

# Papanicolaou smear: A diagnostic aid in sexually transmitted infections

Sabeena Jayapalan, R. S. Bindu

Department of Dermatology and Venereology, Government Medical College, Thiruvananthapuram, Kerala, India

## Address for correspondence:

Dr. Sabeena Jayapalan, Department of Dermatology and Venereology, Government Medical College, Thiruvananthapuram - 695 011, Kerala, India.

E-mail: sabinajayapalan@gmail.com

## Abstract

**Background:** Sexually transmitted infections (STIs) are a major public health problem in developing countries, and treatment with the right medicine at the right time is necessary to reduce transmission and improve sexual and reproductive health. Laboratory diagnosis has undergone changes in the recent years. The new generation tests are not cost-effective in resource-poor settings. Papanicolaou smear (Pap smear) of the cervix is a simple, quick, and inexpensive screening procedure for cervical cancer that can also give a clue to the presence of STIs. **Methods:** A hospital-based cross-sectional design with fifty patients was conducted studied. The initial diagnosis based on clinical findings and routine laboratory results was compared with the final diagnosis incorporating Pap smear results. **Results:** The Pap smear was abnormal in 96%, and the abnormalities were koilocytosis (30%), endocervicitis (24%), *Trichomonas vaginalis* (16%), multinucleated giant cells (10%), low-grade squamous intraepithelial lesion (10%), and clue cells (8%). Pap smear could diagnose an infection in 64%, of which 38% were asymptomatic. Clinical and Pap smear correlation was found only in 26% of the patients. **Conclusion:** Along with the conventional methods, Pap smear can be a valuable tool in diagnosing STIs. This can detect asymptomatic infections, not detected by conventional methods, thus preventing complications and further spread in the community. Asymptomatic human papillomavirus (HPV) infection and trichomoniasis are the two STIs that can be easily detected by Pap smear. By detecting asymptomatic cervical HPV infection, the patients at risk for carcinoma of the cervix can be identified and referred to gynecology department for further management.

**Key words:** Koilocyte, Multinucleated giant cell, Papanicolaou smear, sexually transmitted infections, *Trichomonas vaginalis*

## INTRODUCTION

Sexually transmitted infections (STIs) are a major public health problem. Globally, more than a million STIs are acquired every day and have a direct impact on the reproductive and child health and an indirect impact on the national and individual economies.<sup>[1]</sup> STIs are associated with an increased risk of transmission of human immunodeficiency virus (HIV) infection.<sup>[2-4]</sup> Treating STIs with the

right medicine at the right time is necessary to reduce HIV transmission and improvement of sexual and reproductive health. Timely and appropriate treatment necessitates timely diagnosis.<sup>[5]</sup> The available resources determine how an STI is diagnosed. The different diagnostic approaches adopted are presumptive diagnosis based on the

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clinical findings, etiological diagnosis based on laboratory testing, and syndromic diagnosis based on a constellation of clinical features. The laboratory diagnosis of STIs has improved greatly in the past decade with the introduction of newer technologies. Unfortunately, the newer diagnostic technologies and even many conventional laboratory procedures are beyond the reach of many health-care providers in resource-poor settings. Our institution is a tertiary care center in the government sector, and there are several limitations for sophisticated laboratory investigations. We diagnose STIs on a combination of presumptive, etiological, and syndromic diagnostic approaches. Facilities available in our department are direct microscopy; serology for syphilis, HIV, and hepatitis; and culture for gonorrhea. Papanicolaou smear (Pap smear) of the uterine cervix is a simple, quick, and inexpensive screening procedure for cervical cancer. Pap smear also helps in detecting or at least gives a clue to the presence of STIs such as trichomoniasis, *Candida*, *Actinomyces*, herpes simplex virus (HSV), cytomegalovirus, and human papillomavirus (HPV).<sup>[6-8]</sup> We were interested in finding whether the incorporation of cervical Pap smear findings into our diagnostic armamentarium could improve upon our etiological diagnosis.

## METHODS

Consecutive female patients with symptoms of STIs and asymptomatic female partners of male STI patients, attending the department of dermatology and venereology of a tertiary care center for a period of 6 months, were subjected to the study after getting written informed consent. Nonconsenting patients, pregnant patients, and menstruating patients at the time of examination were excluded. A hospital-based, cross-sectional, descriptive type of study design was used. The laboratory tests performed were as follows: (1) Gram stain from the cervix for *Neisseria gonorrhoeae* and pus cells; (2) Gram stain from vaginal discharge for diagnosing bacterial vaginosis based on Nugent's score,<sup>[9]</sup> clue cells, *Candida*, and pus cells; (3) potassium hydroxide mount from the vaginal discharge for *Candida* species; (4) wet mount from vaginal discharge for *Trichomonas vaginalis* (TV); (5) venereal disease research laboratory test and *Treponema pallidum* hemagglutination test for syphilis; (6) enzyme-linked immunosorbent assay for HIV-1 and HIV-2; and (7) serology for hepatitis B virus and hepatitis C virus. The investigations done from genital ulcer were as follows: (1) Tzanck smear for multinucleated giant cells; (2) Gram stain for *Haemophilus ducreyi*; (3) tissue smear for Donovan bodies; and (4) dark ground microscopy for *T. pallidum*.

A specimen for cervical Pap smear was taken with Ayre's spatula by introducing the spatula into the external os and rotating through 360° with light pressure. The material obtained was smeared on a glass slide, fixed immediately and stained by the Papanicolaou method,<sup>[10]</sup> and reported by the pathology department.

The initial diagnosis of STI was based on the presumptive clinical diagnosis and the routine laboratory test result. The final diagnosis was made after incorporating the Pap smear report. The initial proportion of each STI and the final proportion of each STI were calculated. McNemar's test was used to assess the significance of the difference between the two correlated proportions, i.e., the proportion of a particular STI before incorporating Pap smear and the changed proportion of STI after incorporating Pap smear diagnosis.

## RESULTS

Fifty women, forty-two STI patients, and marital partners of eight male STI patients presenting consecutively to the department of dermatology and venereology of a tertiary care center were studied. The age ranged from 19 to 41. Majority (62%) belonged to 25–34 years. All were married. Twenty-four had syphilis followed by herpes genitalis in five, condylomata acuminata in five, bacterial vaginosis in four, trichomoniasis in two, and candidiasis in two. Among the marital partners, three were partners of men with HIV infection, three were partners of men with syphilis, one was the partner of gonococcal urethritis patient, and one was the partner of nongonococcal urethritis patient.

The Pap smear was abnormal in 48 (96%) patients. The abnormal smear findings [Table 1] were koilocytes, endocervicitis, TV, clue cells, multinucleated giant cells, and mild dysplasia. Table 2 shows the correlation between clinical diagnosis and Pap smear findings.

**Table 1: Papanicolaou smear findings**

Smear finding	n (%)
Koilocytosis	15 (30)
Endocervicitis	12 (24)
Trichomonas vaginalis	8 (16)
Multinucleated giant cells	5 (10)
Dysplasia	5 (10)
Clue cells	4 (8)
Normal smear	2 (4)
Total	51 (102)*

\*The total is more than the total number of patients (50) since one patient had smear evidence of both koilocytes and multinucleated giant cells

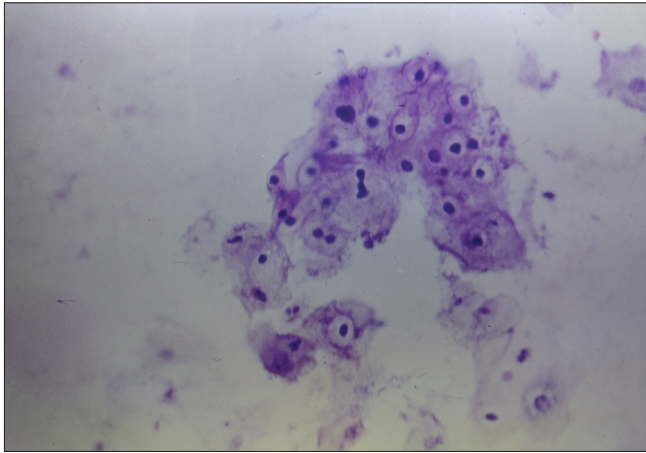


Figure 1: Koilocytes

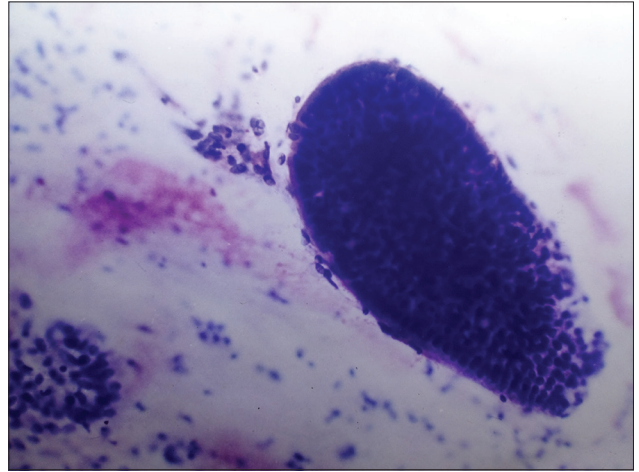


Figure 2: Papillary frond

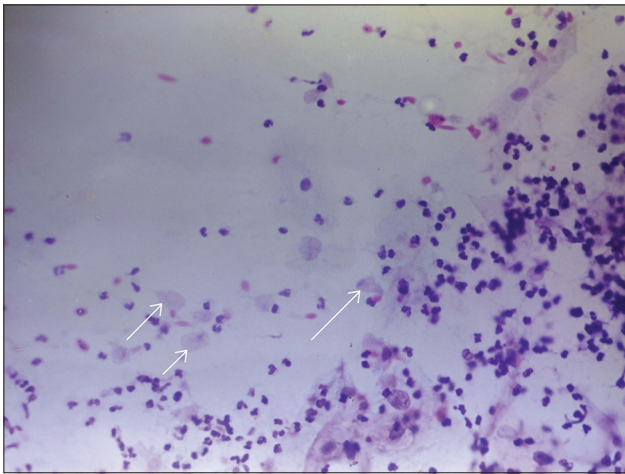


Figure 3: Trichomonas vaginalis

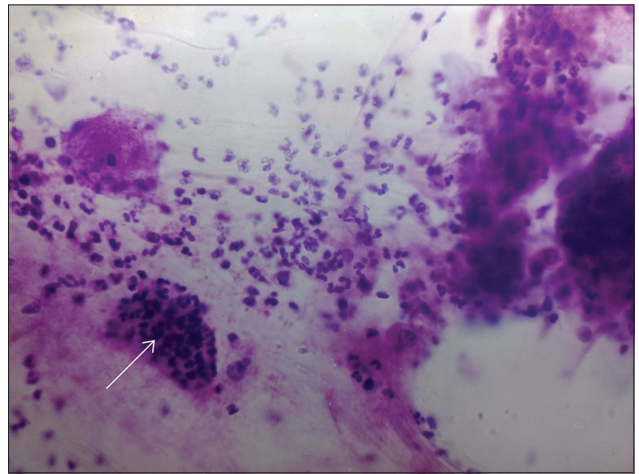


Figure 4: Cannonball appearance

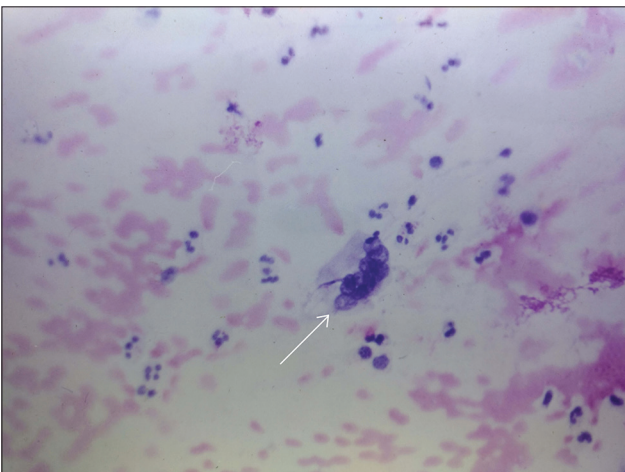


Figure 5: Multinucleated giant cells

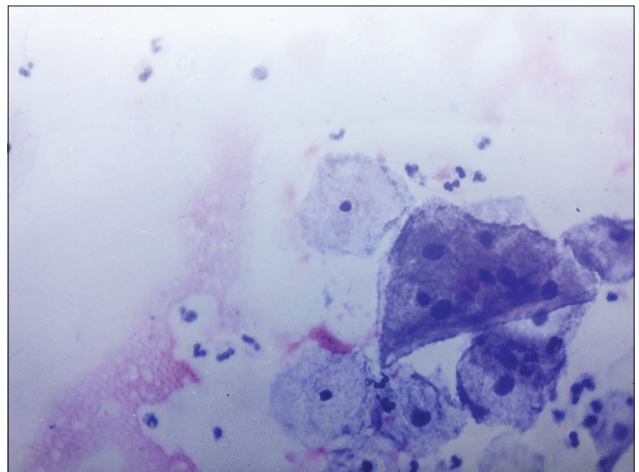


Figure 6: Clue cell

Koilocyte [Figure 1] was observed in the Pap smear of 15 (30%) patients. The clinical diagnosis in these patients was condylomata acuminata in five (10%), syphilis in seven (14%), herpes genitalis in one (2%), bacterial vaginosis in one (2%), and

one (2%) was the marital partner of syphilis patient. One smear also showed papillary frond [Figure 2].

Endocervicitis was reported in 12 (24%) patients. Nine (18%) had syphilis, one (2%) had herpes



**Table 2: Relation between clinical diagnosis and Papanicolaou smear findings**

Clinical diagnosis, n (%)	Koilocyte, n (%)	Endocervicitis, n (%)	TV, n (%)	MNG, n (%)	Clue cell, n (%)	LSIL, n (%)	Normal smear, n (%)	Total, n (%)
Condyloma acuminata: 5 (10%)	5 (10)							5 (10)
Herpes genitalis: 5 (10%)	1 (2)	1 (2)		4 (8)				6 (12)*
Syphilis: 24 (48%)	7 (14)	9 (18)	4 (8)		2 (4)	1 (2)	1 (2)	24 (48)
BV: 4 (8%)	1 (2)		1 (2)		2 (4)			4 (8)
TV: 2 (4%)			2 (4)					2 (4)
Candidiasis: 2 (4%)						1 (2)	1 (2)	2 (4)
Marital partner: 8 (16%)	1 (2)	2 (4)	1 (2)	1 (2)		3 (6)		8 (16)
Total: 50 (100%)	15 (30)	12 (24)	8 (16)	5 (10)	4 (8)	5 (10)	2 (4)	51 (102)**

\*\*The total is 51 instead of 50 because one patient with herpes genitalis, \*Had evidence of both multinucleated giant cell and koilocyte in the smear. LSIL=Low-grade squamous intraepithelial lesion; TV=Trichomonas vaginalis; MNG=Multinucleated giant cell; BV=Bacterial vaginosis

genitalis, and two (4%) had marital partners with syphilis.

Pap smear of eight (16%) patients showed TV [Figure 3]. Only two (4%) had clinical diagnosis of trichomoniasis. The rest four (8%) had syphilis, one (2%) had bacterial vaginosis, and one (2%) was the marital partner of a patient with gonococcal urethritis. Cannonball/pus ball appearance was observed in a smear with TV [Figure 4].

Multinucleated giant cell [Figure 5] was seen in cervical smear of five (10%) patients. Four (8%) had herpes genitalis and one (2%) was the marital partner of nongonococcal urethritis patient. One patient with herpes genitalis had koilocytes along with multinucleated giant cells.

Clue cells [Figure 6] were observed in four (8%) patients. Two (4%) were diagnosed as bacterial vaginosis and the remaining two (4%) had syphilis.

Low-grade squamous intraepithelial lesion (LSIL) was seen in five (10%) patients. Three (6%) were partners of HIV, one (2%) had candidiasis, and one (2%) had syphilis. Normal smear was observed in two (4%) patients. One (2%) had candidiasis and the other (2%) had both syphilis and hepatitis B virus infection.

The specific finding of an infection in Pap smear was seen in 64%. Pap smear findings correlated with the clinical diagnosis in 26%. Thirty-eight percent had evidence of asymptomatic infection. HPV infection was diagnosed initially in 10%, and after Pap smear, this increased to 30% ( $P = 0.002$ ). The proportion of trichomoniasis increased from the initial proportion of 4% to 12% after Pap smear ( $P = 0.03$ ). Initially, 10% had herpes genitalis which increased to 12% after Pap smear ( $P = 1$ ). The 8% clinical diagnosis of bacterial vaginosis increased to 12% after Pap smear ( $P = 1$ ).

## DISCUSSION

The Pap smear, introduced by George Papanikolaou in 1943, is a simple, inexpensive, and painless procedure. Although primarily intended for the screening of carcinoma cervix, it also helps in diagnosing cervical infections.

HPV, the etiological agent of condylomata acuminata, is the most important cause of carcinoma cervix. Koilocytes are squamous epithelial cells infected by HPV, with a central or eccentric hyperchromatic moderately enlarged nucleus and a perinuclear space with irregular sharply defined border. The peripheral cytoplasm is dense and is frequently amphophilic, occasionally basophilic, or eosinophilic. Koilocytosis is graded as atypical squamous cells of undetermined significance if it is mild or LSIL if it is more undifferentiated.<sup>[11]</sup> Both can either regress spontaneously or progress to malignancy. Although only 10% had condylomata acuminata, koilocytosis was observed in 30% of the patients. The cervix of all the patients with koilocytosis was normal, indicating subclinical papillomavirus infection. Pap smear helped in identifying asymptomatic HPV infection in 20%. HPV typing is recommended for follow-up of patients with koilocytotic changes in the cervical smear.<sup>[12-14]</sup> The association of cervical condylomata with moderate squamous atypia and koilocytosis has been reported by Kiviat *et al.*<sup>[15]</sup> Since we do not have the facility for HPV typing and there is a potential risk for malignant transformation, the patients were kept under follow-up and referred for colposcopic studies.

Endocervicitis is observed as vacuolization, decreased staining intensity, karyolysis, and karyorrhexis in the background of inflammatory cells. The most common sexually transmitted agent causing endocervicitis is *Chlamydia trachomatis*. Other agents are *N. gonorrhoeae*, HSV, TV, organisms of bacterial vaginosis, *Mycoplasma hominis*,

*Ureaplasma urealyticum*, etc., Pap smear of 24% of the patients showed endocervicitis similar to a report from Korea.<sup>[16]</sup> The endocervicitis in 2% could be explained by the clinical diagnosis of herpes genitalis. The association of *C. trachomatis* infection with an increased number of histiocytes, polymorphonuclear leukocytes, and transformed lymphocytes and TV infection with increased lymphocytes has been reported in the literature.<sup>[15]</sup> There are reports expressing doubts regarding the predictive value of the inflammatory cervical smear in diagnosing *C. trachomatis* infection.<sup>[17-19]</sup> Bacterial vaginosis and candidiasis were more commonly seen with the inflammatory smear in Iranian women.<sup>[20]</sup> Although with our limited laboratory findings, we were not confident enough to comment upon the cause of endocervicitis, the patients were presumptively treated for *C. trachomatis* infection.

TV is seen in the Pap smear as gray-blue pear-shaped structures with or without neutrophils in the background. Cannonballs or pus balls are neutrophils adherent to the squamous epithelial cells and can be observed in trichomoniasis and *C. trachomatis* infection.<sup>[21]</sup> Based on Pap smear, 16% of our patients had trichomoniasis. Clinically, only 4% had trichomoniasis. Pap smear identified asymptomatic trichomoniasis in 12%. The clinical diagnosis of patients with asymptomatic TV infection was syphilis, bacterial vaginosis, and the marital partner of gonococcal urethritis patients. Although the association of bacterial vaginosis and gonococcal urethritis with trichomoniasis is well established,<sup>[7,22]</sup> the association of trichomoniasis and syphilis is rare. The probability of positive direct microscopy from asymptomatic trichomoniasis is low. Although culture is said to be the most sensitive method in identifying TV, there are reports of culture negativity with Pap smear positivity.<sup>[23-25]</sup> The sensitivity of Pap smear in diagnosing trichomoniasis is 50%–60%.<sup>[7,23]</sup> Combined direct microscopy and Pap smear can identify few more cases as reported from our experience and elsewhere.<sup>[7,23,24]</sup>

Multinucleated giant cells with homogeneous ground-glass appearance of the nucleus are formed by the fusion of individually affected cells in HSV infection.<sup>[6,8]</sup> This was observed in the cervical smear of 10% of the patients. Eight percent had herpes genitalis, and this correlated with the clinical findings. Pap smear helped in identifying asymptomatic herpes infection in 2%, and this was the marital partner of nongonococcal urethritis patients whose nongonococcal urethritis may be due to HSV infection.

Clue cells<sup>[26]</sup> are epithelial cells covered with adherent small uniformly spaced bacilli with a molten waxy appearance to the cytoplasm and were observed in 8% and are one of the criteria put forward by Amsel *et al.* in diagnosing bacterial vaginosis.<sup>[26]</sup> Clinical and Pap smear correlation was found in 4%.

LSIL, characterized by cell atypia with nuclear enlargement and hyperchromasia, was seen in 10%. They were diagnosed with syphilis and candidiasis, and some were partners of HIV patients. Minimal squamous atypia associated with yeast infection was reported by Kiviat *et al.*<sup>[15]</sup> LSIL can either progress to malignancy or revert back to normal.

The STIs that can be diagnosed by Pap smear are trichomoniasis, HPV infection, herpes genitalis, candidiasis, and bacterial vaginosis. The difference in proportion of patients before and after Pap smear was statistically significant for HPV infection and trichomoniasis ( $P = 0.002$  and  $P = 0.03$ , respectively). Although asymptomatic herpes genitalis and bacterial vaginosis could be diagnosed by Pap smear, there was no statistical significance in the difference between before and after proportions. Among the STIs, Pap smear examination is a valuable aid for diagnosing asymptomatic HPV infection and trichomoniasis.

Pap smear identified the presence of a specific STI in 64%. Clinical and Pap smear correlation was found only in 26%. Pap smear enabled us to diagnose asymptomatic infection in 38%, which otherwise would have been left untreated, resulting in complications and spread. Twenty percent of these asymptomatic infections were due to HPV. This raises concern as HPV is a known cause of carcinoma of the cervix. Pap smear also identified LSIL in 10%. These patients were benefitted by Pap smear examination as we could refer the patient with smear suggestive of HPV infection and LSIL to the gynecology department for further management and follow-up who otherwise would have remained undetected.

## CONCLUSION

Laboratory diagnosis has undergone drastic changes in recent years with the introduction of newer technologies. The new generation tests, though very sensitive, specific, and accurate, are not cost-effective in a resource-poor setting. Older conventional assays still remain valid and are being used. Direct microscopy is limited by the low sensitivity. Pap smear is a simple screening

test for cervical cancer. It is being routinely done in all the secondary and tertiary care centers. Our study shows that Pap smear is useful in diagnosing STIs, especially asymptomatic HPV infection and trichomoniasis. Early detection and treatment of these asymptomatic infections can prevent the spread of STIs in the community. By detecting asymptomatic cervical HPV infection and LSIL, patients at risk for carcinoma of the cervix can be identified and followed up. In secondary and tertiary care settings, especially in the government sector, where the facilities for sophisticated laboratory tests are limited, Pap smear, when used along with direct microscopy and other conventional tests, can be of help in identifying asymptomatic infections at no extra cost.

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### Conflicts of interest

There are no conflicts of interest.

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