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Original Article

Pattern of Uterine Cervical Smears using Modified Hematoxylin and Eosin Stain in Pregnant Women Attending STD Clinic at a Tertiary Teaching Institute

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Abstract

Two hundred and sixty pregnant women both married and unmarried attending STD clinic at IOV, Madras Medical College with or without genitourinary symptoms were screened for STIs and underwent uterine cervical cytology stained by modified H and E stain. Inflammatory (acute and chronic) changes were noted in majority (75%) with or without clinical cervicitis followed by neoplastic changes in significant numbers, especially in HSV and HPV infections. We advise early intervention by repeat cytology and regular follow-up for those with abnormal smears (chronic inflammatory and intraepithelial neoplastic) to prevent reproductive morbidity and mortality. Only if a repeat cytology is abnormal, postpartum colposcopy and colposcopically directed biopsies will be highly useful to detect severe dysplasia and early carcinomatous changes.

Key words: Pregnancy, Cervical smear cytology, Sexually transmitted diseases

INTRODUCTION

Sexually transmitted diseases (STDs) affecting mankind since antiquity are an important cause of morbidity and mortality worldwide, especially in woman and children, particularly in resource poor settings of developing world, which are store houses of most of the pathogens causing STDs.[1-3]

Infections in pregnancy are common but few cause fetal infection and damage.[4] Pregnancy is a vulnerable time of women.[5] Hence, STDs in the pregnant women are more serious than in the non-pregnant women.[6] Physiological changes, anatomical changes in genital tract, and immunological alterations (alterations in host defense mechanisms) in a pregnant female have been postulated to influence the course of STDs which pose a special risk of infection for both mother and fetus.[7,8]

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Diagnosing sexually transmitted infections (STIs) in pregnancy can be difficult because most are either asymptomatic or too subtle to recognize clinically as pregnancy modifies the manifestations. The cause for screening and treating for asymptomatic genital infectious disease may be stronger during pregnancy than at other times. Adequate treatment must automatically follow as screening is instituted. Study of the prevalence of pattern of cervical smears with or without STDs is important to know to devise appropriate control measures.[9] Antenatal attendees due to their initial entry and later regular reviews in a reproductive health care settings form an important section of population and are commonly used as a reference point for STD prevalence / early screening for cervical carcinoma in the general population of women.[10]

Cervical Screening for Genital Infections in Pregnancy

In recent decades, screening for genital infections has been an integral part of antenatal care.[11] Wet-fixed

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Papanicolaou-stained preparations are traditional techniques for gynecologic cytology. The components of Papanicolaou stain are hematoxylin (nuclear stain), orange G, and eosin (cytoplasmic stains). Hematoxylin and eosin (H and E) stain is occasionally used on wet-fixed material as a rapid stain, but lacks of advantages of Papanicolaou stain for differential staining of cytoplasmic elements.[12] The squamocolumnar junction from where the cervical smear should be taken is at the external os and therefore easily sampled in young and pregnant women.[13,14]

A cervical smear which is taken properly should include squamous epithelial cells, a few neutrophil polymorphs, histiocytes, endocervical or squamous metaplastic cells, and mucin.[13] The smear during early stages shows gradually increasing numbers of intermediate cells and may show cytolysis. Large numbers of navicular cells (intermediate cells that accumulate intracytoplasmic glycogen with thick cells membranes) are often noted.[13,15]

Degenerative changes of epithelial cells are common in a cervical smear taken during acute phase of cervicitis.[13,16] The mere presence of a few neutrophil polymorphs in a smear does not signify inflammation. Large numbers of neutrophils are associated often with an inflammatory process. They may be not be associated with a specific organism (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, herpes simplex virus (HSV), HPV, *Trichomonas vaginalis*, *Candida* spp., etc.,).[13,16,17] Inflammatory smears are also reported to be associated with bacterial vaginosis (BV) although rare.[17] A bacterial background may be present in association with inflammatory cellular changes, but specific bacteria cannot be reliably identified by cytology alone.[13] Cervical inflammation caused by STI is suspected to promote cervical intraepithelial neoplasia (CIN).[15,18] In chronic cervicitis, inflammatory infiltrate is composed of lymphocytes, plasma cells, and histiocytes. In the presence of cervicitis, the cervix may have red, eroded (cervical erosion') area around the external cervical os.[16] About 40–80% of women with an inflammatory smear have an infection that needs to be treated. Inflammation can accompany CIN.[15]

Candida infection is a common variety seen in cervical smears. It is seen in the form of pink staining pseudohyphae and spores.[13,15] The epithelial cells in the background usually exhibit severe inflammatory changes. Pap smear is unreliable as a diagnostic modality, being positive in only about 25% of culture-positive symptomatic vulvovaginal candidiasis.[7]

Trichomonads are sometimes difficult to identify in smears, since the organisms appear as small ill-defined blobs in the background and they are rounded, oval or pear shaped with the "Mongol eye" nucleus and red cytoplasmic granules.[13] The smear also shows degenerative epithelial changes, leukocytic infiltrate, and an acidophilic reaction in the presence of trichomonads, staining pink irrespective of their maturity.[13,16] Minor epithelial atypia may be associated with trichomoniasis.[16] Using stained cervical smear preparation to diagnose *T. vaginalis* infection is time consuming and cumbersome as compared with vaginal stained smears/wet mounts. The false-negative rate is 30% with Papanicolaou stain.[19,20] *Gardnerella* infection is represented in cervical smear by the clue cell.[13] Gynecologically, BV has been associated with CIN.[21]

HSV type II is believed to be an initiator in the development of carcinoma cervix. In the early stages of infection, only severely inflamed endocervical cells are present. Later, the typical multinucleated giant epithelial cells with red intranuclear inclusions in their molded nuclei are frequently seen.[13,22] HPV is believed to be a promoter of the development of CIN and carcinoma cervix. The initial cytopathic changes in HPV infections are in the form of mild nuclear atypia and koilocytosis. Koilocyte is the most characteristic feature of HPV infection which is a squamous cell with three essential features, namely, abnormal nucleus, a large perinuclear halo or clear space, and a thickened uneven rim of dense cytoplasm.[13]

The precancerous changes are graded histologically as CIN Grades I, II, and III. Each of these grades is reflected by the type of abnormality seen on the cervical smear – mild, moderate, or severe dysplasia.[13] About 90% of the dysplastic lesions fall into the mild and moderate grades of CIN and the rest 10% fall into severe grades of CIN (carcinoma *in situ*).[16] CIN-I lesions are associated with low- and high-risk HPVs, whereas high grades of CIN are more strongly linked to high-risk HPV types.[16,23]

A pregnant woman should have a repeat smear taking after the birth of her baby. Those with high grades of dysplasia should have early surgical intervention after delivery and with few dysplastic changes on the background of chronic inflammation should have uterine cervical cytology with colposcopy-directed cervical punch biopsy at regular interval as a part of regular follow-up.[24,25]

Aims and Objectives

The objectives of the study were as follows:

- To determine the pattern of cervical smears (inflammatory vs. neoplastic) among the pregnant women attending the STD clinic at the institute of STDs.
- To study the characteristics of smears in relation to specific STIs among them.

MATERIALS AND METHODS

It is a cross-sectional study to find out the prevalence of pattern of cervical smears among pregnant women with or without STDs attending the STD clinic at Institute of Venereology, Madras Medical College and Research Institute, Chennai, during the study period of May 1998–July 1999. A total of 260 consecutive pregnant women at various trimesters who attended the STD clinic at the Institute of Venereology, Madras Medical College and Research Institute were included in the study. Majority of the unmarried pregnant women were referred to be screened for STIs including HIV infection, before medical termination of pregnancy. Many attended on their own due to symptoms. A well-structured, pre-tested pro forma was prepared and used for the study. It consists of various information including their age, socioeconomic status marital status, sexual history, and obstetric history apart from detailed clinic history. Pregnant women were screened for STDs including cervical smears and a provisional diagnosis was made. All other investigations were done accordingly to confirm the diagnosis.

RESULTS

In this study, 260 pregnant women were examined and the following results were obtained.

Age Distribution of Pregnant Women in the Study Group

Age group in years	Total no.	Percentage
15–19	48	18.5
20–24	144	55.4
25–29	50	19.2
30–34	16	6.2
35–39	2	0.8

Majority of pregnant women were in the group of 20–24 (55.4).

Socioeconomic Status of Pregnant Women

Monthly income (in rupees)	Total no.	Percentage
Up to 500	116	44.6
500–1000	74	28.5
1000–1500	32	12.3
1500–2000	18	6.9
Above 2000	20	7.7

Majority of pregnant belonged to lower socioeconomic strata.

Marital Status of the Pregnant Women in the Study Group

Marital status	Total no.	Percentage
Married	200	76.9
Single (unmarried)	60	23.1

Majority of the pregnant women were $n = 200$, 76.9%. In women, who were single ($n = 60$, 23.1%), majority were deserted by their known partner after knowing about the present pregnancy ($n = 50$, 83.3%), others forming the group were kept mistresses ($n = 6$, 10%), and commercial sex workers ($n = 4$, 6.6%).

Presenting Complaints of Pregnant Women

Complaints	Total no.	Percentage
Checkup	124	47.7
Genital discharge	72	27.7
Genital sore	34	13.1
Itching in genitalia	30	11.5
Growth in genitalia	20	7.7
Burning micturition	12	4.6
Lower abdominal pain	12	4.6
Fever	6	2.3
Frequency of micturition	4	1.5
Swelling in genitalia	4	1.5
Loss of weight	4	1.5
Cough	4	1.5
Loose stools	4	1.5
Loss of appetite	2	0.8
Dysuria	2	0.8

Majority of the pregnant women had visited the STD clinic for checkup ($n = 62$, 47.7%). Genital discharge and genital sore were the most common presenting complaints in 72 (27.7%) and 34 (13.1%) patients, respectively followed by itching in genitalia in 30 (11.5%) and growth in genitalia in 20 (7.7%) patients. Some pregnant women also had other symptoms such as burning micturition ($n = 12$,4.6%), lower abdominal pain ($n = 12$, 4.6%), fever ($n = 6$, 2.3%), cough ($n = 4$, 1.5%), low stools ($n = 4$, 1.5%), loss of weight ($n = 4$, 1.5%), frequency of micturition ($n = 4$, 1.5%), swelling of genitalia ($n = 4$, 1.5%), loss of appetite ($n = 2$, 0.8%), and dysuria ($n = 2$, 0.8%).

Premarital/extramarital Contacts among Married Pregnant Women ($n = 200$)

When married pregnant women were interviewed for this study, around 4% gave a history of (H/O) extramarital contact ($n = 8$) and 2% gave H/O premarital contact ($n = 4$). One woman (0.5%) admitted having both pre- and extra-marital contacts.

Number of Partner(S) among Unmarried/Single Pregnant Women ($n = 60$)

Group	Total no.	No. of partners	Percentage
Deserted	50	Single	83.3
Kept mistress	6	Single	10.0
Commercial sex workers	4	Multiple	6.7

Among unmarried pregnant, 25 women who were otherwise single gave H/O contact with a known

partner (83.3%). Four women (6.7%) who were commercial sex workers gave H/O contacts with multiple males. Six women (10%) who were kept mistress for a known partner denied H/O exposure outside.

Past History of STDs

Past history of STDs	Total no.	Percentage
Present	22	8.5
Absent	238	91.5

STDs: Sexually transmitted diseases

Distribution of Previous STDs (*n* = 22)

Disease	Total no.	Percentage
Genital ulcer	14	63.6
Genital discharge	6	27.3
Genital warts	2	9.1

STDs: Sexually transmitted diseases

BOH among the Pregnant Women (*n* = 68)

BOH	Total no.	Percentage
Spontaneous abortion	40	58.8
Still birth	16	23.5
Neonatal death	6	8.8
Spontaneous abortion and still births	6	8.8

Clinical Signs in the Study Group of Pregnant Women (*n* = 260)

Clinical sign	Total no.	Percentage
Cervical erosion	152	29.2
Soddening of vulva	100	19.2
Genital ulcer	72	13.8
Genital wart	40	7.7
Bilateral inguinal lymphadenopathy	28	5.4
Condylomata lata	16	3.1
Skin rash	12	2.3
Excoriations of vulva	8	1.5
Intertrigo groin	8	1.5
Molluscum contagiosum	4	0.8
Scabies	4	0.8
Tinea cruris	4	0.8

Cervical erosion was the most common clinical sign seen followed by soddening of the vulva.

Nature of the Genital Discharge among the Study Group (*n* = 260)

Nature of the discharge	Total no.	Percentage
Mucopurulent	104	40
Mucoid	96	36.9
Curdy white	52	20
Frothy	6	2.3
Serosanguinous	2	0.8

Majority of the pregnant women had mucopurulent discharge.

Results of Investigations in the Study Group (*n* = 260)

Endocervical and urethral smear gonococcus (Gram stain), and endocervical culture for gonococcus were negative in all pregnant women.

Investigations	Positive results (total no.)	Percentage
Culture of *Candida* spp. in SDA medium	124	47.7
Gram stain for candida	78	30
KOH mount for candida	56	21.5
Wet film for *Trichomonas vaginalis*	28	10.8
Gram stain for clue cells	6	2.3
Urine culture (routine)	6	2.3

VDRL Reactivity/TPHA

Serological test	Patients with reactive VDRL/ TPHA	Percentage
VDRL	66	25.4

Prevalence of HBs Ag

Serological test	Patients positive for HBs Ag	Percentage
HBs Ag	46	17.7

Prevalence of HIV Infection

Serological test	Total no. of patients positive for HIV antibodies	Percentage
HIV	16	6.2

Distribution of Infections among Pregnant Women

Infections	Total no.	Percentage
Vulvovaginal candidiasis	124	47.7
Syphilis	66	25.4
Hepatitis-B	46	17.7
Trichomoniasis	28	10.8
Genital herpes	26	10
Chlamydia cervicitis	21	8.1
Genital warts	20	7.7
HIV	16	6.2
Non-gonococcal urethritis	12	4.6
Bacterial vaginosis	6	2.3
Non-specific genital ulcer	4	1.5
Chancroid	2	0.8
Molluscum contagiosum	2	0.8
Scabies	2	0.8

Out of 66 patients with syphilis, 2 had primary syphilis (3%), 10 had secondary syphilis (15.2%), and 54 had yearly latent syphilis (81.8%).

Total Number of Cervical Smears Examined = 260

Cytological findings	Total no.	Percentage
Normal smear	60	23.1
Neutrophilic (acute) inflammatory smear with *Candida*	50	19.2
Neutrophilic inflammatory smear with microorganisms in the background	46	17.7
Neutrophilic inflammatory smear with Grade 1 dysplasia	22	8.5
Neutrophilic inflammatory smear with degenerative epithelial cell changes	16	6.2
Neutrophilic inflammatory smear with Grade 1 dysplasia with *Candida*	14	5.2
Neutrophilic inflammatory smear with degenerative epithelial cells+*Candida*	6	2.3
Neutrophilic inflammatory smear with intranuclear inclusion bodies and giant epithelial cell with polynucleation	6	2.3
Neutrophilic inflammatory smear with degenerative epithelial cell changes with Grade 1 dysplasia	4	1.5
Neutrophilic inflammatory smear+Grade 1 dysplasia+koilocytosis	4	1.5
Neutrophilic inflammatory smear+Grade 1 dysplasia+*Candida*+koilocytes	4	1.5
Neutrophilic inflammatory smear with trichomonads	4	1.5
Neutrophilic inflammatory smear with trichomonads and *Candida*	4	1.5
Mixed inflammatory smear with Grade 1 dysplasia	4	1.5
Clue cell alone	4	1.5
Clue cells with Grade I dysplasia	2	0.8
Chronic inflammatory smear with microorganisms in the background	2	0.8
Chronic inflammatory smear with Grade I dysplasia with koilocytes	2	0.8
Chronic inflammatory smear with Grade II dysplasia	2	0.8
Mixed inflammatory smear with microorganisms in the background	2	0.8
Neutrophilic inflammatory smear with herpetic nuclear inclusion bodies and *Candida*	2	0.8

DISCUSSION

Majority of the pregnant women had visited the STD outpatient department for checkup. Most of them were referred cases. A good number of pregnant women presented with various genitourinary symptom on their own along with their partners. This increase in number of self-referral by these women could be as a result of campaigns conducted for generating awareness about STDs including HIV infection.

Nearly half of the pregnant women reported only for checkup. However on clinical examination and completion of investigations, it was found that 230 (88.5%) pregnant women were found to be suffering from one or other infections. This shows that majority of the pregnant women were asymptomatic. Genital infections often remain unnoticed during pregnancy as their signs and symptoms may be seen as part of the normal discomfort of pregnancy. The asymptomatic nature of infections in females and their frequent consultation in gynecological clinics instead of STD clinics may have been responsible for a low prevalence of STD reported in women.[26] Genital discharge, genital sore, and itching in genitalia were the most common symptoms noticed among the study group. Genital ulcer was the most common among the previous STDS noted. We also noted the increased rate of recurrence of genital herpes during pregnancy, similar to the observers.[19,27] In the study group, cervical erosion, soddening of vulva, genital ulcer, and genital warts were the important clinical signs noted. Erosion of cervix was seen in nearly one-third of pregnant women. Erosion or cervical ectopy enhances the entry of a variety of pathogenic agents.

In this study, mucopurulent vaginal discharge was commonly seen. Vaginal discharge apart from vaginal infection may also be caused by mucopurulent cervicitis, so it is essential to examine cervix of all patients with vulvovaginal complaints using speculum. Patients with *T. vaginalis* vaginitis (n = 14) had predominantly mucopurulent discharge and the typical frothy discharge of trichomoniasis was seen only in three. The usual description of discharge in vulvovaginal candidiasis is curdy white and adherent. Only 20% of the pregnant women had the above said clinical sign. Majority of these patients had no symptoms while the rest had itching in genitalia. The clinical presentation is similar in both HIV-infected and seronegative pregnant women although severity of symptoms was noted in the former.

Three patients (2.3%) had BV and their genital discharge was mucoid in nature. Prevalence of BV in our study was low compared to an American study which quotes prevalence of 15–20%. Serosanguineous discharge was noted in a pregnant woman with extensive genital herpes. Culture from the endocervical canal for gonococcus was negative in all the study subjects. Non-gonococcal urethritis was seen in twelve pregnant women (*Escherichia coli* was the most common isolate).

The prevalence of genital herpes in the study population was 10%. Most of these cases were due to recurrences of HIV infection, especially in the third trimester. Low prevalence of genital herpes is comparable with other studies.[28] The prevalence of genital warts in the group of pregnant women studied was 7.7%. This was almost comparable to the existing data available.[29] Extensive huge, anogenital warts were noted in the HIV-positive pregnant women. Only one in the study had previous the history of condyloma acuminata.

Prevalence of trichomoniasis in the study population was 10.8%. This corroborates with the reported prevalence data.[2,7,30] About 50% of the pregnant women had genital candidiasis.

Among the cervical smears of pregnant women studied (n = 260), majority of them were inflammatory in nature (n = 194, 76.4%). Dysplastic changes could be noticed in 58 (22.3%) and degenerative epithelial cell changes could be found in 26 (10%) smears. Features of acute inflammation were seen in 182 (93.8%) smears [Figure 1] and chronic inflammation in 6 (3.1%). Both features were found in 6 smears (3.1%). Among the inflammatory smears (n = 194), *Candida* spores/pseudohyphae were seen in 80(41.2%), trichomonads in 8(4.1%), herpetic intranuclear inclusion bodies and multinucleated giant epithelial cells in 8 (4.1%), and koilocytes in 10 (5.2%) smears [Figure 2]. Clue cells were seen in 6 cervical smears (2.3%). Out of these, one smear showed Grade 1 dysplasia (33.3%). Those showing dysplastic features, Grade 1 dysplasia [Figure 3] was noted in 54 (27.8%) and Grade 2 dysplasia was found in 2 smears (1%). Acute inflammatory and chronic inflammatory pictures were noted in this study. Grades 1 and 2 dysplastic changes were noticed in chronic HSV/HPV infections [Figure 4]. Severe dysplastic changes in few areas of the smear were also noticed. Normal smear was also observed in significant numbers in the study group. Hence, we suggest that colposcopic-directed cervical biopsy can be used to further investigate and follow-up in the postpartum period due to high persistent rate of CIN.

Cytologic Findings in Patients with HPV Infection

The cervical smears (H and E stain) showed koilocytosis (n = 10), inflammatory changes (acute in 12, chronic in 4, and both in 4), and degenerative epithelial cell changes (n = 4). Dysplastic changes were noted in 12 smears (Grade 1 in 10 and Grade 2 in two).

Cytologic Findings in Patients with HSV Infection

The cervical smears (H and E stain) showed multinucleated giant epithelial cells and intranuclear bodies (n = 8), inflammatory changes mainly acute in majority. Degenerative epithelial changes were noted in four and dysplastic changes in 10 patients.

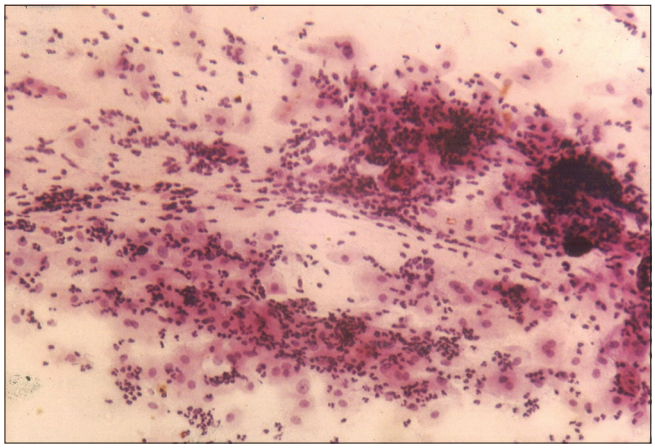

Figure 1: Cervical smear (H and E stain) showing sheets of polymorphs indicative of acute inflammation in a woman with candidiasis and genital herpes

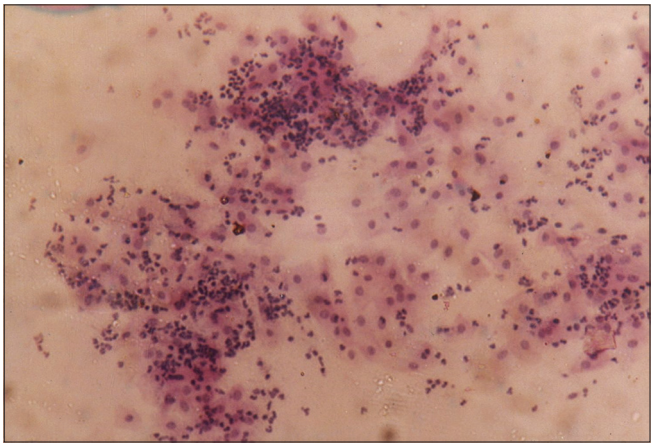

Figure 3: Cervical smear (H and E stain) showing Grade 1 dysplasia in low magnification in a pregnant woman with genital warts

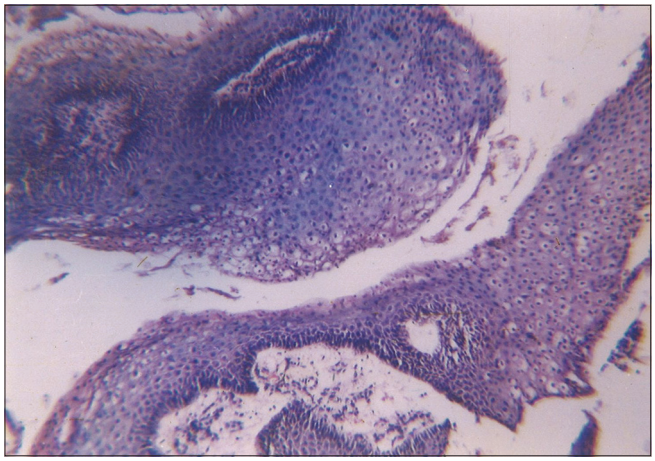

Figure 2: Koilocytosis in HPE of genital warts

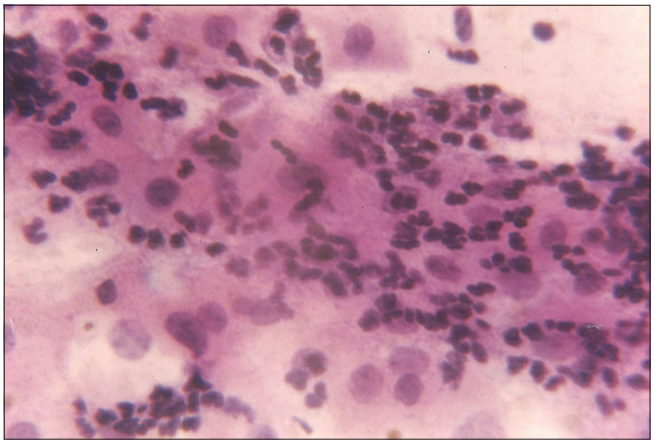

Figure 4: Cervical smear (H and E stain) with dysplastic changes

Cytologic Findings in Patients with Bacterial Vaginosis

The cervical smears (Hand E stain) showed clue cells and did not show any inflammatory changes. Two patients had features of Grade 1 dysplasia.

Cytologic Findings in Patients with Trichomoniasis

The cervical smears (Hand E stain) showed mainly inflammatory cells consisting of polymorphs. Dysplastic changes were noted in four study subjects.

Cytologic Findings in Patients with Candidiasis

The cervical smears (Hand E stain) showed mainly inflammatory cells consisting of polymorphs. Degenerative changes were noted in three study patients.

Cytologic Findings in Patients with Syphilis

Majority of the smears (Hand E stain) showed acute inflammatory changes. Two patients who had coexisting HIV infection had a chronic inflammatory picture. Six patients showed features of mild dysplasia.

Cytologic Findings in Patients with Chancroid

Majority of the smears (Hand E stain) showed acute inflammatory changes.

CONCLUSION

The large numbers of pregnant women with an STD provide an important opportunity to reduce the reservoir of infection in the broader community. Hence, systematic screening of STIs which may include cervical smears to detect early neoplastic changes in pregnancies combined with adequate treatment and follow-up (colposcopic-directed cervical biopsy later on) will reduce the risk of adverse consequences. Appropriate antenatal, intrapartum, and postnatal care should be provided for those with chronic inflammatory and early carcinomatous changes in cervical study. Chronic viral infections such as warts and herpes should be actively managed with frequent clinical follow-up for warning signals as they often predispose to cervical cancer. Chronic cervicitis cases should have cytology at constant intervals to detect early atypia. Colposcopically directed biopsy which is one of the safe and reliable methods can be employed to further evaluate those with abnormal cervical cytology.

To conclude, there is an urgent need to mount effective, rational, and feasible intervention programs to combat the raising prevalence of carcinoma cervix in Indian women with early screening programs in antenatal mothers (first regular visits to any medical care facilities) so as to enroll them for regular care and follow-up, making optimum use of the existing health and social welfare services.

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