CERVICAL CYTOLOGICAL CHANGES IN HIV INFECTED PATIENTS ON HAART ATTENDING CARE AND TREATMENT CLINIC AT MUHIMBILI NATIONAL HOSPITAL

BY

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CERTICICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled: *Cervical Cytological Changes in HIV Infected Patients on HAART Attending Care and Treatment Clinic at Muhimbili National Hospital,* in partial fulfillment of the requirements for the degree of Masters of Medicine in Anatomical Pathology of the Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, Liset Maria Menéndez Torres, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature..... Date...22nd Nov, 2007

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DEDICATIONS

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ABSTRACT

Background: Cervical cancer is one of the most common malignancies in women worldwide and the commonest female cancer in Sub-Sahara Africa. Human Papilloma Virus (HPV) is the primary sexually transmitted etiologic agent involved in the development of cervical carcinoma and its precursor lesions. Human Immunodeficiency Virus (HIV) infected patients present with persistent HPV infection which may alters the natural history of HPV and initiate squamous intraepithelial lesions (SIL). HIV associated cervical lesions are multicentric, aggressive and tend to recur after treatment. However, the introduction of highly active antiretroviral therapy (HAART) may reduce of HSIL and carcinoma in HIV infected individuals, but this needs confirmation.

Objectives: To determine cervical cytological changes, age distribution and risk factors in HIV infected patients with cervical lesions attending care and treatment clinic (CTC) at Muhimbili National Hospital (MNH) from 2005-2006.

Study design: An analytical cross-sectional study

Material and Method: This study included 120 HIV infected patients attending the CTC at MNH and 50 HIV negative controls recruited from Cervical Cancer Screening Unit (CCSU) at Ocean Road Cancer Institute (ORCI). Serological results of all patients were sought from the clinical files in the medical record department of MNH and ORCI. Materials were obtained from the squamocolumnar junction by using Ayres Spatula. The cytological smears were classified using the Bethesda system.

Results: The study included 170 patients recruited randomly from the two different centers mentioned earlier. Out of this 120 were HIV infected patients on HAART and 50

were HIV negative controls. In HIV infected patients the age ranged from 20-66 years with a mean age of 40.5 years while among HIV negative controls the age ranged from 20-69 years with a mean age of 41.6 years. SIL was the commonest type of cervical lesion found among HIV infected patients, which constituted 38.3% (n=64), followed by cervicitis (28.3%; n=34) while carcinoma constituted 5.8% (n=7). The prevalence of SIL and carcinoma was higher among HIV infected women older than 45 years while cervicitis was most common found in young women.

Conclusion: This study has shown that elderly HIV infected women have a higher prevalence of SIL and carcinoma compared to young women. The increased prevalence is most likely a result of HIV infection which increases the likelihood of HPV persistence and progression of SIL. The small number of HSIL and carcinoma in HIV infected patients detected in this study could be due to the use of HAART which improves the immune system and retards the progression of SIL.

Recommendations: HIV infected women on HAART must still receive careful cervical screening follow-up for the assessment of cervical lesions.

LIST OF ABBREVIATIONS

ABC	-	Avidin-Biotin Complex
AIDS	-	Acquired Immune Deficiency Syndrome
CCSU	-	Cervical Cancer Screening Unit
CD	-	Cluster of Differentiation
CFC	-	Chronic Follicular Cervicitis
CIN	-	Cervical Intraepithelial Neoplasia
CIS	-	Carcinoma in-situ
CSCC	-	Cervical Squamous Cell Carcinoma
CT	-	Chlamydia Trachomatis
CTC	-	Care and Treatment Clinic
DAB	-	3,3'-diamonobenzidine tetrahydrochloride
DNA	-	Deoxyribonucleic acid
DPX	-	Dextrene Polystyrene Xylene
HAART	-	Highly Active Antiretroviral Therapy
HIV	-	Human Immunodeficiency Virus
HPV	-	Human Papilloma Virus
HSIL	-	High Grade Squamous Intraepithelial Lesion
HSV	-	Human Simplex Virus
H_2O_2	-	Hydrogen Peroxide
LSIL	-	Low Grade Squamous Intraepithelial Lesion
MNH	-	Muhimbili National Hospital

MUCHS	-	Muhimbili College of Health Sciences
NG	-	Nisseria Gonorrhea
NHS	-	Normal Horse Serum
OC	-	Oral Contraceptive
ORCI	-	Ocean Road Cancer Institute
PAP	-	Papanicolaou smear
RNA	-	Ribonucleic acid
SCC	-	Squamous cell Carcinoma
SIL	-	Squamous Intraepithelial Lesion
STD	-	Sexually Transmitting Disease
TBS	-	Tris-buffered saline
TV	-	Trichomona Vaginalis
UK	-	United Kingdom
USA	-	United States of America

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INTRODUCTION AND REVIEW OF LITERATURE

Cancer of the uterine cervix is one of the most common malignancies in women worldwide and the commonest female cancer in Sub-Saharan Africa (1). Approximately 500,000 new cases of cervical cancer are diagnosed yearly, and about 200,000 women die of the disease (2). The introduction of Papanicolaou (PAP) smear in 1930's made early detection and treatment of pre-invasive disease possible which has significantly contributed to the reduction of morbidity and mortality related to cervical cancer in developed countries (3). The developing countries have however, experienced an increase in both, the prevalence and incidence of precursor lesions and cervical cancer during the last two decades due to the absence of effective screening programs (4, 5, 6). It is now well known that invasive cervical cancer is the end result of progressive consecutive changes, which begin with precursor lesions namely cervical dysplasia, cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesion (SIL) and carcinoma in-situ (CIS) (7). However if early detection is made, these early lesions are reversible and cure is achievable.

Epidemiological studies have identified a number of risk factors that contribute to the development of cervical cancer, including low socioeconomic class, sexual intercourse at an early age, multiple sexual partners, multiparity, long term oral contraceptive use, smoking, vitamin deficiency and sexually transmitted infection including *Chlamydia trachomatis* and *Herpes virus type II* (8, 9, 10). Several recent studies have implicated *Human papilloma virus* (HPV) as a primary sexually transmitted aetiologic agent in the development of cervical squamous cell carcinoma (CSCC) and its precursors (11). HPV

infection and latency are necessary for initiation of precancerous lesions (12), but not sufficient for the development of cervical cancer (13).

Human Papilloma Virus (HPV)

HPV is a double-stranded DNA tumor virus that belongs to the Papovavirus family. Twenty-three of the 80 known types of HPV may affect the genital tract (14), especially the cervical transformation zone which is highly susceptible to HPV carcinogenicity (15). HPV is transmitted presumably through microscopic tears in the surface of the epithelium that commonly occur during sexual intercourse and binding to specific receptors on epithelial cells for infection to occur (16).

HPV can be divided into risk groups based on their genomic association with the host cell and association with different types of benign lesions and invasive carcinoma. The low risk viruses (types 6 and 11) are associated only with condyloma and mild dysplasia in the vast majority of instances and the virus exists in episomal form in the host cell. The intermediate risk group, which was recently combined with the high risk viruses, is associated with all levels of CIN including CIS and with approximately 10% of invasive cervical carcinomas and the viral genome is linearly integrated (4). The most frequent of these are types 31, 33, 35. The high risk virus types 16 and 18 are also associated with all levels of intraepithelial neoplasia but account for majority of high grade lesions and most of invasive cervical carcinomas. HPV type 16 was found 2.2 fold more often in HIV infected patients in U.K (17). However, a study done in Tanzania showed that HPV type 18 is more prevalent in HIV infected patients (18). HIV-seropositive patients represent the largest population with persistent genital HPV infection (40-60%). A low CD4 cell

count and high HIV viraemia levels increase the risk of harboring oncogenic HPV types (19).

The term low grade squamous intraepithelial lesion (LSIL) of the Bethesda classification system encompasses mild dysplasia/cervical intraepithelial neoplasia-1 (CIN-I) and koilocytic changes induced by HPV (20). High grade squamous intraepithelial lesion (HSIL) includes moderate dysplasia/CIN-II and severe dysplasia/CIN-III (20). LSIL are attributable to both high and low risk types of HPV, with 30% containing more than one type of HPV and less than 10% harbouring only low risk types (14). It is postulated that HSIL may originate from mild dysplasia or may arise directly after infection by high risk HPV types (21).

The exact mechanism by which HPV infects human epithelial cells remains largely unknown (4). However, some studies suggest that the HPV receptor is located on the basal epithelial cells and belongs to the ά6β4 integrin receptor family (14). Viral replication occurs in the basal epithelial cells, which is a less differentiated layer of the epithelium but late protein synthesis and virus packaging occurs in the mature superficial epithelial layers (14).

Molecular analysis of HPV- associated cervical carcinomas reveals clonal integration of the viral genomes in the host cell DNA (14). This leads to over expression of the E6 and E7 viral proteins, which transform cells by binding to and inhibiting the functions of pRb and p53 tumor-suppressor gene products (4, 14). Integration of the HPV genome is a pre-requisite for transformation and irreversibility eventually progressing to cervical cancer. HPV infection is characterized cytologicaly by the presence of koilocytes which are

superficial or immature squamous cells with large and irregular, well defined perinuclear halos with cookie cutter border and cytoplasmic thickening, enlarged nuclei with undulating (raisin-like) nuclear membrane and rope-like chromatin; often binucleated or multinucleated with variation in nuclear size (4).

Human Immunodeficiency virus (HIV)

HIV was unknown until the early 1980's when successful isolation of a virus believed to cause acquired immunodeficiency syndrome (AIDS) was achieved (24). This virus became know as the Human Immunodeficiency Virus-I (HIV-1). A second less virulent virus, HIV-2, was described in 1986 and has remained mainly confined to West Africa (23). At the end of year 2000 it was estimated that 36.1 million people worldwide, including 1.4 million children were living with HIV/AIDS. More than 70% of these people (25.3 million) live in sub-Saharan Africa, with another 16% (5.8 million) in South and Southeast Asia (23). Tanzania is among the worst HIV/AIDS affected countries in Africa. Estimates suggest that in Tanzania, approximately 1,600,000 people were living with HIV/AIDS at the end of 2003 (22). In the same year, up to 230,000 adults and children were thought to have died from the disease. Women are significantly more affected than men accounting for 60% (22). All HIV infected persons are at risk for illness and death from opportunistic infections and neoplastic complications as a consequence of the inevitable loss of immunosurveillance associated with AIDS (24).

HIV is a retrovirus belonging to the Lentivirus family and consists of a core surrounded by a lipid envelope. Within the viral core are the p24 protein, p7/p9 protein, two copies of genomic RNA, the viral reverse transcriptase and integrase. The HIV-1 provirus encodes

three structural proteins (gag, pol and env) in addition to the six accessory proteins (tat, rev, vif, vpr and vpu) (25). HIV tat protein up-regulates the expression of HPV in tissue, a finding that would be expected to lead to increased frequency of HPV- induced lesions such as SIL and cancer (25). The p24 antigen has been used as a surrogate marker for disease progression and anti-p24 monoclonal antibodies is used to study the distribution of infection in various tissues by morphological methods (25). Numerous studies have suggested that HIV infection increase women's susceptibility to HPV infection or alter the natural history of a pre-existing HPV infection (27). A study done by Xiao- Wei Sun (28) showed that HIV infected women have a high rate of persistent infection by the high risk types of HPV that are strongly associated with the development of HSIL and invasive cervical carcinoma. In the setting of HIV, these pre-invasive cervical lesions are characterized by multifocality, rapid progression, and high recurrence rates despite treatment (2). Although most follow-up studies have shown a spontaneous regression of a large proportion of both low grade and high grade SIL among HIV seronegative women (7), a substantial proportion of dysplastic lesions have been shown to progress to a more advanced stage in HIV infected women (8). Generally in studies done so far the prevalence of cervical lesions among HIV infected women was noted to be 48.2% and the lesions were recorded as inflammatory, pre-malignant and malignant conditions (29).

Pap smear Technique

The Pap smear technique was named after George Papanicolaou, a physician who developed the procedure in the 1930s. Since World War II, Pap smear became the most widely used and most successful cancer screening method in the world. Pap screening

reduced cervical cancer death rates by 74% between 1955 and 1992, and the rate continues to decline by about 2 percent a year (29). However, cervical cancer screening program was only introduced in Tanzania in the 1980's and at Ocean Road Cancer Institute (ORCI) reports show that up to 70% of their patients present with advanced cancer of the cervix (13). Currently, the Pap smear test is the method of choice for mass screening for the early detection of CIN, HPV infection, cervical cancer as well as other lesions (20). The American Cancer Society (20) and the American College of Obstetrician and Gynecologist (7) recommend that screening begin at the age of 18 years regardless of whether the woman is sexually active or not.

Cervicitis

Cervicitis which is inflammation of the cervical epithelium and stroma may be caused by sexually transmitted agents such as C. *trachomatis* (CT), *N. gonorrhoeae* (NG), *Herpes simplex virus* (HSV) and *Trichomonas vaginalis* (TV). However, in a few cases it may be attributed to chemical irritation (deodorants, douching), local trauma from foreign bodies (tampons, pessaries, intrauterine devices), surgical instrumentation, and therapeutic intervention (30). Risk factors include younger age, being single, separated, divorced; using non-barrier contraception and change of sexual partner (31). A study done by Kelly (30) showed that the prevalence of cervicitis in the general population is 26.1%, teenagers and young adult population being the most affected. The cytological features of acute cervicitis depend on the causative agent. The presence of binucleated cells is associated with TV infection; while minimal squamous atypia is associated with yeast infection. Koilocytosis is associated with genital HPV and abundant clue cells are most

likely due to Gardnerella infection (30). The cytological morphology of chronic follicular cervicitis (CFC) in conventional smears includes two main elements. First, both mature and immature lymphoid cells are present. Second, the presence of germinal centre macrophages containing phagocytosed cellular debris, plasma cells, histiocytes and polymorphonuclear leucocytes. An increased number of histiocytes, lymphocytes and the presence of metaplastic cells with vacuolated cytoplasm are associated with CT infection (31).

Squamous Intraepithelial Lesions

Cervical squamous intraepithelial lesions are more common in teenagers, HIV positive women and the socially disadvantaged populations (5). A previous study done in the USA showed that the prevalences of LSIL and HSIL in the general population were 5.7% and 0.6 % respectively (33). Infection with HIV and its related immunosuppression are associated with an increased prevalence, incidence and persistence of SIL (5, 14). However, several studies done elsewhere have shown that long-term use of highly active antiretroviral therapy (HAART) may be beneficial in reducing SIL, and its progression to higher grades (34).

Cervical carcinoma

Invasive cervical carcinoma is the end result of a progressive consecutive changes, which begins with precursor lesions namely SIL (7). Risk factors for cervical cancer have been mentioned above. Several studies have implicated HPV as a primary sexually transmitted etiologic agent in the development of cervical carcinoma (9). In most developed countries cervical carcinoma shows a unique pattern among age groups. After the age 25-30 years

there is a sharply increased rate, reaching a pick after 45-50 years (15). Decrease in screening coverage or lower sensitivity of cytology in the old age groups are plausible explanations but immunosuppression or acquisition of new HPV infection during middle age cannot be ruled out (37).

RATIONALE OF THE STUDY

Tanzania is among the countries in the Sub-Saharan region mostly affected by the HIV/AIDS pandemic, the female population being most vulnerable than male (26). HIV infection and cervical cancer are major public health problems among women in Tanzania. Although the prevalence of cervical cancer in the general population and in HIV/AIDS patients is unknown in Tanzania, hospital-based studies done around the world show that cervical cancer account for approximately 40% of all cancers diagnosed in women. Among women infected with HIV there is a high prevalence of HPV infection (18). HPV infection in HIV-infected women appears to be more persistent and HIV associated cervical lesions are multicentric, aggressive and recurrent after treatment (14). Due to scanty information concerning HIV-associated cervical lesions in our country, rational and efficient management of cervical cancer in the setting of HIV infection is not possible. This study is therefore important, since early detection of pre-cancerous lesions or early cervical neoplasia provides an opportunity to prevent or delay progression by clinical interventions. The early detection of cervical lesions in HIV infected patients through routine screening tests and clinical interventions reduces the prevalence of cervical cancer and also improve the quality of life of the patients. The study will also provide baseline data on the frequency and pathogenesis of cervical cancer in HIV infected patients and form the basis for further studies.

OBJECTIVES

Broad objective

To determine cervical cytological changes in HIV infected patients on HAART attending the Care and Treatment Clinic at Muhimbili National Hospital from 2005 to 2006

Specific objectives

1. To determine the proportion of cervicitis, cervical intraepithelial lesion (CIN/SIL) and cancer in HIV infected patients by Pap smear.
2. To determine age distribution of HIV infected patients with cervical lesions.
3. To determine the risk factors associated with cervical lesions in HIV infected patients.

HYPOTHESIS

There is high prevalence of cervical intraepithelial lesion and cervical cancer in HIV infected patients in Tanzania.

MATERIAL AND METHOD

Study area

The study was conducted at Muhimbili National Hospital (MNH) in Tanzania. The hospital receives referral patients from all over the country which serves as regional hospital for Dar es Salaam and as a teaching hospital for Muhimbili University College of Health Sciences (MUCHS). The Tanzanian population is over 36 million people at present and that of Dar es Salaam is about 3.5 million. The mainstay of the study was in the department of Histopathology and Morbid Anatomy but there were collaboration with the department of Obstetrics and Gynecology at MNH and the Cervical Cancer Screening Unit at Ocean Road Cancer Institute (ORCI).

Sampling

The study population included all known HIV female patients who attended the CTC at Muhimbili National Hospital. It included patients above 18 years of age, who consented to participate in the study. HIV negative controls were recruited from the Cervical Cancer Screening unit (CCSU) at Ocean Road Cancer Institute (ORCI). Patients below 18 years, pregnant women and those who did not give consent for participation were excluded from the study. Patients' identification, biodata and experimental results were entered in a questionnaire (Appendix I).

The sample size was calculated using the following formula:

$$n = \frac{4P(100-P)}{E^2}$$

Where **P** is the estimated proportion of HIV-infected individual with cervical lesion and **E** is the estimated maximum error allowed, which will be 5%

According to Lee KJ, et al.; 2004 (29); 48.2% of HIV infected individuals will present with cervical lesions.

Therefore;

$$n = \frac{4 \times 48.2 (100 - 48.2)}{5^2} = 399$$

This study was conducted for one year.

Therefore;

$$n = \frac{399}{2} = 200$$

Where **2** mean the two years spent in the study (29).

The minimum number of individuals who were included into the study was 170.

Laboratory Methods

Serology

Serological results of all patients were sought from the clinical files in the Medical Records Department of MNH and ORCI.

Pap smear

Pap smears were performed by a Gynecologist at MNH and in the CCSU at ORCI. Patients were put in lithotomy position and a bivalve speculum was introduced. The cervix was exposed under illumination for assessment. Specimens were taken from the

squamocolumnar junction using an Ayres spatula. The specimens were smeared on two glass slides and fixed immediately in 95% alcohol/ether for Pap staining. Slides were examined under light microscope and results were discussed with a Pathologist and entered in data sheet.

Pap Staining Procedure

Alcohol/ether fixed smears were hydrated in graded alcohol from 95%, 85%, and 70% followed, by immersion in water. The smears were stained with Harris Haematoxylin for 5 minutes, differentiated in 1.0% acetic acid in alcohol and blued in running tap water for 3 minutes. Smears were then dehydrated in ascending graded alcohol from 70% to 95% and stained with orange G for 3 minutes, rinsed in 95% alcohol followed by staining with EA 36 for three minutes and then dehydrated in absolute alcohol. Thereafter the smears were placed in xylene to remove alcohol and finally were mounted in DPX and glass cover slips were applied.

Statistical Methods

Study design

This was essentially an analytical cross-sectional study which was carried out on HIV-infected patients attending care and treatment clinic (CTC). The study aimed at determining the proportion of HIV-infected patients that present with cervical cytological changes.

Data Analysis

Data analysis was done using computer package; EPI INFO 6 and SPSS 12. The Chi-square ($\chi 2$) test and the P-value statistical methods were used to test the validity of associations. P-value ≤ 0.05 was considered significant.

Data Collection and Management

Data was collected in structured questionnaires (Appendix II-IV), which were tested and corrected before use. The questionnaires were coded for easy computer analysis.

Ethical Issue

Permission was sought from the College Ethical Clearance Committee to conduct the study. Informed consent was sought from patients to screen them for cervical cancer (Appendix I). All patients' information in this study was treated as being strictly confidential and their identities were not revealed. Before performing Pap smear, pre counseling for cancer was done and patients were at liberty to accept or refuse being screened for cervical cancer. However this had no bearing whatsoever to the quantity and/or quality of the services they expected to receive. All those patients who presented with cervical lesions were referred to the Gynecologist.

Limitations of the study

1. All HIV infected patients were recruited from the Care and Treatment Clinic (CTC), who are not representative of the general population. Therefore, the study was under the effect of a potential selection bias.
2. The assessment of cervical lesions was based on cytologic specimens rather that histologic sample. Therefore, the study can not exclude the possibility that negative cytology may mask persistent histologic disease or that abnormal cytology may reflect non-neoplastic changes.
3. All HIV infected individuals were on HAART which constitute a potential confounding factor in the outcome of the results.
4. Baseline data of CD 4 cell count was not used in the study to make correlation between immunosuppression and type of cervical lesions encountered.

RESULTS:

Demographic data of the patients

The study included 170 patients selected randomly from two centers between June 2005 and December 2006. One hundred and twenty HIV-seropositive patients using HAART were recruited from the CTC at MNH and 50 HIV-seronegative controls were recruited from the CCSU at ORCI. Among HIV positive patients the age ranged from 20 - 66 years with a mean age of 40.5 years, while among HIV negative controls age ranged from 20 - 69 years with a mean age of 41.6 years. The age group 36-45 years was the most affected by HIV (39.2%, n=47) followed by the age group 20-35 years (30.8%, n=37) while those 56 years and above were least affected (5.8%, n=7) as shown in Table 1. Among 120 HIV infected patients on HAART, 77 (64.1%) were single and 43 (35.8%) were married, while in HIV negative controls 26% (13/50) were single and 74% (37/50) were married which is highly statistically significant (P-value=0.00), indicating that HIV infected individuals were more likely to be single as shown in Figure 1.

Distribution of cervical lesions

Cervical lesions were classified according to the modified Bethesda system (20). The lesions were classified as cervicitis, squamous intraepithelial lesions (SIL) and carcinoma. Among HIV infected patients cervicitis constituted 28.3% (n=34), SIL 38.3% (n=46) and carcinoma 5.8% (n=7), while among HIV negative controls 28% (n=14) had cervicitis, 34% (n=17) SIL, and 2% (n=1) had carcinoma. This was however, not statistically significant (χ^2=0.97; df =2; P-value=0.61), which indicate that HIV infected

patients on HAART and HIV negative controls are at the same risk for cervical lesions as shown in Figure 2.

Cervicitis

Cytological subtype of cervicitis: Among 34 HIV infected patients on HAART with cervicitis 32 (94.1%) were acute cervicitis and 2 (5.9%) were chronic cervicitis, while among 14 HIV negative controls (100%) had acute cervicitis as shown in Table 2.

Cytomorphological features of cervicitis: Figure 3 shows the cytomorphological features of cervicitis according to HIV-serostatus. Out of 32 smears of HIV infected patients with acute cervicitis, 100% showed a heavy neutrophilic background, with abundant superficial squamous cells (clue cells) covered by small rod like microorganisms, which are suggestive of bacterial vaginosis, more likely due to *Gardnerella vaginalis* (GV), while among HIV negative controls 85.7% (12/14) of smears showed the same feature (Plate I). Moth eaten appearance is a nonspecific inflammatory change found in 62.5% (20/32) cytological smears of HIV infected patients and 50% (7/14) smears of HIV negative controls. Abundant clusters of endocervical cells with a honeycomb pattern are suggestive of cervical erosion or an underlying neoplastic process, which was found in 46.9% (15/32) smears of HIV infected patients and 35.7% (5/14) smears of HIV negative controls (Plate II). Cytoplasmic inclusions are suggestive of chlamydial or HSV infection, which was present in 9.4% (3/32) cytological smears of HIV infected patients (Plate III). Features suggestive of *Trichomona vaginalis* (TV) were present on 6.2% (2/32) smears of HIV infected patients (Plate IV). CFC was diagnosed in two conventional smears of HIV infected patients. Both smears showed abundant

lymphocytes and tingible body macrophages which were smeared across the slide and few neutrophils (Plate V).

***Squamous Intraepithelial Lesions* (SIL)**

Cytological subtypes of SIL: Out of 46 HIV infected patients on HAART with SIL, 27 (58.6%) were LSIL and 19 (41.3%) were HSIL, while out of 17 HIV negative controls 13 (76.4%) were LSIL and 4 (23.5%) were HSIL as shown in Table 3.

Cytomorphological features of SIL: Figure 4 shows the cytomorphological features of SIL according to HIV-serostatus. Out of 27 smears of HIV infected patients on HAART with SIL 100% showed abundant individual and clusters of superficial squamous cell with mild nuclear enlargement and fined granular chromatin, while among HIV negative controls 69.2% (7/13) showed abundant amount of squamous cells with the same degree of cellular atypisms, and 4 (30.8%) smears exhibited few clusters with cellular atypisms; 89.9% (24/27) smears of HIV infected patients had a heavy neutrophilic background, while among HIV negative controls 15.4% (3/13) smears showed the same cytological features, which are suggestive of the presence of a persistent inflammatory agent (Plate VI). Binucleated cells suggestive of HPV or TV infections were present on 5 (18.5%) smears of HIV infected patients on HAART and 1 (7.7%) smears of HIV negative control (Plate VII). Three smears (11.1%) of HIV positives patients on HAART showed dyskeratosis. Among 19 conventional smears of HIV positive patients on HAART with HSIL, 14 (73.7%) smears showed abundant clusters of superficial and intermediate squamous cells with a moderated to severe nuclear enlargement, granular chromatin and regular nuclear membrane, while among HIV negative controls 75% (3/4)

had the same features (Plate VIII). A neutrophilic background was seen in 100% of smears of HIV infected patients on HAART and in 25% (1/4) smears of HIV negative controls. One smear (3.7%) of HIV infected patient showed abundant dendritic cells suggestive of mucosal exposure to HIV (Plate IX).

Cervical carcinoma

Cytological subtypes of carcinoma: Of the 7 HIV positive patients on HAART with carcinoma 6 (85.7%) were squamous cell carcinoma and 1 (14.3%) was adenocarcinoma (Plate X), while the single HIV negative control (100%) was squamous cell carcinoma.

Cytomorphological features of carcinoma: Smears of HIV infected patients on HAART with squamous cell carcinoma exhibit a neutrophilic background, with abundant clusters of pleomorphic cells, containing hyperchromatic nuclei with irregular nuclear membrane and few mitosis (Plate XI), the same features were seen on smear of the single HIV negative control. Orangeophilic cells are suggestive of keratinization, which was found in 66.6% (4/6) conventional smears of HIV infected patients on HAART.

Koilocytic changes

Koilocytic changes and HIV-serostatus: Out of 87 smears of HIV infected patients on HAART with cervical lesions 27 (31.3%) showed scattered superficial squamous cells with binucleated perinuclear halo (Plate XII), while out of 32 smears of HIV negatives controls with cervical lesions 9 (28%) showed the same cytological findings which are suggestive of HPV or HSV infection. However, these findings were not statistically significant (P-value=0.76), suggesting that HIV positive patients on HAART and HIV negative control are at the same risk for HPV or HSV infection.

Koilocytic changes and age: Of the 27 smears of HIV infected patients on HAART with koilocytosis 6 (22.2%) smears were from patients between 20-35 years and 21 (77.8%) smears were from patients older than 35 years, while among 9 smears of HIV negative controls 4 (44.4%) were from patients between 20-35 years and 5 (55.6%) smears were from patients older than 35 years.

Koilocytic changes and sexual partners: Among 27 smears of HIV infected patients on HAART with koilocytic changes 19 (70.4%) were from patients with multiple lifetime sexual partners and 8 (29.6%) were from those who reported a single sexual partner, while among 9 smears of HIV negative controls 6 (66.7%) were from patients with multiple lifetime sexual partners and 3 (33.3%) were from those with a single partner. These results are statistically significant (P-value=0.01), indicating that HIV infected patients on HAART with multiple sexual partners are at risk for HPV or HSV infection

Koilocytic changes and sexual debut: Out of 27 smears of HIV infected patients on HAART with koilocytic changes 26 (96.3%) were from patients who had sexual intercourse before 17 years and 1 (3.7%) from those who had sexual intercourse at older age, while among 9 smears of HIV negative controls 7 (77.8%) were from patients who had sexual intercourse before 17 years and 2 (22.2%) from those who had sexual intercourse at older age. These results are statistically significant (P-value= 0.02), implying that early age of sexual debut predisposes HIV positive patients on HAART to HPV or HSV infection.

Distribution of cervical lesions according to age group

Distribution of cervicitis: Among 34 HIV infected patients on HAART with cervicitis the age ranged from 28-55 years with mean age of 38.2 years, the most affected age group was 36-45 years (52.9%), while among 14 HIV negative controls the age ranged from 20-46 years with a mean age of 33.2 years and the most affected age group was 20-35 years (64.3%) as shown in Table 4.

Distribution of SIL: In 46 HIV infected patients on HAART with SIL the age ranged from 20-63 years with a mean age of 41.3 years, the most affected age group was 46-55 years which constituted 34.7%, while in 17 HIV negative controls the age ranged from 30-69 years with mean age 52.2 years and the most affected age group was 36-45 years which constitute 23.5% as shown in Table 5.

Distribution of Carcinoma: Of the 7 HIV infected patients on HAART with carcinoma the age ranged from 32-66 years with a mean age of 47.6 years. The age group most affected was 56 years and above which constituted 71.4%, while a single HIV negative control with carcinoma was in the age group of 46-55 years which constituted 100% .

Cervical lesions and risk factors

Cervicitis

Distribution of cervicitis and marital status: Of the 34 HIV infected patients on HAART with cervicitis 32 (94.1%) were single and 2 (5.9%) were married, while among 14 HIV negative controls 9 (64.3%) were single and 5 (35.7%) were married. This result was statistically significant (P-value=0.02), indicating that HIV infected patients on HAART

who are single are at increased risk for cervicitis when compared with HIV negative controls.

Distribution of cervicitis and number of sexual partners: Among 34 HIV infected patients on HAART with cervicitis 9 (26.4%) had a single sexual partner and 25 (73.5%) had multiple lifetime sexual partners, while among 14 HIV negative controls 9 (64.3%) had a single sexual partner and 5(35.7%) had multiple sexual partners. This trend was statistically significant (P-value=0.01), indicating that HIV positive patient on HAART with multiple lifetime sexual partners are at increased risk for cervicitis when compared with HIV negative controls.

Distribution of cervicitis and age of first sexual intercourse: Out of 34 HIV infected patients on HAART with cervicitis 27(79.4%) had sexual intercourse before 17 years and 7 (20.6%) at older age, while out of 14 HIV negative controls 7 (50%) had sexual intercourse before 17 years and 7 (50%) at older age. The difference of proportion was statistically significant (P-value= 0.05), which indicate that HIV infected patients on HAART with sexual debut at early age are at increased risk for cervicitis when compared with HIV negative controls.

***Squamous intraepithelial lesions* (SIL)**

Distribution of SIL and marital status: In 46 HIV infected patients on HAART with SIL 34 (73.9%) were single and 12 (26.1%) were married, while in 17 HIV negative controls 5 (29.4%) were single and 12 (70.6%) were married. The finding was statistically significant (P-value= 0.00), indicating that single HIV infected individuals on HAART were at increase risk for SIL compared to HIV negative controls.

Distribution of SIL and number of sexual partners: Of the 46 HIV infected patients on HAART with SIL 10 (21.7%) had a single sexual partner and 36 (78.3%) had multiple lifetime sexual partners, while of the 17 HIV negative controls 8 (47.1%) had a single sexual partner and 9 (52.9%) had multiple sexual partners. This results are statistically significant (P-value=0.05), indicating that HIV infected patients on HAART with multiple sexual partners are at increased risk for SIL compared to HIV negative controls.

Distribution of SIL and age of first sexual intercourse: Out of 46 HIV infected patients on HAART with SIL 37 (80.4%) had sexual intercourse before 17 years and 9 (19.6%) at older age, while among 17 HIV negative controls 9 (52.9%) had sexual intercourse before 17 years and 8 (47.1%) at older age. These results were statistically significant (P-value= 0.03), indicating that HIV infected patient on HAART with sexual debut at early age were at increased risk for SIL compared to HIV negative controls.

Distribution of SIL and parity: Among 46 HIV infected patients on HAART with SIL 32 (69.6%) reported four or more full term pregnancies and 14 (30.4%) had less than four full term pregnancies, while among 17 HIV negative controls 7 (41.2%) had four or more full term pregnancies and 10 (58.8%) had less than four full term pregnancies. This results were statistically significant (P-value=0.04), implying that HIV infected patients on HAART who had four or more full term pregnancies are at increased risk for SIL compared to HIV negative controls.

Cervical carcinoma

Distribution of carcinoma and marital status: Out of seven HIV infected patients on HAART with carcinoma 2 (28.6%) were married and 5 (71.4%) were single, while the

only HIV negative control with carcinoma (100%) was single. There were not correlation between marital status and carcinoma (P-value=0.75).

Distribution of carcinoma and number of sexual partners: Of the 7 HIV infected patients on HAART with carcinoma 2 (28.6%) had a single sexual partner and 5 (71.4%) had multiple lifetime sexual partners, while the only HIV negative control with carcinoma had multiple lifetime sexual partners. The results did not showed statistically significant difference (P-value=0.75), which indicate that all individuals are at the same risk for cervical carcinoma regardless lifetime number of sexual partners.

Distribution of carcinoma and age of first sexual intercourse: Among 7 HIV infected patients on HAART with carcinoma 5 (71.4%) had sexual intercourse before 17 years and 2 (28.6%) at older age, while the single HIV negative control reported sexual intercourse at older age. These results are not statistically significant (P-value=0.37) but also the sample was too small. This can however suggest that both groups of individuals are at the same risk for carcinoma regardless of sexual debut.

Distribution of carcinoma and parity: In 7 HIV infected patients on HAART with carcinoma 3 (42.8%) reported less than four full term pregnancies and 4 (57.1%) had four or more full term pregnancies, while the single HIV negative controls reported four or more full term pregnancies. This however, is not statistically significant (P-value=0.62) but also the sample was too small for meaningful conclusions.

DISCUSSION

Cervicitis

In the present study the prevalence of cervicitis among HIV infected patients was28.3% which is slightly elevated when compared to a study done in USA by Kelly et al., (30), where the prevalence of cervicitis in the general population was 26.1%. The slight difference in the results is more likely due to the fact that all patients in this study were HIV infected and it is known that HIV infected women are at an increased risk for persistent infections with different microorganisms due to impairment of the immune system (41) which may also contribute to cervicitis. The proportion of HIV positive patients and HIV negative controls with cervicitis in this study was the same (28%). This is more likely due to the fact that all HIV infected patient recruited in this study were using HAART which can reduce opportunistic causes of cervicitis. This has also been suggested by researchers that HAART lowers the incidence of various opportunistic diseases associated with immunosuppression but its possible impact on cytological lesions is unclear (34).

A previous study showed that inflammatory changes on cervical cytological smears often indicate the presence of sexually acquired infection and masked underlying premalignant disease of the cervix (31). In this study 9.4% inflammatory smears of HIV infected patients showed few cells with cytoplasmic inclusions, which is suggestive of chlamydial infection. *Chlamydia trachomatis* is the most common cause of sexually transmitted infection and cytological changes in Pap smears have been documented (36). However, a study done by Forster et al., (43) reported that some chlamydial negative patients had a

Pap test which was reported to contain inclusions consistent with chlamydial infection. The author speculated that the inclusions were probable aggregates of bacteria, cell debris or other artifacts thus concluding that Pap test has low sensitivity compared to chlamydial culture. Despite the fact that this study could not confirm the presence of CT infection, it showed that features suggestive of CT were more common on smears of HIV infected patients, which corroborates with several studies (36, 42) in which researchers reported that there was a highest prevalence of CT in HIV infected individuals. Follicular cervicitis was identified in two conventional smears of HIV infected patients. Halford J (32) reported that a large number of cases are associated with chlamydial infection. The conventional smears showed scattered lymphoid cells, tingible body macrophages and few neutrophils. These cytomorphological features are similar to previous findings (32) which reported that lymphocytes and macrophages lie singly and usually smeared across the slide. This contrast with Thin Prep (liquid based cytology) smear in which follicular aggregates can be distinguished.

In this study the age group mostly presenting with cervicitis among HIV infected patients were between 36-45 years which constituted 38.3%. These findings were different to those of previous study (30), which reported cervicitis in adolescents and young adults attributed to highest risk of sexually transmitted diseases (STD) in these groups. However, the discrepancy in the results can be due to a selection bias during sampling since all our patients were recruited from the Care and Treatment Clinic (CTC), which is mainly attended by patients older than 25 years on ART and they are not representative of the general population in Tanzania.

The likelihood of cervicitis was significantly higher with increasing number of sexual partners and early sexual debut. The same report was obtained by others (31), who speculated that the risk of cervicitis is high in individuals with multiple sexual partners due to sexual exposure to potential genital pathogens. Another study (44) showed that there was no association between number of sexual partners and risk of cervicitis. However, the researcher identified other risk factors for cervicitis such as increased age and absence of H_2O_2 producing lactobacilli, which suggest that the development of cervicitis may be promoted by factors associated with persistent or prolonged disruption of normal viginal flora such as immunosuppression.

Squamous intraepithelial lesions

Infection with HIV alters the clinical course of HPV infection and SIL by increasing the likelihood of viral persistence and progression of lesions (24). The prevalence of SIL among HIV infected patients in this study was 38.3%, highly elevated when compared to a cohort study conducted by Schuman et al., (39), who found that the prevalence of SIL among HIV-seropositive women was only 19%. Another study done by Marte et al., (42) reported that the prevalence of SIL among HIV infected patients who were receiving care from ambulatory clinic was 33% in contrast with other HIV infected women in the community in which the prevalence of SIL was 4%. The prevalence of SIL among HIV positive patients and HIV negative control were 38.3% and 34% respectively. Although there was no statistically significant difference a slightly higher prevalence of SIL among HIV-seropositive patients when compared to HIV-seronegative controls was noted. This is due to the fact that HIV infected individuals are more likely to have persistent HPV

infection and confirms findings of similar previous studies (4, 8). LSIL was the most common subtype of SIL in both HIV serostatus groups, which suggests that rapid progression to HSIL is relatively rare regardless of HIV-serostatus as also seen in a previous study (39). Low prevalence of HSIL in HIV infected patients in this study could also be due to regression of the lesions, as a result of HAART. Although limited data is available on the natural history of HPV infection and dysplasia in patients treated with HAART, a study done by Foppa et al., (34) showed that long-term use of HAART leads to regression of cervical intraepithelial lesions, while Schuman et al., (39) found that HAART had no effect on the progression of SIL. The majority of conventional smears of HIV infected patients showed a heavy neutrophilic background compared to HIV negative controls. These findings corroborate those seen by Wilson et al., (31) and suggest that inflammatory smears are due to an underlying premalignant disease of the cervix resulting from persistent HPV infection and other agents which give rise to a prolonged cervical irritation. Indeed this study had shown koilocytic changes accompanied by others cytological features such as binucleation of superficial squamous cells, dyskeratosis and mild nuclear hyperchromasia with fine granular chromatin (LSIL), while other group of cells showed moderated to severe nuclear enlargement with granular chromatin and regular nuclear membrane (HSIL), all suggesting persistent HPV infection. Although immunohistochemistry failed to demonstrate HPV infection on cytological smears. A study done by Bollmann et al., (38) showed that the classical cytopathic effect of HPV encompasses koilocytosis including partial koilocytosis,

dyskeratosis, spindle nuclei and binucleation which were associated with high risk HPV types.

In this study the group which had the highest prevalence of SIL among HIV infected patients was 46-55 years. These findings were different to those of previous study (33) which the author reported SIL in teenagers attributed to highest risk of HPV infection in this group. However, the discrepancy in the results can be due to a selection bias mentioned early.

Regarding risk factors associated with SIL those patients who were single and had early sexual debut, were at an increased risk for SIL, due to increased likelihood of HPV exposure. Patients who have multiple lifetime sexual partners and those who reported four or more full term pregnancy were also at increased risk for SIL. Similar findings were reported by Bosh et al., (35) who suggested that some risk factors that reflect sexual behavior such as the number of sexual partners reflect the probability of HPV exposure. The same author corroborated the relationship between high parity, SIL and carcinoma, but the physiological reason for the association is not clear.

Carcinoma

Invasive cervical cancer is the end result of progressive consecutive changes of SIL (7). The nature of the association between HPV and cervical cancer has been exhaustively investigated and since the early 1990s, all reviews have consistently concluded that the evidence fulfils most of the established criteria of causality (37). Although the findings were not statistically significant there was a high frequency of cervical carcinoma in HIV infected patients compared to HIV negative controls in this study. The same trend has

been seen in another study (14) which reported that HIV alters the natural history of HPV infection, thus causing rapid progression of SIL to carcinoma. Cervical carcinoma was the least common type of cervical lesion detected in this study. This may in part be explained by the possibility that progression from SIL to invasive cancer may exceed the mean survival time for HIV positive females (19) or may be due to HAART as documented by others (34). Squamous cell carcinoma was the most predominant type of carcinomas found in HIV infected patients. However this finding differs from those of Bosh (35) who reported a high frequency of adenocarcinoma.

Among HIV infected patients with carcinoma the most affected age group was 56 years and above. The same results were obtained by Bosh (35) who suggested that decrease in screening coverage or lower sensitivity of cytology in the old age group are plausible explanations, but acquisition of new HPV infection during middle age cannot be ruled out. Cervical cancer in women younger than 25 years is extremely rare (8). HIV infected women with cervical cancer before the age 35 years is considered an acquired AIDS-defining illness by the Center of Disease Control (20).

Despite the fact that the risk factors associated with cervical carcinoma were not statistically significant in this study, they corroborate with several studies (2, 5, 7) where the researchers reported that age of sexual debut, lifetime number of sexual partners, marital status, and other characteristic of sexual activity increase the risk of becoming infected with the HPV, and thus development of cervical cancer. The relationship between high parity and cervical cancer has been seen before (39), where it was

suggested that hormonal factors related to pregnancy or cervical trauma associated to delivery may be contributory.

CONCLUSION

A reduction of HSIL and carcinoma in HIV infected patients were detected in this study. This could be due to the use of HAART which has a profound effect on improving the immune system and may also have a beneficial effect in the regression of SIL by decreasing the likelihood of HPV persistence and improving the quality of life of the patients. HIV infected individual at older age had a higher prevalence of SIL and carcinoma compared to young one. The increased prevalence may be due to infection with HIV which alters the clinical course of HPV infection and SIL by increasing the likelihood of viral persistence and progression of lesions. Several risk factors such as number of sexual partners, early sexual debut and other characteristic of sexual activity increase the risk of becoming infected with the HPV, and thus development of SIL and cervical cancer.

RECOMMENDATIONS

1. The cervical screening for SIL and carcinoma should also include cervical swab for culture and sensitivity for identification and treatment of possible etiologic agents associated with cervical lesions.
2. HIV infected women on HAART must still receive careful cervical screening and follow-up for the assessment of cervical lesions.
3. Additional studies are needed to comprehensively examine how HAART impacts on the incidence of cervical abnormalities, the persistency of high-risk HPV infection and occurrence of SIL and cancer.

Table 1: Distribution of HIV status according to age group.

Age Group (years)	HIV Status		Total
	Negative (%)	Positive (%)	
20-35	19(38)	37(30.8)	56
36-45	14(28)	47(39.2)	61
46-55	9(18)	29(24.2)	38
≥56	8(16)	7(5.8)	15
Total	**50**	**120**	**170**

Table2: Relationship between cytological subtypes of cervicitis and HIV status.

HIV status	Cervicitis		Total
	Acute (%)	Chronic (%)	
HIV +ve	32(94.1)	2(5.9)	34
HIV -ve	14(100)	-	14
Total	46	2	48

Table 3: Relationship between cytological subtypes of SIL and HIV status.

HIV status	LSIL (%)	HSIL (%)	Total
HIV +ve	27(58.6)	19(41.3)	46
HIV -ve	13(76,4)	4(23.5)	17
Total	40	23	48

Table 4: Distribution of cervicitis according to HIV serostatus and age group.

Age Group	Cervicitis		Total
(years)	HIV-ve (%)	HIV+ve (%)	
20-35	9(64.3)	11(32.3)	20
36-45	4(28.6)	18(52.9)	22
46-55	1(7.1)	5(14.7)	6
≥56	-	-	-
Total	**14**	**34**	**48**

Table 5: Distribution of SIL according to HIV serostatus and age group.

Age Group (years)	SIL		Total
	HIV-ve (%)	HIV+ve (%)	
20-35	2(11.7)	14(30.4)	16
36-45	4(23.5)	14(30.4)	18
46-55	3(17.6)	16(34.7)	19
≥56	8(47.1)	2(4.3)	10
Total	**17**	**46**	**63**

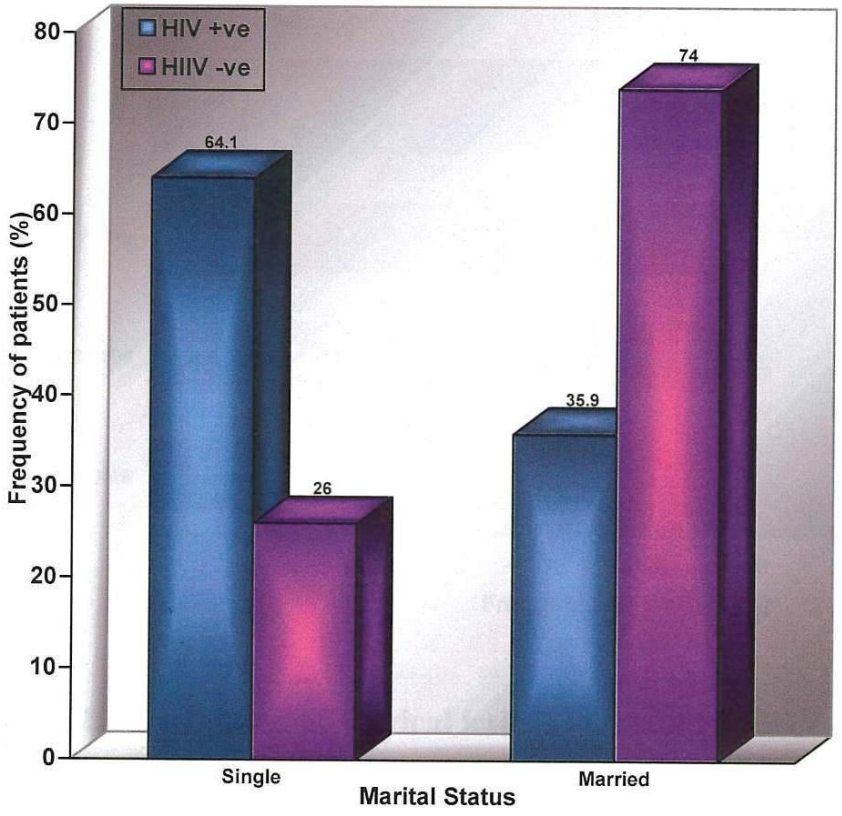


Figure 1. Distribution of patients according to marital status and HIV infection.

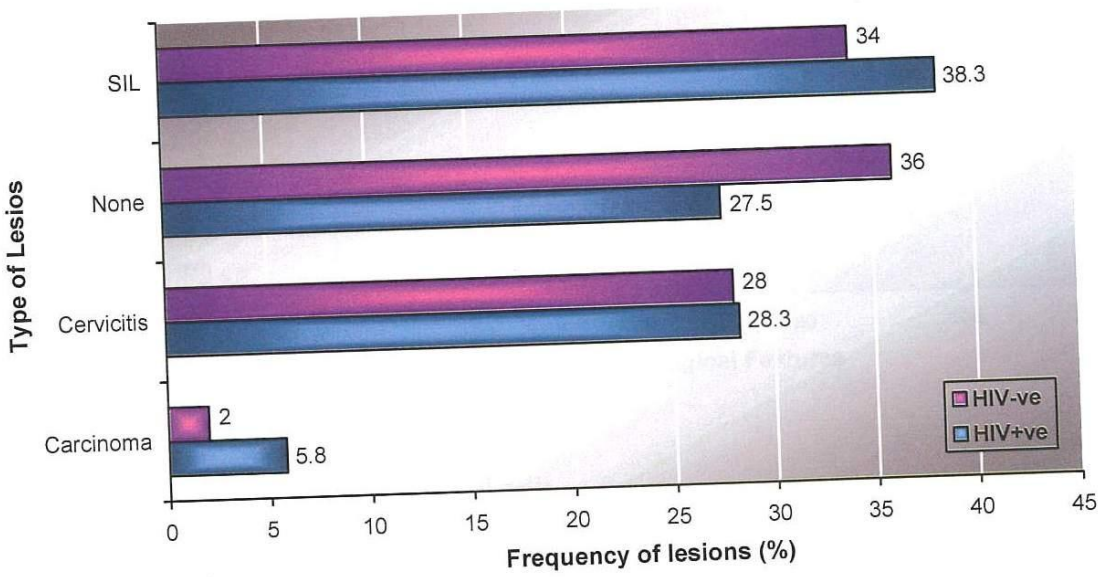
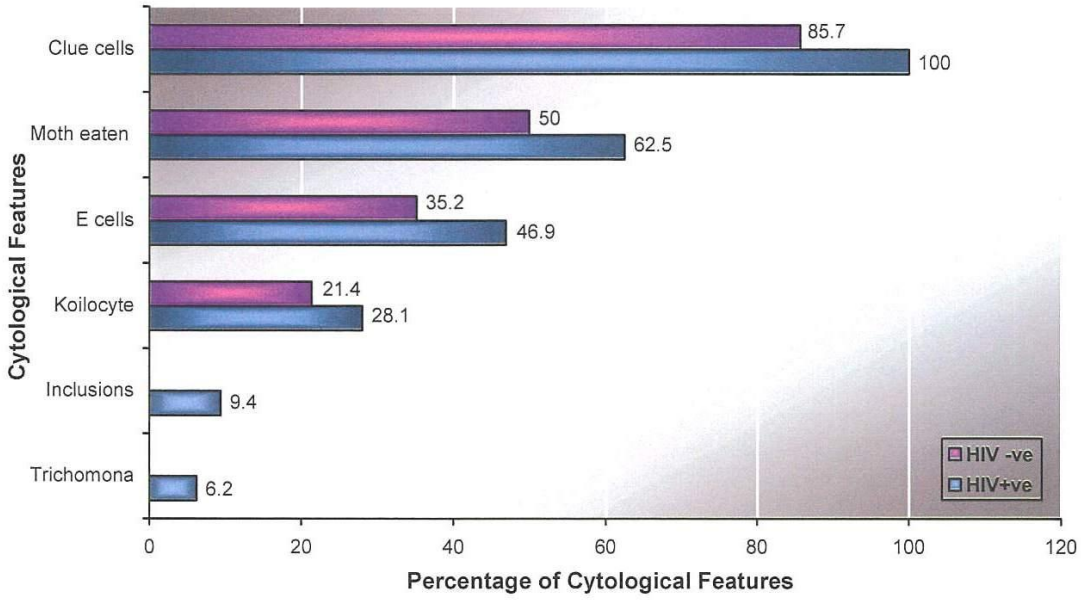


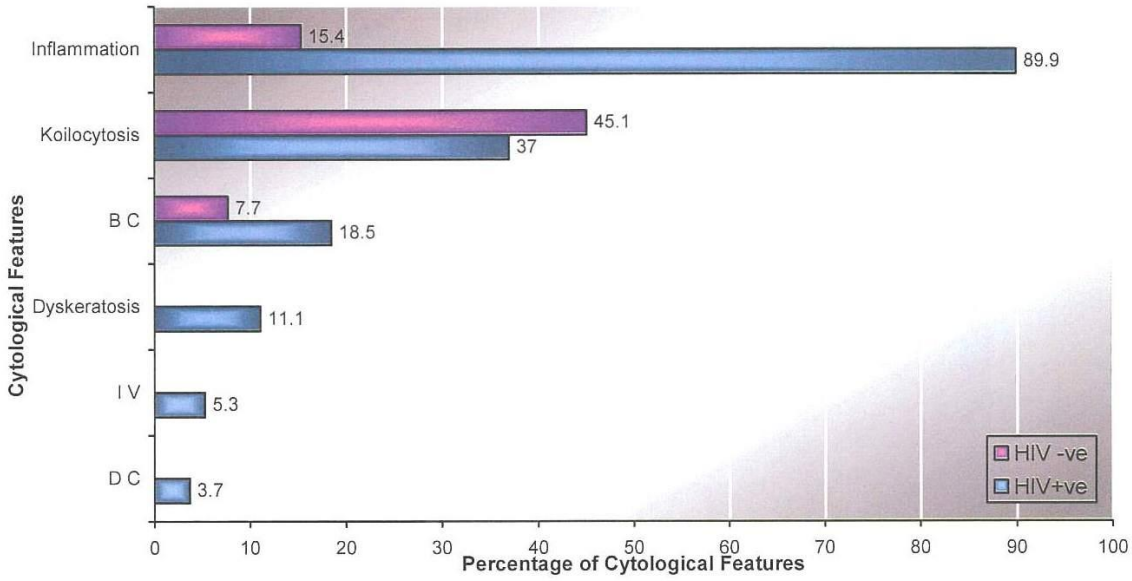
Figure 2. Distribution of cervical lesions according to HIV status



Key: E cells = Endocervical cells

Inclusions = Cytoplasmic inclusions

Figure 3. Relationship between cytomorphological features of acute cervicitis and HIV-serostatus.



Key: BC = Binucleated cells

IV = Intranuclear vacuolation

DC = Dendritic cells

Figure 4. Distribution of cytomorphological features of SIL according HIV-serostatus.

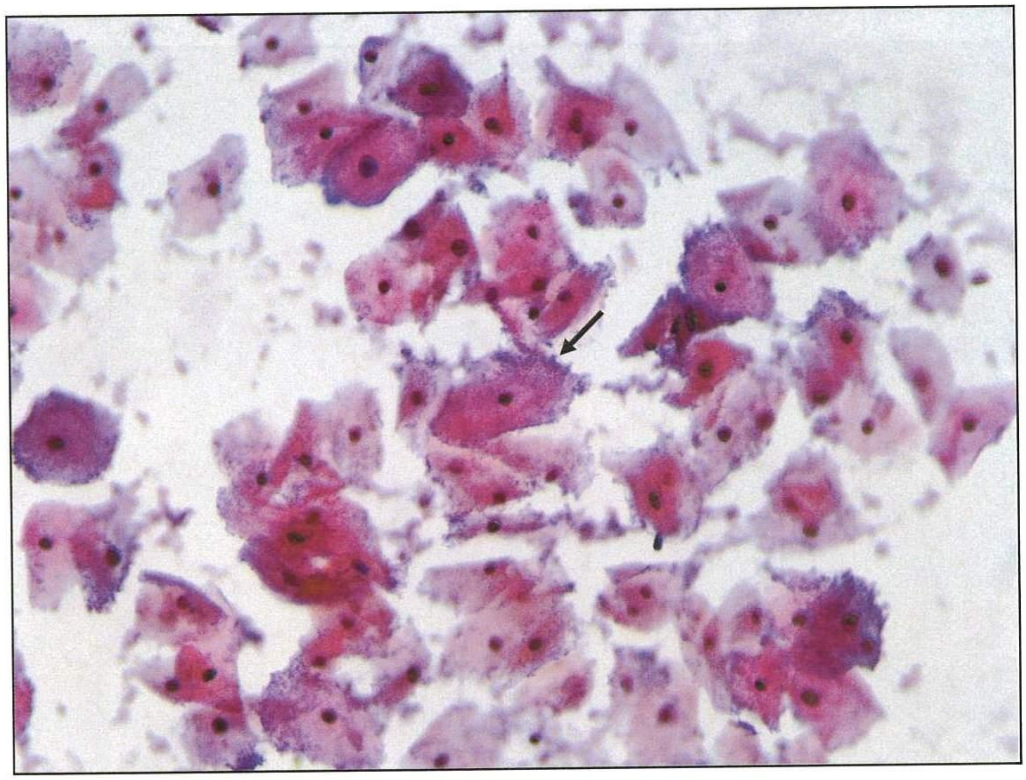


Plate 1: Cervical Pap smear staining showing abundant clue cells (arrow) suggestive of bacteria vaginosis more likely due to *Gardnerella vaginalis* infection (X 40).

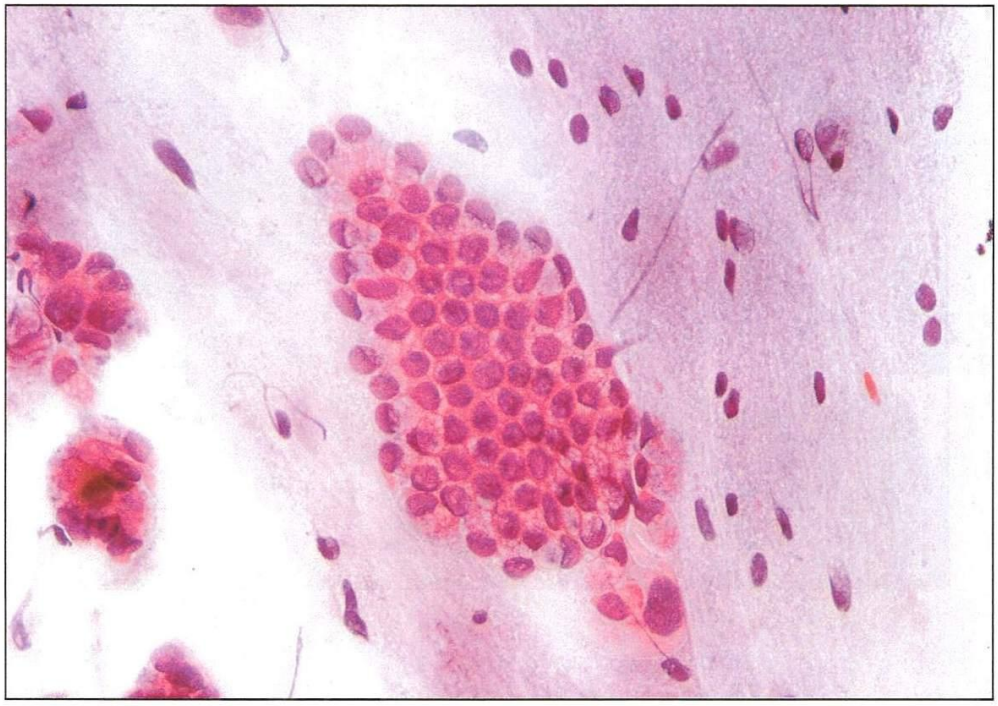


Plate II: Cervical Pap smear staining in a patient with acute cervicitis with abundant clusters of endocervical columnar cells with a honeycomb pattern (X 40).

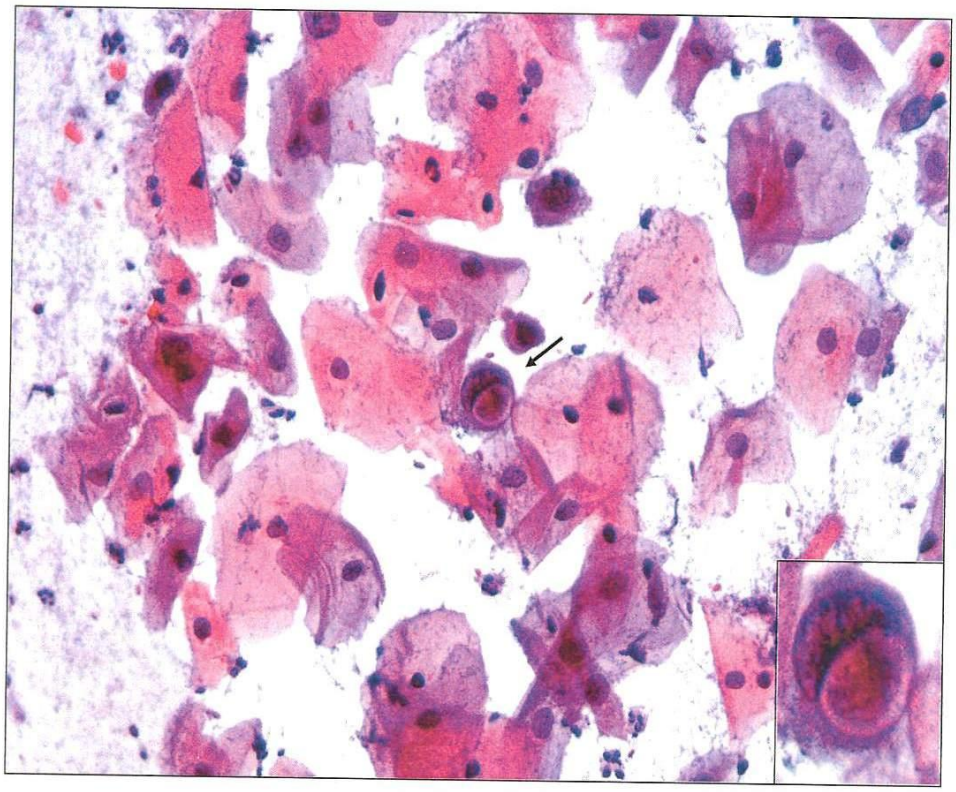


Plate III: Cervical Pap smear staining in a patient with acute cervicitis showing a squamous cell with cytopathic changes characterized by the presence of a cytoplasmic inclusion body (arrow and inset) indicating chlamydial infection (X 40).

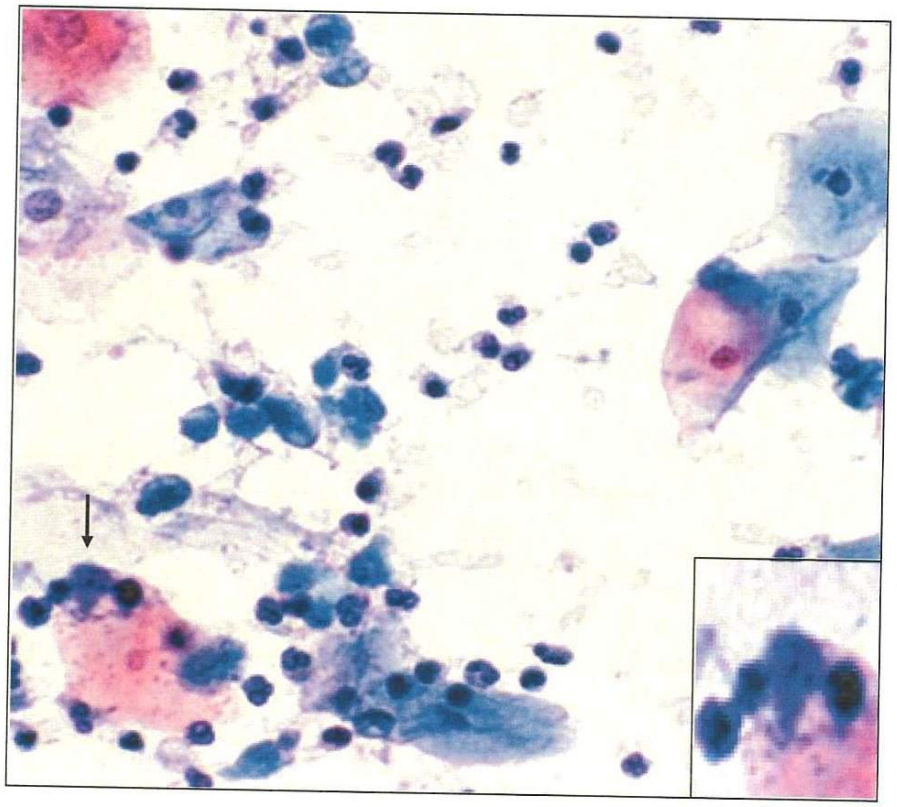


Plate IV: Cervical Pap smear staining showing acute cervicitis with abundant neutrophils. The arrow and inset showed a superficial squamous cell with a *Trichomona vaginalis* (X 40).

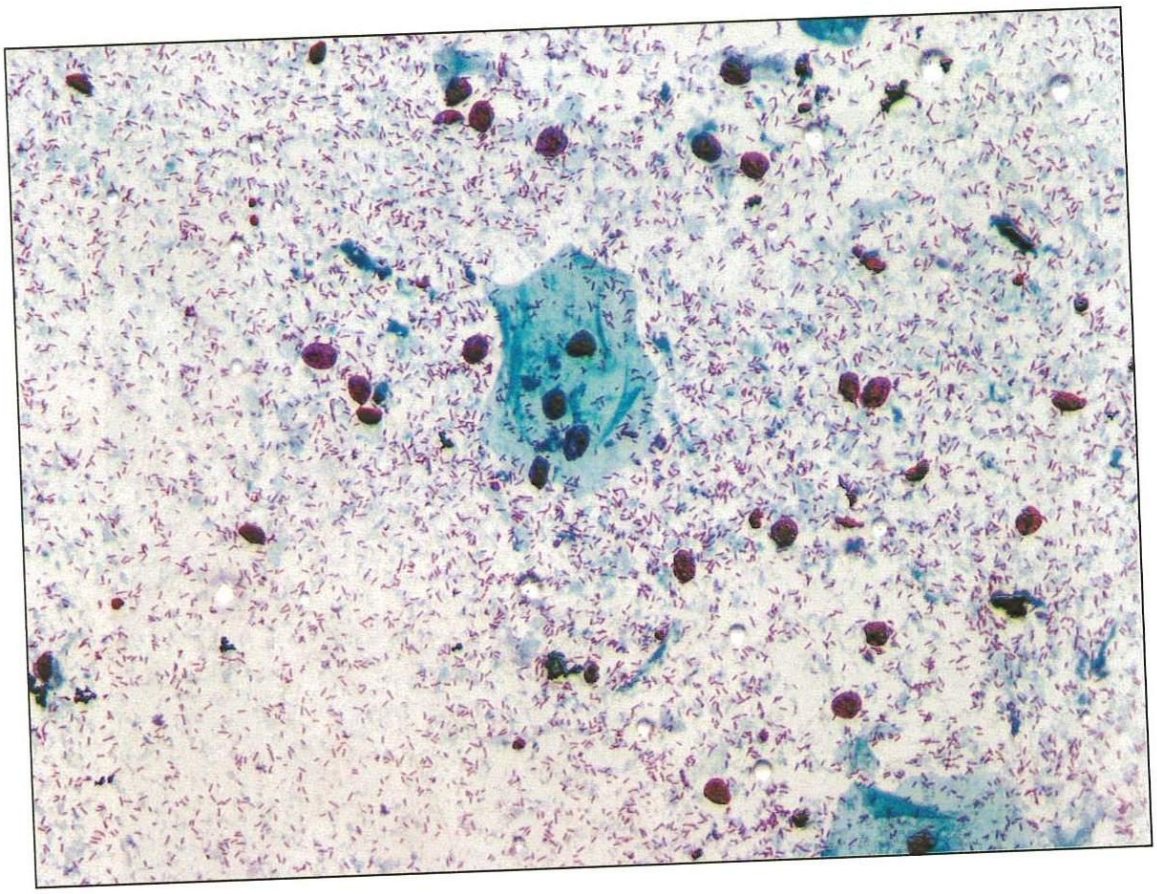


Plate V: Cervical Pap smear staining showing chronic cervicitis with scattered lymphocytes (X 40).

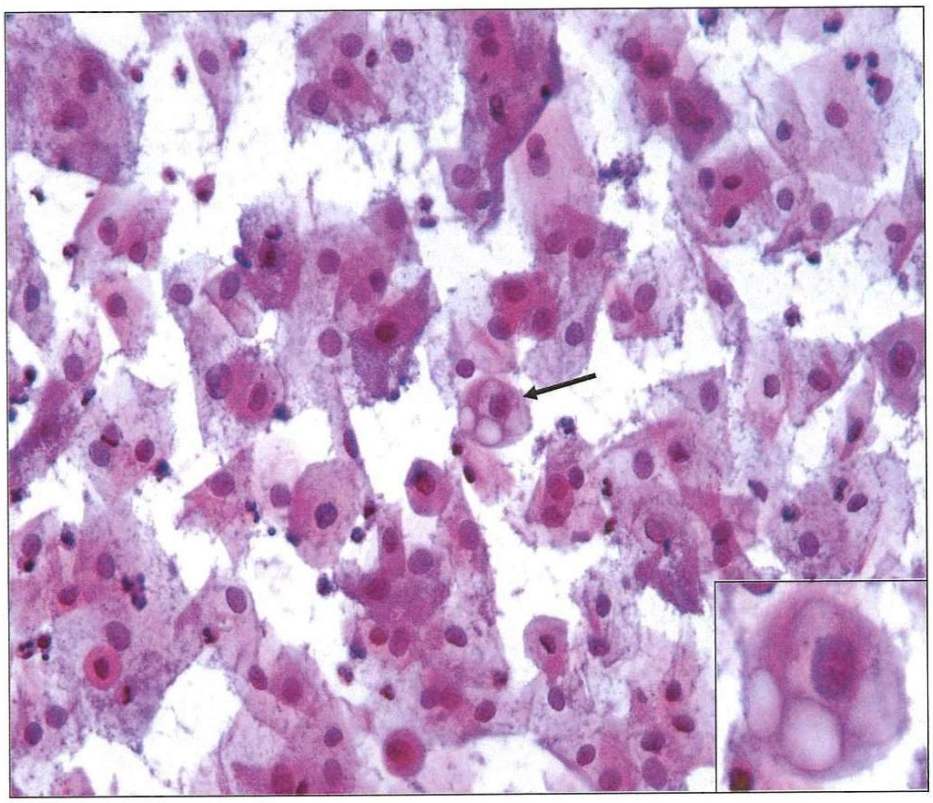


Plate VI: Cervical Pap smear staining showing LSIL with an inflammatory background and a cell with multiple cytoplasmic vacuolation (arrow and inset) suggestive of viral infection (X 40).

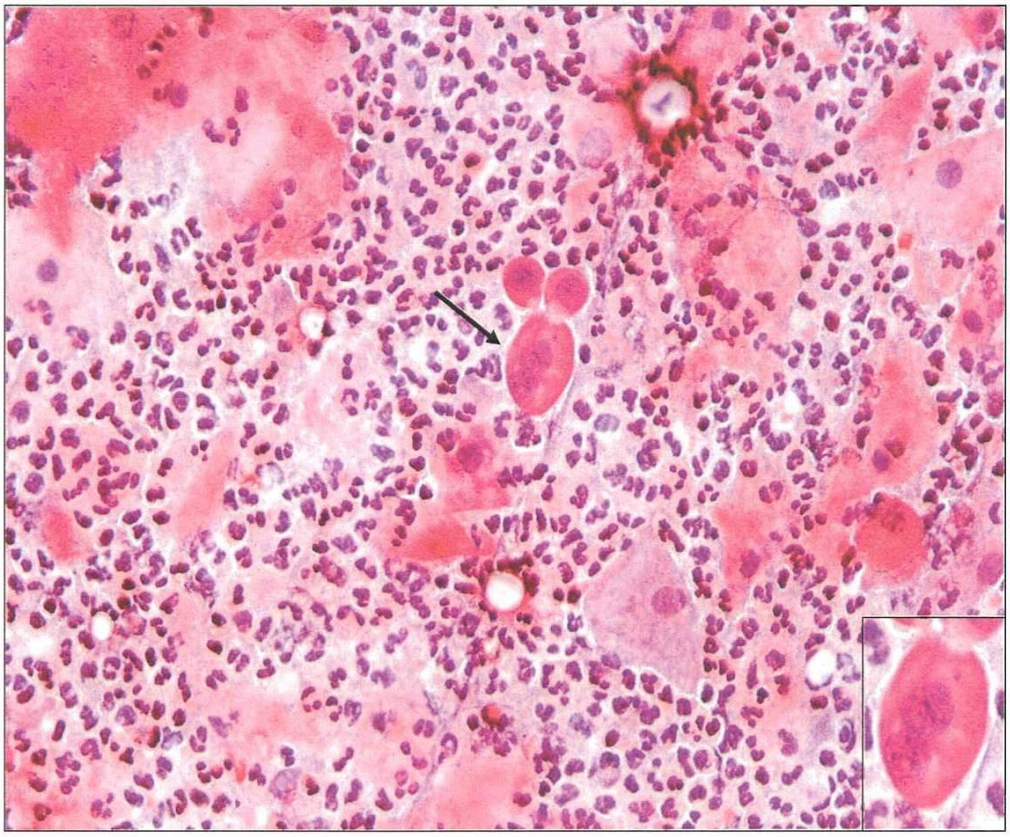


Plate VII: Cervical Pap smear staining in a patient with LSIL showing extensive polymorphnucler leukocytic background and a binucleated parabasal squamous cell with mild nuclear enlargement and fine granular chromatin (arrow and inset) suggestive of viral infection (X 40).

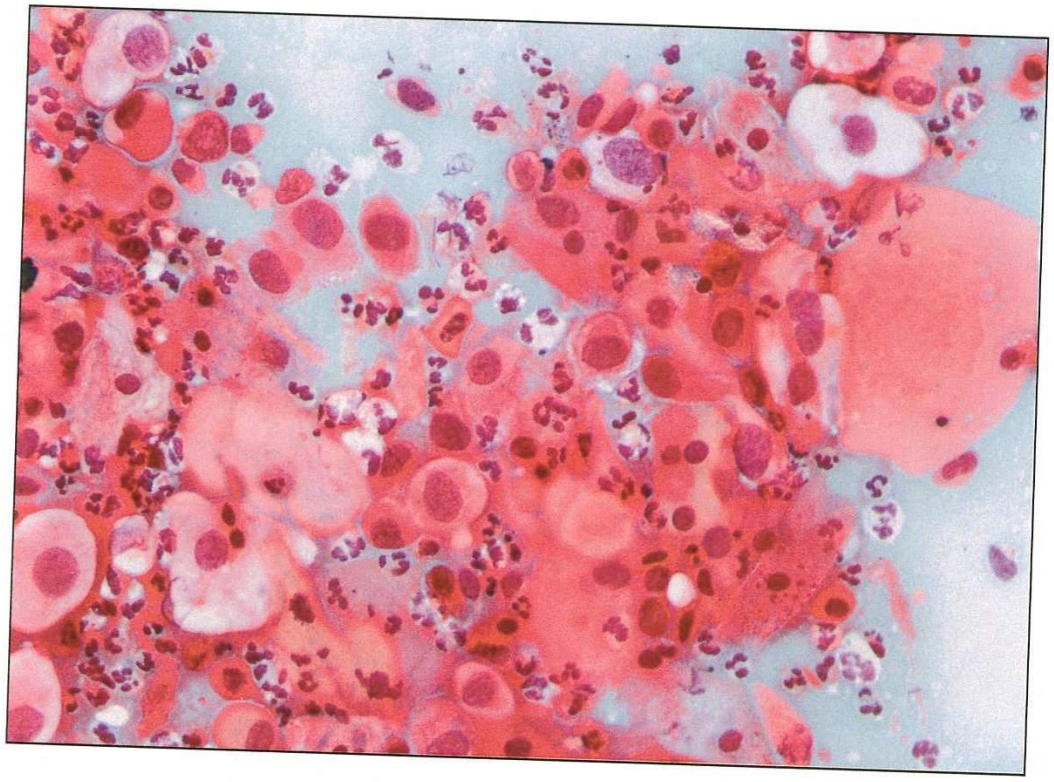


Plate VIII: Cervical Pap smear staining in a patient with HSIL showing moderated to severe nuclear enlargement and orangeophilic cells in an inflammatory background (X 40).

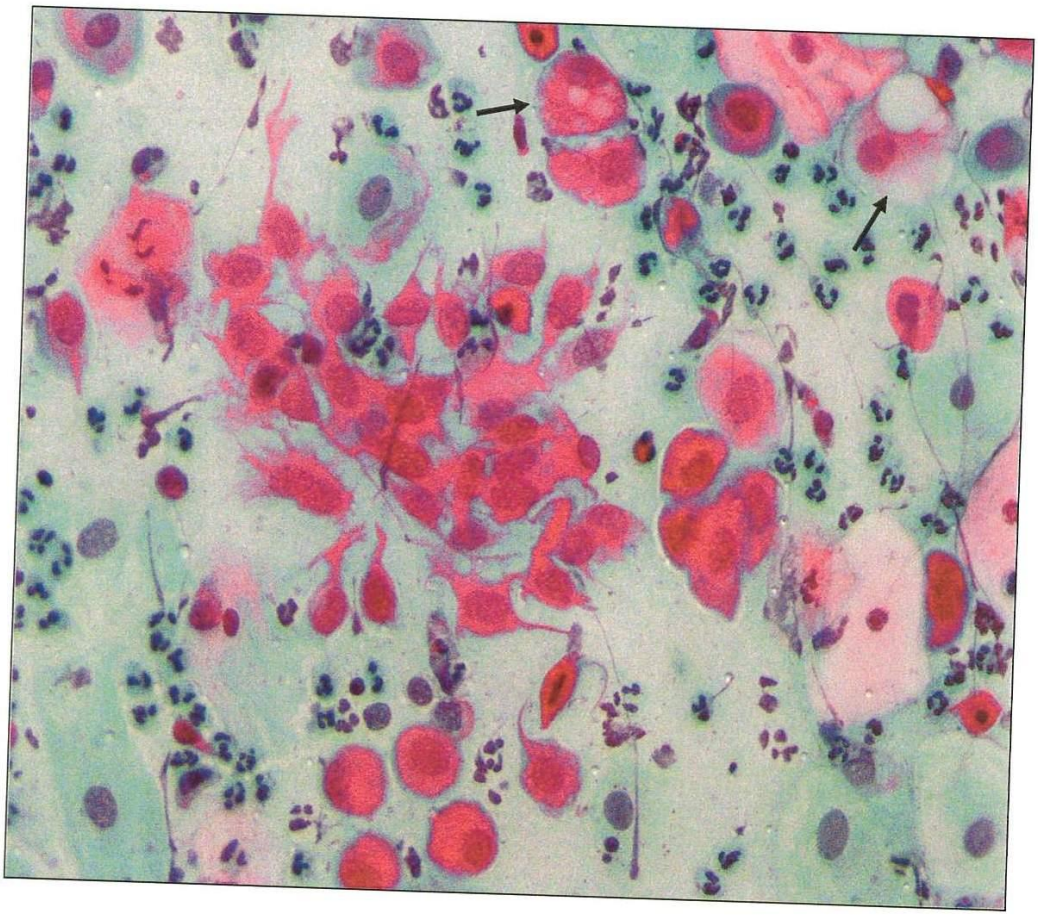


Plate IX: Cervical Pap smear staining in a patient with HSIL showing abundant neutrophils and in the center there is a cluster of dendritic cells. The arrows show a parabasal squamous cell with multiple cytoplasmic vacuolation suggestive of viral infection (X 40).

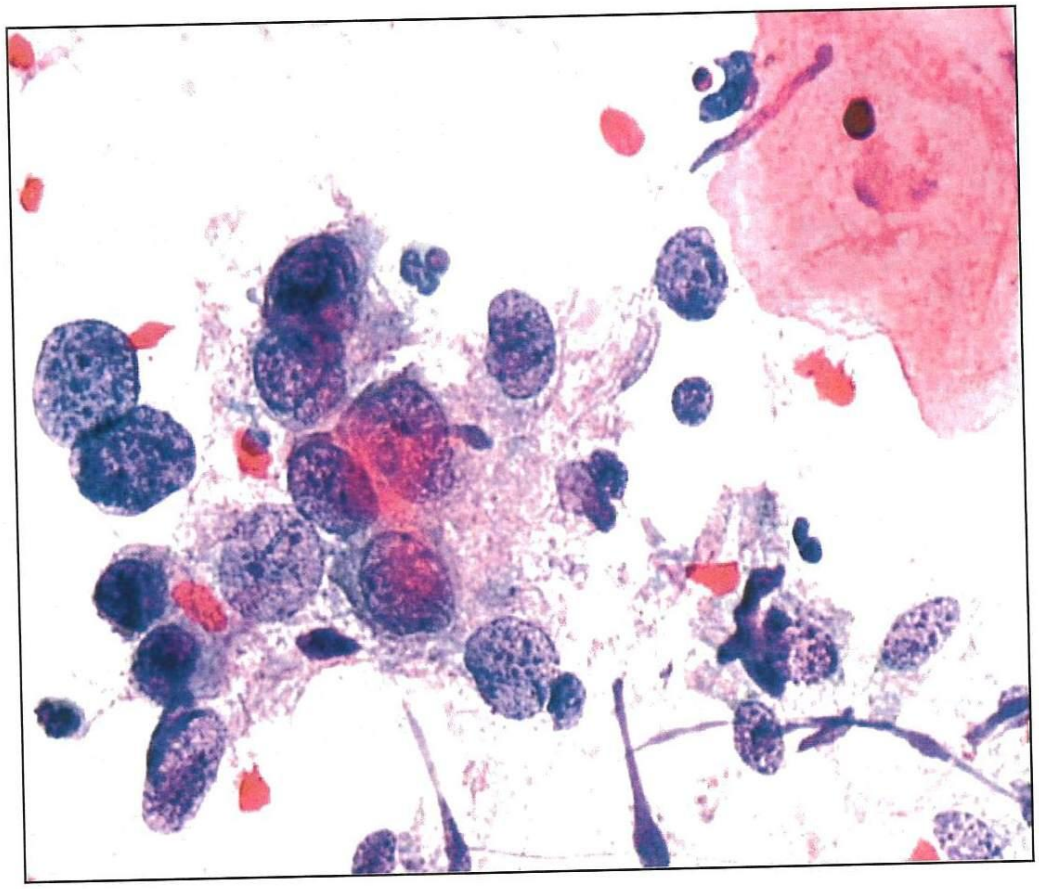


Plate X: Cervical Pap smear staining in a patient with adenocarcinoma showing atypical clusters of columnar cells (X 40).

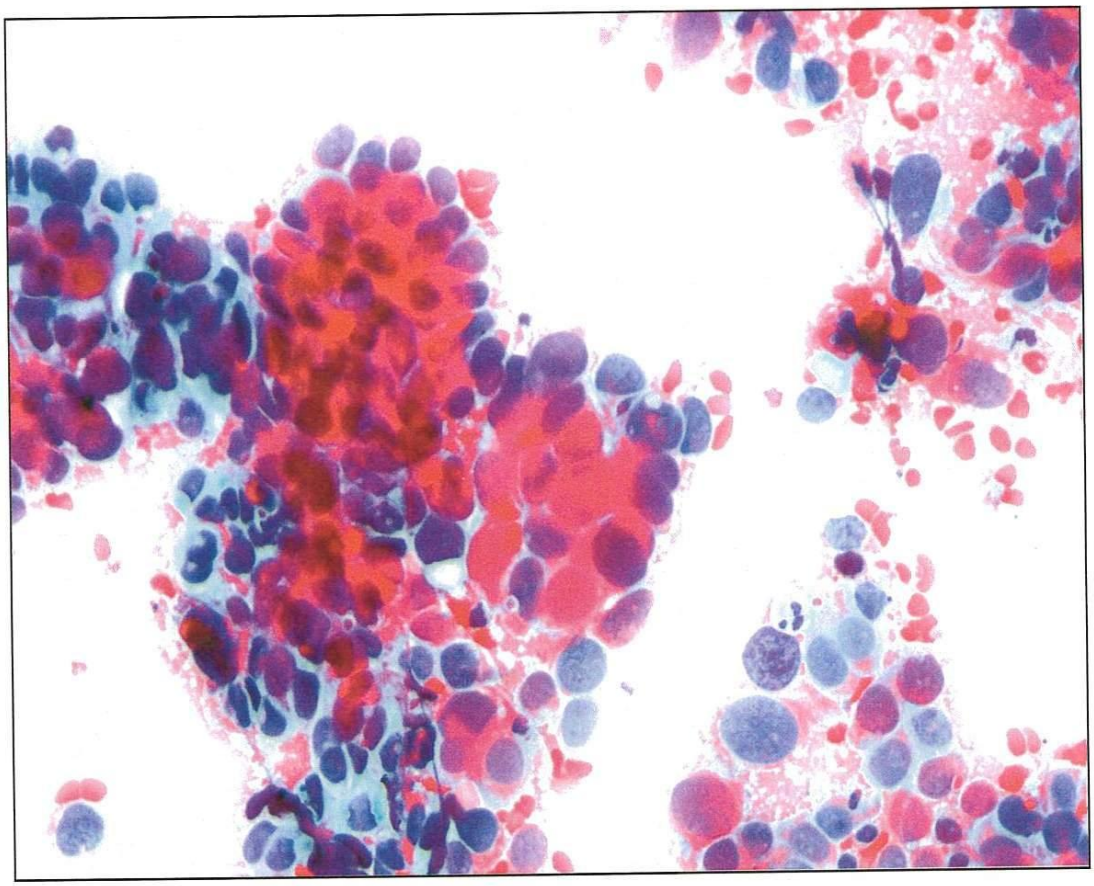


Plate XI: Cervical Pap smear staining in a patient with squamous carcinoma, which exhibits clusters of cohesive cells with marked nuclear enlargement, pleomorphisms, hyperchromatism and keratinization (X 40).

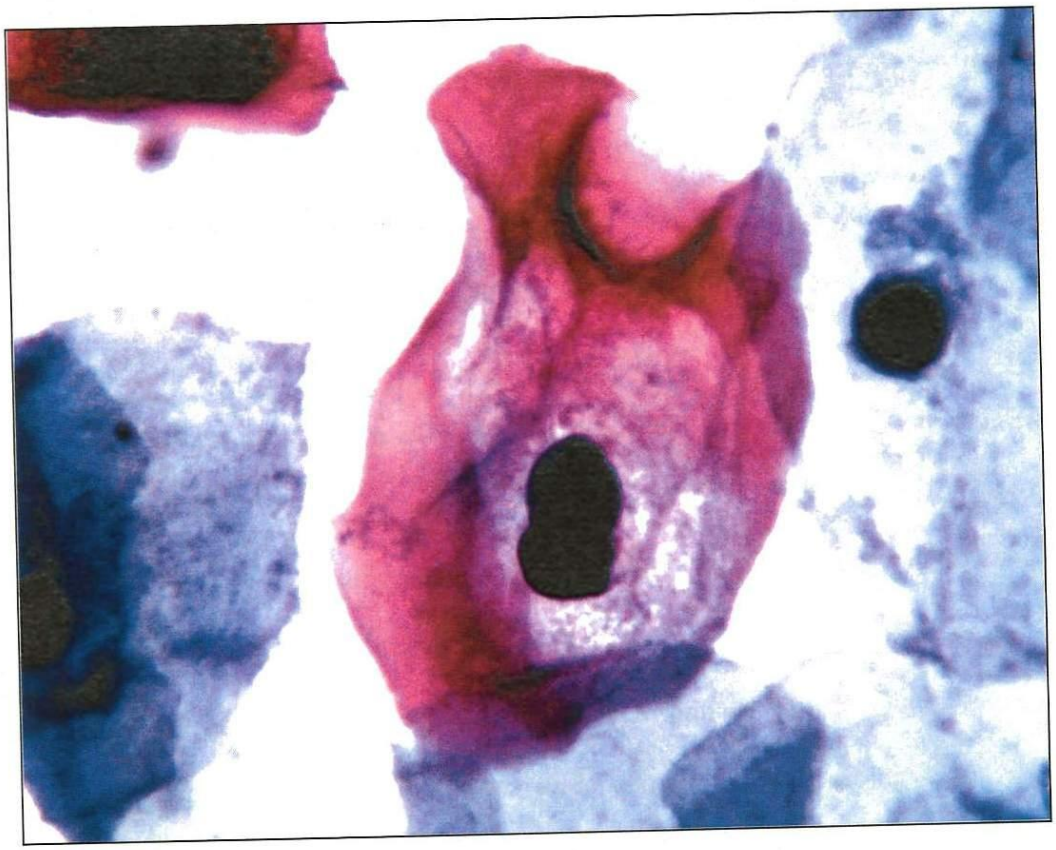


Plate XII: Cervical Pap smear staining showing a cell with binucleation and perinuclear halo indicative of HPV infection (X 40).

Plates Legends

PLATE I: Acute cervicitis displaying abundant clue cells, Pap staining X 40.

PLATE II: Acute cervicitis with clusters of endocervical cells, Pap staining X 40.

PLATE III: Acute cervicitis with chlamydial changes, Pap staining X 40.

PLATE IV: Acute cervicitis with trichomoniasis, Pap staining X 40.

PLATE V: Chronic Follicular Cervicitis with scattered lymphocytes, Pap staining X 40.

PLATE VI: LSIL with cytological changes suggestive of viral infection, Pap staining X 40.

PLATE VII: LSIL with binucleated cell, Pap staining X 40.

PLATE VIII: Cytological features of HSIL, Pap staining X 40.

PLATE IX: HSIL with abundant dendritic cells, Pap staining X 40.

PLATE X: Cytological features of cervical adenocarcinoma, Pap staining X 40.

PLATE X1: Cytological features of cervical squamous cell carcinoma, Pap staining X 40.

PLATE XII: Cytological features suggestive of HPV infection, Pap staining X 40.

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