



EFFICACY OF DIAGNOSIS OF HERPES SIMPLEX VIRUS INFECTION IN PAP SMEAR - A CASE SERIES

Obstetrics & Gynaecology

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ABSTRACT

Background: Cervical infections are a common problem in women of reproductive age group. Pap smear, a test used for screening cervical cancer, is a quick test which can be also used for diagnosing cervico-vaginal infections. However, diagnosis of HSV infection using PAP smear is scarcely reported in the literature. **Methods:** A retrospective data of 1300 subjects of pap smears in our institution, which is a tertiary care hospital in Bilaspur. Samples obtained were stained with Papanicolaou stain (Pap) and examined for HSV infection. **Results:** Among all studied smears, HSV infection was detected in 10 women, age ranging from 32-60 yrs. The studied cases depicted cytomorphological findings of multinucleation, margination and moulding. **Conclusion:** Cervical smear is one of the most simple and quick tests that is beneficial in diagnosing cervical infections. Early and accurate diagnosis of HSV infection is crucial for women of childbearing age, to prevent complications like neonatal herpes and to avoid unnecessary treatments for cervical cancer.

KEYWORDS

INTRODUCTION

Sexually transmitted infections (STIs) are a major public health problem in developing countries, and timely and appropriate treatment is necessary to reduce transmission and improve sexual and reproductive health.⁽¹⁾ HSV is an extremely common STI responsible for approximately 500 million genital infections and billions more oral infections.⁽²⁾ Most reported cases involve the age groups between 16-40 years, which coincides with an increase in sexual activity among this age group.⁽³⁾ At the level of the individual patient, genital herpes is associated with significant psychosocial morbidity and complications such as neonatal herpes, the result of transmission of HSV from mother to baby.⁽⁴⁾ Therefore health literacy is very important for prevention, early diagnosis and treatment of STI.⁽⁵⁾

It has been already proven that human papillomavirus (HPV) is clearly linked to cervical cancer and a hypothesis that HSV may facilitate carcinogenesis in HPV-infected cervical cells, is still under discussion.⁽⁶⁾ Evidence supporting a role for HSV in the pathogenesis of cervical cancer indicates a likely 'hit and run' mechanism, in which HSV infection is presumed to initiate the oncogenic process but not involved in the later steps.⁽²⁾

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 like Herpes Simplex Viral infection.⁽¹⁾ The purpose of this study is to assess the efficacy of PAP smear examination in conclusive diagnosis of Herpes Simplex Virus Infection.

MATERIALS AND METHODS

A retrospective analysis was performed of cases showing genital herpes simplex virus infection on PAP smear over the past 2 years (July 2023- August 2025). A total of 10 cases were found from the total 1300 pap smears received. PAP smear slides stained by the Papanicolaou method¹ were retrieved and reviewed. Clinical history and physical examination findings were recorded from the cytopathology registers of the Pathology Department. The initial diagnosis was based on the presumptive clinical diagnosis. The final diagnosis was made after incorporating the PAP smear report. Diagnostic cytopathological findings suggestive of HSV infection were noted in every individual case. Associated cytological finding were graded as Negative for Intraepithelial Lesion or Malignancy (NILM), Atypical Squamous Cells – Undetermined Significance (ASC-US), Low grade Squamous Intraepithelial Lesion (LSIL), High grade Squamous Intraepithelial Lesion (HSIL) or Carcinoma as applicable as per Bethesda System.^{7,8}

OBSERVATION

Five women, who presented to the Obstetrics and Gynaecology OPD, with complaints ranging from abdominal pain, itching and erosions in genitals to bleeding per vagina and were diagnosed HSV infection positive, by PAP smear method were studied (Table 2). The age ranged from 32 to 60. Majority (40%) belonged to 40-50 years (Table 1). All were married. Among the HSV infection diagnosed cases, most patients (80%) presented with complaints of lower abdominal pain (Table 2). The specific findings of an HSV infection in PAP smear like moulding and margination was found in 100% cases, while multinucleation, intranuclear grooving and intranuclear inclusion was seen in 60-80% cases (Table 3). Pap smear findings correlated with the clinical diagnosis in 40% cases.

Table 1: Case Series Of HSV Infection Based On Pap Smear Findings

Case	Age	Gender	Testing site	Clinical symptoms	Genital examination findings	Provisional diagnosis
1.	32	Female	Cervix	Pain in the lower abdomen	cervical erosions present	Non specific Cervicitis
2.	36	Female	Cervix	Pain in the lower abdomen	cervical erosions present, genital vesicles present	Herpes Genitalis
3.	40	Female	Cervix	Pain and itching in vulval region	Bulky cervix, cervical erosions present	Herpes Genitalis
4.	42	Female	Cervix	Menorrhagia and pain in the lower abdomen	Bulky, irregular cervix, cervical erosions present	Abnormal Uterine Bleeding
5.	42	Female	Cervix	Pain in the lower abdomen	cervical erosions present, genital vesicles	Herpes Genitalis

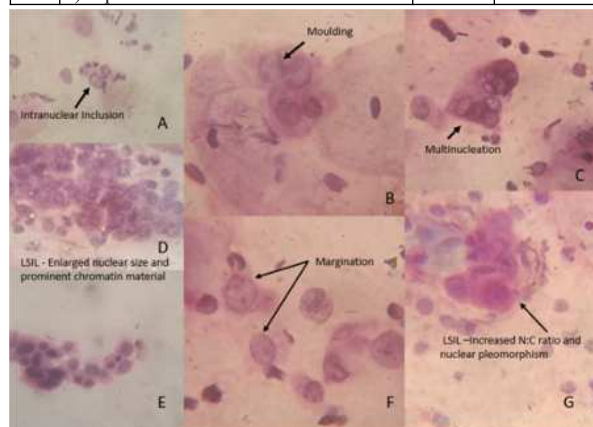
					and ulcers present	
6.	48	Female	Cervix	Pain in the lower abdomen and postmenopausal bleeding	Bulky cervix	Abnormal Uterine Bleeding
7.	55	Female	Cervix	Pain in the lower abdomen and menorrhagia	Bulky, irregular cervix	Abnormal Uterine Bleeding suspected Endometrial Adenocarcinoma
8.	56	Female	Cervix	Pain in the lower abdomen	cervical erosions present	Non specific Cervicitis
9.	60	Female	Cervix	Pain in the lower abdomen and postmenopausal bleeding	Normal	Abnormal Uterine Bleeding
10.	60	Female	Cervix	Pain and itching in vulval region	Bulky cervix, cervical erosions present	Herpes Genitalis

Table 2: Clinical Presentation

Sl. No.	Complaints	No. Of Cases	% Of Cases
1.	Discharge per vagina	0	0%
2.	Itching in genital area	2	20%
3.	Abdominal pain	8	80%
4.	Bleeding per vagina	4	40%
5.	Vesicles and pustules around genitals	2	20%
6.	Inguinal lymphadenopathy	0	0%
7.	Fever/ Flu	0	0%

Table 3: Microscopic Findings

Sl. No.	MICROSCOPIC FEATURES	NO. OF CASES	% OF CASES
1.	Moulding	10	100%
2.	Margination	10	100%
3.	Multinucleation	8	80%
4.	Intranuclear Inclusion	6	60%
5.	Intranuclear Grooving	8	80%
6.	Background Inflammation chronic acute-on-chronic	8 2	80% 20%
7.	Other associated infection (cocci/bacilli – Bacterial Vaginosis)	2	20%
8.	Cervical cytomorphological changes: 1) NILM including squamous metaplasia 2) ASCUS 3) LSIL 4) HSIL 5) Squamous cell carcinoma	8 1 1 0 0	80% 10% 10% 0% 0%



PAP smear photomicrograph of cases showing: (A) Intranuclear

inclusion; (B) Moulding; (C) Multinucleation; (D,E) Enlarged nuclear size and prominent chromatin material; (F) Margination; (G) Increased N:C ratio and nuclear pleomorphism.

DISCUSSION

Age Distribution:

According to Jaishankar D et al, most reported cases involve the age groups between 16-40 years, which coincides with an increase in sexual activity among this age group.^(3,9) According to Srikant S et al, majority of the affected belonged to the 20-39 age group.⁽¹⁰⁾ The seroprevalence of HSV-1 and HSV-2 showed increasing trend with age which reflects the higher cumulative exposure risk with age, as expected based on global data.⁽¹¹⁾ In the study conducted by Thappa D et al, there was a wide age range affecting females of both extremes of age (mean age = 32.38 years) with the maximum number of patients in the age group of 21- 30 years.⁽¹²⁾ In contrast, our study was representing an elder population, may be because screening for cervical cancer and other pathology using pap smear is done usually after 30 years of age or because the clinical symptoms for genital herpes appear at a later stage of life.

Clinical Presentation

The classical clinical presentation of the first episode of symptoms of primary genital HSV infection is characterized by bilateral clusters of erythematous papules, vesicles or ulcerations on the external genitalia, in the perianal region or on the buttocks, occurring 4–7 days after sexual exposure.⁽¹³⁾ According to Jaishankar D et al, primary and recurrent genital herpes infections most commonly result in lesions and inflammation around the genital area. In women, the sites of infection are mainly the vulva and the vagina, with some cases involving cervical and perianal regions.⁽³⁾ Most people infected with HSV are asymptomatic or have very mild symptoms like fever, headache, body aches or swollen lymph nodes which neither patient nor clinician can correlate with viral infection. Specific symptoms like fluid filled blisters in the genital region may be present while nonspecific symptoms such as pain, itching, irritation, and excoriations of genital region, urethritis, cervicitis etc. may cause delay in diagnosis. Sometimes it can present with a completely unrelated sign like aseptic meningitis.^(14,15,16,17) After infecting the surface, the virus travels up the nearest nerve to the ganglion away from the reach of immune system and remains there which explains why the infection can stay dormant for so long with few or no symptoms, and then have a recurrent outbreak with noticeable symptoms several years later.^(18,19) In our study, 20% cases presented with genital ulcers and 20% with vaginal pain and itching which correlates with the findings of other studies,^(15,17) while majority of patients (80%), in contrast to other studies presented with lower abdominal pain as their major complaint.

HSV And Cervical Cancer

The possible association of HSV and cervical dysplasia has been hypothesized for decades and facilitation of carcinogenesis by HSV in HPV-infected (HPV 16 and HPV 18) cervical cells have been extensively studied.⁽⁵⁾ The carcinogenic potential of HSV has been conclusively established by detection of HSV-2 RNA in cytologically abnormal cervical biopsies and a DNA fragment in a cervical cancer specimen.⁽²⁾

Multiple studies done by McDougall et al²⁰, Li et al²¹ and Bahena-Roman et al²² have found an association between HSV and cervical cancer and it was concluded that HSV-2 may be involved in early stages of cell transformation but is not required for future progression to squamous cell carcinoma. This is consistent with the 'hit and run' hypothesis proposed by G. R. B Skinner in 1976, in which HSV-2 plays a role in initial cell transformation only.⁽²⁾

Koanga et al², assessed the frequency of inflammation and cervical abnormalities in HSV-2 positive and negative patients which concluded that HSV-2 may induce cervical inflammation, which could act as a co-factor in cervical cancer formation. This is similar to our study in the fact that all our cases (10/10 cases) had features of inflammation, among which two had atypical features. Furthermore, it is entirely possible that patient factors may influence the relationship between HSV and cervical cancer in some patients.⁽²⁾

Papanicolaou smear (Pap smear) is gaining its popularity as the new generation tests are not cost-effective in resource-poor settings.⁽¹⁾ Coleman DV⁽²³⁾ and Naib et al⁽²⁴⁾ extensively documented the herpes

cytopathic effect which shows the “3 M”s – multinucleation, moulding, and margination of chromatin. Nuclei have a “ground-glass” appearance due to intranuclear viral particles and enhancement of the nuclear envelope caused by peripheral margination of chromatin, found in 100% of our cases (10/10 cases), similar to the study by Coleman D V.⁽²³⁾ Dense eosinophilic intranuclear (Cowdry) inclusions surrounded by a halo or clear zone were variably present, 60% cases (6/10 cases) similar to the study conducted by Naib et al.⁽²⁴⁾ Large multinucleated epithelial cells with moulded nuclei are characteristic but may not always be present.⁷ In our study 100% cases (10/10 cases) had moulding and 80% cases (8/10 cases) had multinucleation similar to findings of both Naib et al and Coleman D V. Naib and his colleagues (1969) also drew attention to the fact that, of 245 women they studied whose cervical smears contained the multinucleate giant cell typical of genital herpes infection, 58 (23.7%) were found to have coexisting neoplastic changes in the cervical epithelium, which supports our findings of 2/10 cases having coexisting atypical features (ASCUS- 1 case, LSIL-I case).⁽²⁴⁾

CONCLUSION

Patients with HSV-2 seropositivity and having symptoms of genital herpes may be missed during clinical and/or cytological (Pap smear) examination and such patients may become reservoirs for transmission. Therefore, clinicians and patients must be aware of the subclinical presentation of genital herpes and the potential these patients have for transmitting HSV.⁽²⁵⁾ The underdiagnosis of genital herpes seems largely due to misinterpretation of atypical genital and extragenital lesions.⁽²⁶⁾

As genital HSV infection has substantial public health implications, increased awareness of the disease and its prevalence, together with better use of diagnostic tests, may be one step towards more effective management and infection control.⁽⁴⁾ Our study shows that Pap smear is useful in diagnosing STIs like Herpes Simplex Virus infection. Early detection and treatment of these asymptomatic infections can prevent the spread of STIs in the community. 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.⁽¹⁾

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