Section: Cytopathology

Original Research Article

STUDY OF CONVENTIONAL PAP SMEARS IN HIV POSITIVE FEMALES AT A TERTIARY HEALTHCARE CENTRE IN GUJARAT

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ABSTRACT

Background: According to the WHO, cervical cancer is the fourth most common cancer in females worldwide and the second most common in India. The immunocompromised state of HIV is associated with a host of other viral infections, including the Human Papillomavirus (HPV). Cervical cancer is the commonest HPV related disease and is considered an "AIDS defining illness". HIV infected women are more likely to develop faster, more aggressive, invasive cervical cancer, which is less responsive to treatment and has a poorer prognosis. It is therefore very essential to routinely screen HIV infected females for cervical intraepithelial lesions and malignancy, to provide early diagnosis and timely treatment. **Materials and Methods:** A cross-sectional study of Conventional Pap smears of 51 females who were known cases of HIV was done at a tertiary health care centre. The Rapid-PAP and Haematoxylin & Eosin-stained smears were evaluated and reported according to the Bethesda reporting system, 2014. **Results:** The cases ranged from 21 to 64 years, with most in the 31-40 age group. The majority of the cases had complaints of discharge, followed by 'bleed on touch' or abnormal bleeding. The study found that 27.45% of cases showed epithelial cell abnormalities, ranging from ASCUS to SCC. **Conclusion:** HIV positive women are more likely to have epithelial cell abnormalities. Screening for these with the help of a conventional Pap smear is an easy, cost-effective, relatively less invasive, and fast method for early diagnosis of such cases. Therefore, routine screening is vital in these patients.

INTRODUCTION

According to the data of 2022 by the Global Cancer Observatory, WHO, the fourth most common cancer in females worldwide is the cancer of the cervix uteri.[1] In India, it is second only to breast cancer, with over one lakh estimated cases [2] and more than 79000 deaths.[3] There has been a substantial decline in the incidence of cervical cancer in India over the years,[4] which may be attributed to regular screening and eventual early detection of cervical intraepithelial lesions by Pap smear, vaccination, and advances in treatment modalities.

With the implementation of various awareness programs and an increase in the number of HIV (Human Immunodeficiency Virus) patients accessing ART (Anti-Retroviral Therapy),[5] there is an increase in the life expectancy of People Living With HIV-AIDS (PLWHA). The immunocompromised state of HIV is known to be a facilitator in acquiring a host of other viral infections, including the Human Papillomavirus (HPV). The prevalence of HPV infection is much higher in HIV patients as compared to those who are not infected with HIV.[5]

Cervical cancer is the commonest HPV related disease [6] and is considered an "AIDS defining illness".[7] Besides, it has been studied that HIV infected women are more likely to develop faster, more aggressive, invasive cervical cancer, which is less responsive to treatment and has a poorer prognosis.[8] It is therefore very essential to routinely screen HIV infected females for cervical intraepithelial lesions and malignancy, to provide early diagnosis and timely treatment.

MATERIALS AND METHODS

The present study is a cross-sectional study of Conventional Pap smears of 51 females who are known cases of HIV, performed at a tertiary health care center. Two unstained smears prepared using the conventional Pap smear technique were received. These were stained with the Rapid-PAP staining procedure and Hematoxylin and Eosin stain,

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respectively. The smears were evaluated and reported according to the Bethesda reporting system, 2014. Observations were tabulated with the help of Microsoft Excel.

RESULTS

Of the 51 cases studied, the age of the youngest case was 21, and that of the eldest was 64. Most females were in the age group of 31-40 (41.18%), closely followed by those in the age group of 41-50 (35.39%). Table 1 shows the frequency of cases according to age groups.

The following line graph shows the observed trend in the age of the studied cases.

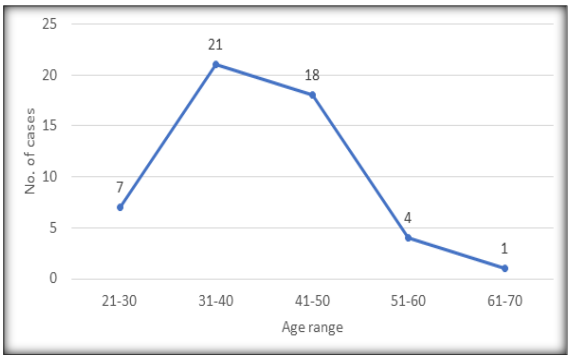

Chart 1: Frequency according to age range

As seen in Table 2, of the 51 cases, 18 (35.29%) were relatively asymptomatic. Discharge was the commonest presenting symptom in 21 cases (41.18%), followed by bleeding in 5 cases (9.8%). 3 cases each had a history of mixed infection and prolapse, and one case had a history of treatment with chemotherapy and radiotherapy for a previously diagnosed cervical malignancy.

Chart 2 depicts the frequency distribution of the presenting features

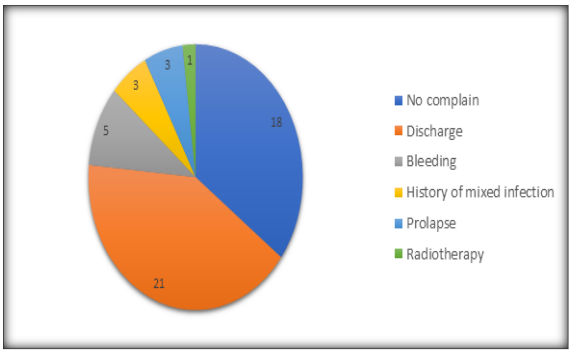

Chart 2: Frequency distribution of presenting signs/symptoms

On studying the smears, 37 out of 51 (72.55%) cases were diagnosed as NILM (Negative for Intraepithelial Lesion or Malignancy), whereas 12 of the 51 cases (23.53%) showed epithelial cell abnormalities, and 2 (3.92%) were unsatisfactory for evaluation.

Of the 12 cases with epithelial cell abnormalities, 4 were diagnosed as ASC-US (Abnormal Squamous Cells of Undetermined Significance), 4 as LSIL (Low-grade Squamous Intraepithelial Lesion), 3 as HSIL (High-grade Squamous Intraepithelial Lesion), and one was diagnosed as SCC (squamous cell carcinoma).

Table 3 and Chart 3 show the frequency distribution of cases according to the diagnosis, according to the 2014 Bethesda System for Reporting Cervical Cytology.

Table 1: Frequency according to age

Age	No. of cases	Percentage
21-30	7	13.73%
31-40	21	41.18%
41-50	18	35.29%
51-60	4	7.84%
61-70	1	1.96%
Grand Total	**51**	**100.00%**

Table 2: Frequency of presenting signs/symptoms

Presenting signs/symptoms	No. of cases	Percentage
No complaint	18	35.29%
Discharge	21	41.18%
Bleeding	5	9.80%
History of mixed infection	3	5.88%
Prolapse	3	5.88%
Radiotherapy	1	1.96%
Grand Total	**51**	**100.00%**

Table 3: Frequency according to diagnosis

Diagnosis	Number of cases	Percentage
NILM	37	72.55%
ASC-US	4	7.84%
LSIL	4	7.84%
HSIL	3	5.88%
SCC	1	1.96%
Unsatisfactory	2	3.92%
Grand Total	**51**	**100.00%**

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Cases that were diagnosed as NILM were further categorized as NILM without inflammation, NILM with inflammation (Inflammatory smears), and NILM with Trichomonas Infection, as shown in Table 4.

Table 4: Frequency distribution of subcategories of NILM

Diagnosis	No. of cases	Percentage
NILM without inflammation	29	56.86%
NILM - Inflammatory smears	6	11.76%
NILM - Trichomonas	2	3.92%
Total	**37**	**72.55%**

As shown in Table 5, when the age groups were clustered according to the different diagnoses, most cases with epithelial cell abnormalities were seen in women over the age of 30.

Table 5: Age groups clustered according to diagnoses

Age groups clustered according to diagnosis.	No. of cases
NILM	**37**
21-30	6
31-40	17
41-50	11
51-60	3
ASC-US	**4**
21-30	1
31-40	1
41-50	2
LSIL	**4**
31-40	1
41-50	2
61-70	1
HSIL	**3**
41-50	2
51-60	1
SCC	**1**
41-50	1
Unsatisfactory	**2**
31-40	2
Grand Total	**51**

As presented in Table 6, on clustering the presenting findings of the cases concerning the diagnosis, it was observed that 5 out of 8 patients with intraepithelial lesions or suspicion for malignancy had a history of excessive bleeding/ bleed on touch/ postmenopausal bleeding.

One case with a history of radiotherapy and chemotherapy for a previously diagnosed cervical malignancy was diagnosed as having HSIL (High grade Squamous Intraepithelial Lesion), one case diagnosed as having LSIL (Low grade Squamous Intraepithelial Lesion) presented with uterovaginal prolapse, while the other had no presenting complaint.

Table 6: Presenting features clustered according to diagnoses

Presenting signs/symptoms	No. of cases
NILM	**29**
Discharge	12
History of mixed infection	1
No complaint	14
Prolapse	2
NILM - Trichomonas	**2**
Discharge	1
History of mixed infection	1
NILM - Inflammatory smears	**6**
Discharge	4
No complaint	2
ASC-US	**4**
Discharge	2
History of mixed infection	1
No complaint	1
LSIL	**4**
Bleeding	2
No complaint	1
Prolapse	1
HSIL	**3**

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Bleeding	2
H/o – Radiotherapy	1
SCC	**1**
Bleeding	1
Unsatisfactory	**2**
Discharge	2
Grand Total	**51**

DISCUSSION

The results of the present study were compared with 10 other studies that have evaluated and described the results of conventional Pap smear in HIV positive females.

The age range of the population in the present study is 21-64 years, which was comparable to the age range in the studies conducted by Bagga et al,[9] Chalermchockcharoenkit et al,[10] Gaym et al,[11] Jha et al,[12] and Jennifer et al,[13] as shown in Table 7.

Table 7: Age range of the population in different studies

Study	Age Range
Bagga et al. [9]	21-58 years
Chalermchockcharoenkit et al. [10]	14-65 years
Gaym et al. [11]	15-55 years
Jha et al. [12]	18-71 years
Jennifer et al. [13]	20-60 years
Present study	21-64 years

In the present study, epithelial cell abnormalities (ECA) are seen mainly in cases over 30 years. When compared to the different studies, it was seen that the age group with the majority of cases of ECA in studies by Devi & Priya,[14] Gaym et al,[11] and Jha et al,[12] were younger than the present study; those studied by Gupta et al,[15] were older; and the majority of cases of ECA in studies by Getinet et al,[16] and Madan et al,[17] belonged to a similar age group as the present study. (Table 8)

Table 8: Age group showing the majority of cases of ECA in different studies

Study	The majority of cases with ECA
Devi and Priya [14]	26-30 years
Gaym et al. [11]	<30 years
Getinet et al. [16]	30-45 years
Gupta et al. [15]	35-49 years
Jha et al. [12]	26-35 years
Madan et al. [17]	31-40 years
Present study	31-40 years

The most common clinical presentation in the present study was abnormal vaginal discharge (seen in 41.18% cases) and abnormal bleeding/ bleed on touch was seen in 9.8% cases. Other studies that have described the clinical presentation show the following findings: (Table 9)

Table 9: Clinical findings in different studies

Clinical findings	Bagga et al. [9]	Getinet et al. [16]	Jha et al. [12]	Present study
Most prevalent Clinical finding	Abnormal vaginal discharge	Abnormal vaginal discharge	Abdominal pain	Abnormal vaginal discharge
Discharge	35.30%	14%	13.78%	41.18%
Abdominal pain	-	-	15.85%	-
Cervical erosion	32.35%	-	13.52%	-
Hypertrophied cervix	-	-	3.32%	-
Prolapse	-	-	2.55%	5.88%
Bleeding	-	2%		9.8%

Reporting was done according to The Bethesda System for Reporting Cervical Cytology [18]. The diagnoses of the studied cases of the present study were compared with those of the other studies and tabulated as under (Table 10)

Table 10: Comparison of diagnoses of studied cases of different studies

Diagnosis	Bagga et al. [9]	Chalermchockcharoenkit et al. [10]	Devi and Priya [14]	Gaym et al. [11]	Getinet et al. [16]	Gupta et al. [15]	Jha et al. [12]	Jennifer et al. [13]	Leibenson et al. [19]	Madan et al. [17]	Present study
ECA	34.60 %	15.40%	7.14 %	16.90 %	17.80 %	12%	8.34 %	66.30 %	20.24%	12.60 %	23.53 %

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NILM	65.40 %	84.60%	92.86 %	83.40 %	82.20 %	88%	87.89 %	34.50 %	79.76%	87.39 %	72.55 %
ASC-US	1.50 %	2.80%	0.80 %	10.52 %	5.60%	0.67 %	3.19 %	15.30 %	-	4.62%	7.84%
ASC-H	-	0.60%	-	-	-	2%	0.73 %	1%	-	1.68%	-
LSIL	5.90 %	8.50%	4.37 %	5.15 %	6.10%	0.67 %	2.70 %	40%	19.05%	2.10%	7.84%
HSIL	2.20 %	3.50%	1.20 %	4.36 %	5.10%	7.33 %	1.47 %	10.20 %	1.19%	3.36%	5.88%
SCC	0.70 %	0.10%	-	-	1%	1.33 %	-	-	-	0.80%	1.96%
AGUS	-	-	0.80 %	-	-	-	0.25 %	-	-	-	-

The above findings show that the percentage of cases with Epithelial cell abnormalities (ECA) in the present study is comparable to most of the studies. Whereas, the study by Jennifer et al,[13] shows a very high percentage (66.30%) of the cases to have epithelial cell abnormalities.

The most common diagnosis in all studies except Jennifer et al,[13] (34.50%), NILM (Negative for Intraepithelial Lesion or Malignancy), which is similar in the present study, with 72.55% cases diagnosed as NILM.

The most common diagnosis by Jennifer et al,[13] was HSIL (40%), which was seen in 5.88% of cases in the present study.

Image below (Image 1) shows a crowded cluster of dysplastic cells with a high N:C ratio, irregular nuclear border, and immature cytoplasm in a case of HSIL in the present study. The cells show uneven nuclear enlargement.

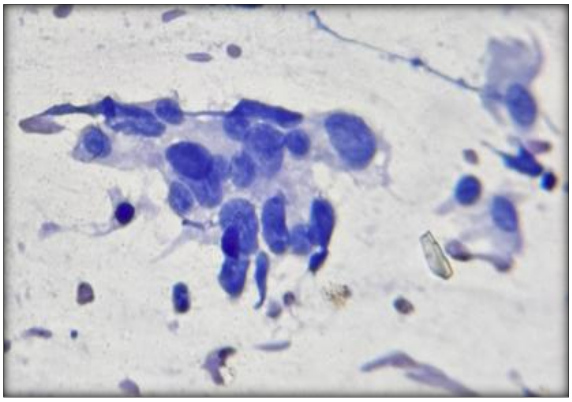

Image 1: High-grade Squamous Intraepithelial Lesion (HSIL)

After NILM, 7.84% of cases of ASCUS and LSIL were noted in the present study. ASCUS was the second most common diagnosis in studies by Gaym et al. [11] (10.52%), Jennifer et al. [13] (15.30%), Jha et al. [12] (3.19%), and Madan et al. [17] (4,62%).

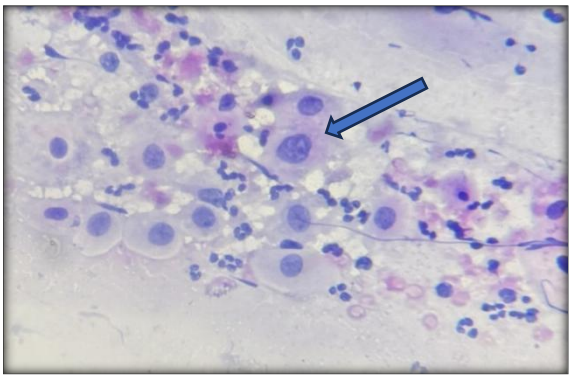

Image 2: Atypical Squamous Cells of Undetermined Significance (ASC-US)

Image 2 shows Atypical squamous cells (marked by an arrow) with 2.5 to 3 times nuclear enlargement, irregular nuclear contour and chromatin, with adjacent normal-looking intermediate squamous cells as seen in a case diagnosed as ASC-US in the present study.

Image 3 shows a cluster of atypical cells with 3 times nuclear enlargement, nuclear hyperchromasia, and irregular nuclear contour with perinuclear clearing (Koilocytic changes) and mature cytoplasm of a case of LSIL in the present study.

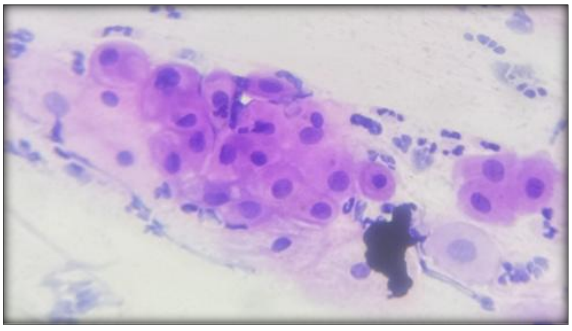

Image 3: Low-grade Squamous Intraepithelial Lesion (LSIL)

LSIL was the second most common diagnosis in studies by Bagga et al,[9] (5.905), Chalermchockcharoenkit et al,[10] (8.50%), Devi & Priya,[14] (4.37%), Getinet et al,[16] (6.10%), and Leibenson et al,[19] (19.05%).

The present study has one case (1.96%) reported as SCC (squamous cell carcinoma), which is

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comparable to the studies by Bagga et al,[9] (0.70%), Getinet et al,[16] (1%), Gupta et al,[15] (1.33%), and Madan et al,[17] (0.80%).

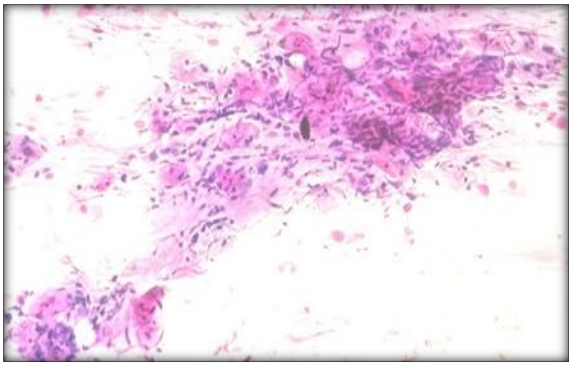

Image 4: Squamous Cell Carcinoma (SCC) on low power

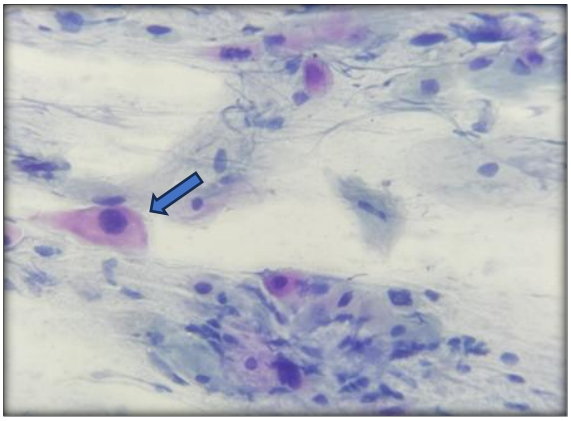

Image 4: Squamous Cell Carcinoma (SCC) on high power

The above images (Image 4 and Image 5) depict singly scattered and clusters of pleomorphic malignant cells with high N:C ratio, variable nuclear chromatin (vesicular to pyknotic), and a few "tadpole cells" (marked by an arrow) are also seen with tumor diathesis in the background.

The present study has not identified any case of ASC-H (Atypical Squamous Cells, cannot rule out HSIL), which has been reported by Chalermchockcharoenkit et al,[10] (0.60%), Gupta et al,[15] (2%), Jha et al,[12] (0.73%), Jennifer et al,[13] (1%), and Madan et al,[17] (1.68%).

Studies by Devi & Priya,[14] and Jha et al,[12] have also described Atypical glandular cells of Undetermined Significance (AGUS) as 0.80% and 0.25% of the cases, respectively. The present study has not identified any case of AGUS.

On comparing the studies for NILM with inflammation or specific infections, the following results were observed: (Table 11)

The present study identified 37 of the 51 cases (72.55%) as NILM, of these, 6 cases (11.76%) were NILM with non-specific inflammation. Madan et al,[17] has identified 14.28% cases as NILM with non-specific inflammation, and Jha et al,[12] have identified 1.52% cases in the same category.

The present study has identified 2 cases (3.92%) with Trichomonas infection, and no other organism was detected. Trichomonas infection was seen in 1.60% of cases in the study by Chalermchockcharoenkit et al,[10] 0.50% of the cases in the study by Jha et al,[12] and 1.68% of cases in the study by Madan et al.[17]

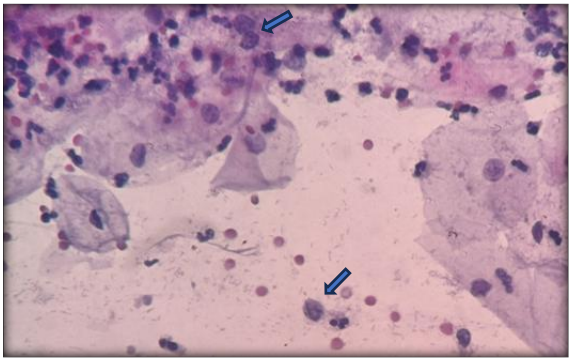

Image 6: NILM with Trichomonas vaginalis

Image 6 is of a case diagnosed as being infected with Trichomonas vaginalis organism (marked by arrow), the pear-shaped, cyanophilic organism seen in the present study, with a background of acute inflammatory cells.

The most common organisms identified by Chalermchockcharoenkit et al,[10] and Madan et al,[17] were Candida and budding yeasts, and that by Jha et al,[12] was Bacterial vaginosis.

Table 11: Comparison of subcategories of NILM

Parameter	Chalermchockcharoenkit et al. [10]	Jha et al. [12]	Madan et al. [17]	Present study
NILM	84.60%	87.89%	87.39%	72.55%
NILM with nonspecific inflammation	-	1.52%	14.28%	11.76%
Candida and budding yeasts	19.40%	1.78%	2.52%	-
Trichomonas vaginalis	1.60%	0.50%	1.68%	3.92%
Bacterial vaginosis	0.20%	29.39%	2.10%	-
Mixed infection	-	2.01%	-	-
Herpes simplex virus	1.50%	0.24	-	-
Reactive cellular changes associated with inflammation	11.70%	-	-	-

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CONCLUSION

The present study and all the comparative studies taken into consideration show that a high number of cases with HIV have epithelial cell abnormalities. Screening with the conventional Pap smear helps in the early detection of these abnormalities and, therefore, early intervention. It is therefore necessary that more such studies be conducted, and screening by Pap smear be regularly done in HIV positive patients.

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