the directed punch biopsies might have missed the target areas. Comparing the speculoscopy and histopathology results with another parallel study in whom a colposcopic diagnosis of HPV infection was made confirmed by histology evidence and was present in 83% of cases which is much higher than our reported results.⁸ Among women attending sexually transmitted diseases (STD) clinics, reported studies showed that 8-13% of the pap smears had signs of HPV infection.¹⁰ Comparing these results with our control group, 5% of the pap smears and biopsies showed signs of HPV infections. During the last few years the relationship between HPV and cervical neoplasia has been intensively studied. The precursors of carcinoma of the cervix are regarded as a continuum, CIN. Cervical intraepithelial neoplasia has a variable course: registration is commoner in early (grade 1) lesions and progression is more common in grade II and III and above, many cervical lesions, which were formerly classified as CIN, are really subclinical HPV infection.¹¹ Therefore women with vulval warts with cytological and histological evidence of HPV infection and CIN should be marked as a group requiring special care. The development of new techniques such as the Polymerase Chain Reaction (PCR) has indicated that HPV infections are more prevalent than might be expected from the clinical findings of visible warts. However, PCR techniques are not yet clinically available, and the clinician has to rely on the identification of genital warts, acetowhite changes of

the cervix and cytology. Although cervical cytology is practical and a well established screening method for detecting subclinical HPV infection and dysplasia, there has been some concern in recent years regarding its sensitivity. False negative results have been reported from women with biopsy proven CINI and CINII.⁹ Colposcopy allows direct visualisation of the cervix and is considered a more sensitive method identifying cervical pathology than cytology. However colposcopy would be unsuitable as a screening procedure owing to the cost involved and shortage of colposcopists.¹² Speculoscopy as an adjunctive visual screening measure was found to be sensitive (88%) for high grade CIN and 100% for invasive cancer and most effective when used as a triage instrument.¹³

A combination of both cytology and speculoscopy was used to triage our 20 female patients with vulval warts, and was found to be simple, effective and also helped in directing the punch biopsies. Concerns have been raised regarding the importance and practical significant of diagnosing both clinical and subclinical genital HPV infection. In light of the available data it seems rational to screen and investigate women with genito-anal warts diagnosed by gross appearance to ascertain the full extent of the multifocal disease. Women with CIN may benefit from examination and treatment of their clinical and subclinical lesion, aiming at optimizing the cure rates of CIN, and decreasing the risk of cancer transformation. Diagnosis and treatment of such lesions may aid in reducing the risk of transmission of HPV from the pregnant mother to their babies during delivery. Screening for, and treating subclinical lesions may appear to have an additional importance with advent of the new immunomodulating drugs and the rising hope of eradicating the virus.

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