A Study of Cervical Cytological Changes in Hiv Positive Patients

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Abstract :

Background: Gynaecological conditions are extremely common among women infected with Human Immunodeficiency Virus. These include pelvic inflammatory disease, Sexually Transmitted Diseases like syphilis, Herpes, Cytomegalo virus, Vulvovaginal Candidiasis etc.

Objectives: The purpose of the study is to characterize the cervical cytological changes in HIV seropositive women.

Materials & Methods: This cross sectional study was based on 200 patients with HIVinfection /AIDS, who attended the department of pathology and Gynaecology, Guntur medical college, Guntur.

Results: The study revealed maximum number of inflammatory smears (81.3%), followed by smears showing squamous intra epithelial lesion (13.82%).

Conclusion: PAP smear can be recommended as a part of routine medical examination in gynaecological practice as it is a simple, cost effective, low risk procedure.

Keywords: HIV/AIDS, papanicolaou smear, cervix, inflammatory lesions.

I. Introduction

Over the past decade, the Acquired Immuno Deficiency Syndrome epidemic in India has grown from a handful of Human Immunodeficiency Virus infected persons to a major public health problem with tremendously growing medical, psychological and economic consequences for the country. In India around 22% of total reported Acquired Immuno Deficiency syndrome cases and around 30% of newer Human Immunodeficiency Virus infections were among women. Gynaecological conditions are extremely common among women infected with Human Immunodeficiency Virus. These include pelvic inflammatory disease, Sexually Transmitted Diseases like syphilis, Herpes, Cytomegalovirus, Vulvovaginal Candidiasis etc. The HIVseropositive women have higher chances of cervical intra epithelial neoplasia, vaginal intraepithelial neoplasia and vulval intra epithelial neoplasia. Occurence of cervical cancers associated with Human Immunodeficiency Virus infection can be easily prevented if detected early. The cytological findings obtained in this cohort have been presented. The study highlights and ascertains the utility of cervical smear cytology in picking up early premalignant conditions of cervical cancer and associated changes in various infections.

It is thought that the cervical intraepithelial neoplasia is about 15-20 years progresses to invasive cancer. This prolonged natural history along with the exfoliative nature of precancerous lesions, easy accessibility of cervix to inspection, Palpation and application of cytological procedures have lead to extensive screening programmes for early detection and treatment of disease. Thus cytological screening should gain much popularity and should be accessible to all. It is recommended as a part of routine medical examination in gynaecological practice.Papanicolaou smear screening is an adequate tool for detecting cytologic changes in women with Human Immunodeficiency Virus, a group at increase risk for squamous intraepithelial lesion. Cervical cancer is a preventable disease if women receive regular screening and timely followup and treatment after abnormal papanicolaou smear result.

II. Materials And Methods

This cross sectional study was based on the patients with HIVinfection /AIDS, with Gynaecological problems, pelvic inflammatory diseases, who were referred to the department of pathology and Gynaecology, Guntur medical college,Guntur from September 2013 to August2015. In the present study 200 cases were included. Pregnant women and post hysterectomised women were excluded from the study.

A thorough history was taken with particular reference to age, socioeconomic status, parity, menstrual history, duration of HIV infection, husband health status, STDs, personal and genital hygiene, opportunistic infections, vaginal discharge, CD4 cell count and ART treatment. Cervix was visualized with Sims speculum and associated conditions like erosion, ectropion, hypertrophy, endocervicitis, suspicious growth on cervix and

elongation of cervix were noted. After a thorough vaginal examination pap smears were taken. Smears were immediately fixed in isopropyl alchohol and stained according to the Papanicolaou' s technique. Haematoxylin and Eosin staining also used for some cases. The cytological changes observed in the cervical smears were graded according to The Bethesda system of 2001. Smears taken mainly from women of various age groups ranging from 15-49 years.

III. Observation And Results

In this study 200 HIV positive women were studied for cervical cytological changes in Papsmears who attended the department of pathology and Gynaecology, Government General Hospital, Guntur .According to the Clinical history ,Nulliparaous Women were 15(7.5%), Para 1-2 were -148 (74%), Para 3-4 were 36(18%) and Paras 5 and above were 1 (0.5%).According to the Socioeconomic status, women who were below the poverty line 173(86.5%), women above were 27(13.5%). According to the Nativity ,Urban patients were about 61(30.5%) and Rural patients were about 139(69.5%).According to the age at marriage, Women <19 years were 158(79%) and Women at 20-29 years were 42(21%) Total Number of patients screened is 200.satisfactory smears were studied according to The Bethesda System in 123 satisfactory smears.

The observations were shown in the following tables.

Age in Years	No.of Patients	Percentage
<19	-	-
20-29	58	47.15%
30-39	55	44.71%
40-49	10	8.13%
Total	123	100

Table I: Shows Distribution of patients according to the age groups.

Table	Ii: Shows 1	10.of	patients	with Signific	ant cytologica	l changes in smears
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Age in Years	-ve for dysplasia /malignancy	With epithelial cell abnormalities
<19	-	-
20-29	49 (84.48%)	9(15.52%)
30-39	50(91%)	5(9%)
40-49	7(70%)	3(30%)
	106(86.18%)	17(13.82%)

Table Iii: Shows Analysis of Abnormal Cy	vtological Findings in Relation to A	ge According to The Bethesda
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	System						
Age in Yrs	No.of Patients	ASCUS	AGC Endo	LSIL	HSIL		
			cervical				
<19	-	-	-	-	-		
20-29	58	2(3.49%)	1(1.72%)	5(8.62%)	1(1.72%)		
30-39	55	-	1(1.82%)	4(7.27%)	-		
40-49	10	-	-	2(20%)	1(10%)		
Total	123	2(1.63%)	2(1.63%)	11(8.94%)	2(1.63%)		

Table Iv : Shows Distribution Of Patients According To Known Duration Of Hiv Infection

Duration of infection in years	No.of Patients	Percentage %
0-1	24	19.51
1-2	16	13.0
2-3	15	12.2
3-4	17	13.82
4-5	19	15.45
5-6	9	7.32
6-7	8	6.5
7-8	4	3.25
8-9	4	3.25
9-10	4	3.25
10-11	1	0.81
11-12	2	1.62

 Total
 123
 100

More number of patients seen with less than 1 year duration of infection, i.e., 24 (19.51%).

Table – V: Shows Analysis Of Abnormal Cytological Findings In Relation To Duration Of Infection

Duration of	No.of	ASC-US	AGC endocervical	LSIL	HSIL
Infection in	patients				
years					
0-1	24	-	-	2(833%)	1(4.16%)
1-2	16	-	-	2(12.5%)	-
2-3	15	-	-	1(6.66%)	-
3-4	17	-	2(11.76%)	2(11.76%)	-
4-5	19	-	-	1(5.26%)	1(5.26%)
5-6	9	-	-	2(22.22%)	-
6-7	8	2(25%)	-	-	-
7-8	4	-	-	1(25%)	-
8-9	4	-	-	-	-
9-10	4	-	-	-	-
10-11	1	-	-	-	-
11-12	2	-	-	-	-
Total	123	2	2	11	2

Table – Vi: Shows Analysis Of Abnomral Cytological Findings In Relation To Pv Findings

PV findings	No.of	ASC-US	AGC	LSIL	HSIL	Total +ve for
-	patients		- Endocervical			SIL
With erosion	52	2(3.84%)	2(3.84%)	7(13.46%)	2(3.84%)	13
Without	71	-	-	4(5.63%)	0	4
erosion						
Total	123	2	2	11	2	17

Table – V	/ii:	Shows Distribution	of cases	according to	cytological findings
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S.No.	Cytological changes	No.of cases	Percentage
1	Inflammatory smears with squamous	22	17.88%
	metaplasia		
2	Inflammatory smears with Trichomonas	24	19.51%
	vaginalis		
3	Inflammatory smears with candidiasis	1	0.81%
4	Inflammatory smear with squamous	1	
	metaplasia and Trichomonas vaginalis		
5	Smears +ve for SIL	17	13.82%
6	Infalmmatory smears with metaplasia	1	
	and AGC		

Table – Viii: Shows Distribution of patients in relation to STD

Cytological findings	Positive for STD	Negative for STD	Total
+ve for SIL	9	8	17
-ve for SIL	67	39	106
Total	76 (62%)	47(38%)	123(100%)

 Table – Ix:
 Shows Distribution Of Patients In Relation To Antiretroviral Treatment

Cytological Findings	With ART	Without ART	Total
With abnormality	9	8	17
Without abnormality	47	59	106
Total	56(46%)	67(54%)	123(100%)

IV. Discussion

The aim of this study was to characterize cytological changes in cervical smears of 200 HIV seropositive women who attended the Departments of pathology and gynaecology, Government General Hospital, Guntur. The cases belonged to different reproductive age groups, different socio-economic classes and of urban and rural areas in Guntur District. Cytological evaluation was carried out on 123 satisfactory cervical smears. The study revealed maximum number of inflammatory smears (81.3%), followed by smears showing squamous intra epithelial lesion (13.82%) and smears with no significant cytological changes (5.69%) were seen. Number of unsatisfactory smears were 77 (38.5%). These smears were stained and examined but

unsatisfactory for evaluation of epithelial abnormality because of inadequate squamous component, endocervical cells or squamous metaplastic cells and were obscured with blood and inflammatory cells. **Inflammatory smears:-**

In their study Joshi et al¹ showed that out of 104 papsmears obtained from HIV seropositive women 38.5% (40/104) were inflammatory and 19.2% (20/104) were abnormal. This study also revealed women presenting with STD and HIV infection both, were 2.8 times more likely to have inflammatory papsmears and 3.5 times more likely to have abnormal papsmears compared to HIV seronegative women presenting without Sexually Transmitted Diseases. It was also observed that women at the age of 30 yrs i.e active sexual and reproductive age group and above 30 yrs had only half the risk of having inflammatory cytology compared to those below 30 yrs. Mbakop A et al² reported the incidence of inflammatory smears as 95.5% (62/65) in HIV seropositive women. Micheletti et al³ stated that in a study conducted with 153 HIV seropositive women and 169 HIV seronegative women. The frequency of candidiasis, Trichomoniasis and bacterial vaginosis in cervical smears was almost equal in two groups. In our study the incidence of inflammatory smears with specific and nonspecific infections was about 81.3% (100/123).

Non Specific inflammatory smears:-

Archana Sharma et al⁴ have reported the incidence of nonspecific inflammatory smears as 64% (53/83) in HIV seropositive women. In their study Anuvat Roongpisuthipong et al⁵ reported the incidence of nonspecific inflammatory cases as 10.6% (51/482) in HIV women. Rashmi Bagga et al⁶ reported the incidence of inflammatory smears as 20% in their study. Anorlu RI et al⁷ have reported the incidence of inflammatory smears as 15.7%. A study done by O Fernandez-Limia et al⁸ showed the incidence of bacterial vaginosis with nonspecific inflammatory changes as 31.86%. In our study the incidence of Nonspecific inflammatory smears was 42.27% (52/123). In our study smears show superficial and intermediate squamous epithelial cells with focal vacuolations in the cytoplasm. In focal areas nuclear enlargement with margination of chromatin seen. The smear shows plenty of neutrophils in the background.

Inflammatory smears with candidiasis:-

Archana Sharma et al⁴ reported the incidence of candidiasis as 34% in HIV positive women.

Anuvat Roongpisuthipong et al⁵ study showed that the incidence of vulvo-vaginal candidiasis as 20.3%. The study done by O Fernandez Limia et al⁸ stated that the incidence of candidiasis was 13.27%. Edenilson Eduardo Galore et al⁹ reported the incidence of candidiasis as 12.9% (19/147) in HIV positive women. Nimisha D Shethwala et al¹⁰ have reported the incidence as 17.14% (6/35) in HIV +ve women. In those studies diagnosis was done by gram staining, KOH smear and wet mount methods. In our study only one case reported as inflammatory smear with candidiasis. The incidence was 0.81% (1/123). Only cervical smears were evaluated in our study. In the present study papanicolaou stained smear shows basophilic hyphae. The squamous epithelial cells show perinuclear halo along with margination of chromatin. Focal areas show neutrophilic back ground in the vicinity of hyphae.

Inflammatory smears with Trichomoniasis:-

In their study, Frank Sorvillo et al¹¹ stated that the incidence of Trichomoniasis was about 17.4% (37/212) in HIV seropositive women. They also observed that Trichomonas infection was the most frequently identified sexually transmitted disease. The infection was confirmed through wet film examination or by cytology smear study. Archana Sharma et al⁴ reported that the incidence was about 12%. The study done by Susan et al¹² showed the incidence of Trichomoniasis as 45%.Edenilson Edurado Galore et al⁹ stated that the incidence was 8.4% (13/147) in HIV positive women. Clara Menendez et al¹³ reported the Trichomoniasis incidence as 31%, where as O Fernandez-Limia et al as 43.36%. In our study the incidence of Trichomoniasis was about 19.5% (24/123). This was reported basing on indirect evidence of Trichomonas vaginalis infection like perinuclear halos and BB shots or cannon balls. In this study the smears show squamaous cells with increased eosinophilia. Trichomonads not seen in the smear. Aggregates of leukocytes covering the surfaces of the isolated mature squamous epithelial cells, known as BB shots or cannon balls and perinuclear halo are seen which are indirect evidence of Trichomonas vaginalis infection.

Inflammatory smears with squamous metaplasia:-

The authors Downes J and Wallace II^{15} , stated that the incidence of squamous metaplasia was about 28% (11/39) in HIV positive women. In our study the incidence of smears with squamous metaplasia was about 17.88% (22/123). In this study smears shows small mature squamous cells and also immature squanous like cells. Cells show increased N/C ratio with tails of cytoplasm. These are metaplastic squamous cells.

Squamous intra epithelial Neoplasia (SIL):-

Incidence of squamous intra epithelial neoplasia varies according to different studies done by many authors. Incidence ranges from 2.9% to 42%, in HIV seropositive women. Conti M et al ¹⁵study showed that the incidence was 42%. LaRuchi et al¹⁶ stated that the incidence of SIL was 11.7%.Edenilson et al ⁹study showed that as 12.6%. In our study the incidence of squamous intra epithelial neoplasia was about 13.82%. Our study was consistent with that of Edenilson et al study and differ from study of Conti M et al.

Atypical squamous cells of undetermined significance:- (ASC-US)

Anuvat Roong Pisuthipong et al⁵ have reported that the incidence of ASC-US was 4.8%. Out of 123 cases studied 2 cases showed ASC-US in our study (1.63%) we have not come across the cases of ASC-H in our study. In this study smears show squamous cells with mild increase in size of nucleus. Nuclear margins are with relative decrease in cytoplasm.

Atypical glandular cells- Endocervical:-

Adiha Misson Rua Micheletti et al³ reported the incidence of ASC-US and AGUS together as 7.8%. In our study the incidence of AGC- endocervical was about 1.63% (2/123). In our study smear diagnosed as AGC show sheets and small clusters of endocervical cells with enlarged nuclei and few oval nuclei are also seen. Overlapping of nuclei with hyperchromasia, granular chromatin and nucleoli are seen. Cells show very scanty cytoplasm.

Low grade squamous intra epithelial lesion (LSIL):-

In their study pimpika Tansupswatdikul et al ¹⁷reported that the incidence of LSIL was 6.4% (18/280) in HIV seropositive women. Anuvat Roongpisuthipong et al⁵ study showed that 11.2% had LSIL in their study. A study done by La Ruche et al¹⁶ stated that the incidence of LSIL was 7.6%. In the present study the incidence of low grade squamous intra epithelial lesion was about 8.94% (11/123) and correlated with the studies of La Ruche et al. In our study smears show large mature polygonal squamous cells with enlarged nuclei (3-5 times).Nuclei shows hyperchromasia and evenly distributed finely granular chromatin. A few cells show pyknosis with chromatin smudging and wrinkling of nuclear contours. Perinuclear halo, thickening of cytoplasmic border and nuclear atypia seen.

High grade squamous intraepithelial lesion (HSIL):-

Pimpika Tansupswatdikul et al ¹⁷have reported that the incidence of High grade squamous intraepithelial lesion was 12.1% (34/28). Anuvat Roongpisuthipong et al⁵ 64study showed the incidence of HSIL as 4.1%. In the present study the incidence of HSIL observed is 1.63% (2/123). In our study, smear shows smaller and less mature cells resembling parabasal and basal cells with abundant keratinized thin rim of immature cytoplasm. Nuclei are larger showing hyperchromasia, increased N/C ratio, irregular nuclear contours with granular chromatin. Nucleoli are absent. The cells are distributed singly and in small sheets. In our study we have not come across a case of Squamous cell carcinoma.

Cases of squamous intraepithelial lesion in association with age:-

La Ruche G et al^{16} study showed that LSIL seen in below 24 yrs of age and squamous cell carcinoma above 33yrs of age in HIV seropositive women. In the present study squamous intra epithelial neoplasia found in 17 cases (17/123) i.e; 13.82%. In the age group 20-29 yrs 9 out of 58 showed SIL (15.52%) ,in 30-39 yrs 5 out of 55 (9%) showed SIL, in 40-49 yrs 3 out of 10 (30%) showed SIL. Peak incidence was noted in higher age group 40-49 yrs. This was consistent with the above studies. Sadeghi SB et al¹⁸ study showed SIL decreases with increasing age, because of decrease in risk factors in post menopausal women. Present study was done with women in reproductive age group (15-49yrs) only.

Associaton with duration of HIV infection:-

In their study Hluangdansakul W et al ¹⁹ reported that there was statistically significant association between the duration of HIV infection and occurrence of SIL and squamous cell carcinoma. In our study more number of women seen with less than 1 yr duration of infection 19.51% (24/123). We observed increased incidence of squamous intraepithelial neoplasia in women with longer duration of HIV infection. 25% incidence seen with 8yrs duration, 25% incidence seen with 7 yrs duration and 22.2% seen with 6yrs of duration. This was consistent with Hluangdansakul et al²⁷ study.

Association with cervical erosion:-

52 women (42-27%) presented with cervical erosion out of which 13 women show squamous intra epithelial neoplasia (25%). The incidence of SIL was much lesser in women without cervical erosion i.e; 5.63% (4/71). This revealed increased incidence of SIL among patients with cervical erosion.

Association with presence of Sexually Transmitted Diseases (STD) :-

Study done by S.Joshi et al¹ stated that women presenting with STD and HIV both were 3.5 times more risk of having SIL compared to HIV seronegative women without STD. In the present study according to the presence of other sexually transmitted disease (like history of white discharge, genital ulcers, warts etc) the women with STD were 76 (61.78%) and without STD were 47 (38.21%). In patients with STD, out of 76, 9 cases showed SIL (11.84%) and in patients without STD out of 47, 8 cases showed SIL (17.02%). There was no association observed between STD and SIL in our study.

Association with Antiretroviral therapy:-

Joel M. Palefsky²⁰ in his article stated that Highly active anti retroviral therapy (HAART) appears to have limited ability to clear HPV infection and induce regression of intra epithelial neoplasia in HIV positive women. In our study according to ART, women with ART were 56 (45.52%) and women without ART were 67 (54.47%). Cervical abnormal cytology seen in 9 out of 56 (16.07%) in women with ART and 8 out of 67 (11.94%) in women without ART. This showed increased incidence of SIL in women with ART. This was mainly due to less CD_4 counts (Immunosuppression).

Association with CD₄ count:-

In their study Cynthia et al $(2009)^{22}$ revealed the risk of squamous intraepithelial lesion was more with CD₄ < 200 cells/µl. In our study women with less than 200 cells/µl were 29 (23.57%). Women with more than 200 cells/µl were 69 (56.09%). Women with unknown CD₄ counts were 25 (20.32%). Among these more incidence of squamous intra epithelial lesions were seen in women with <200 cells/µl, i.e; 6 out of 29 (20.68%) where as 7 cases out of 69 shows SIL (10.14%) in women with >200 cells/µl. So this study reflects more incidence of SIL associated with decreased CD₄ cell counts (<200/µl) was consistent with the above studies. The other parameters observed in our study were Parity, socio economic status, nativity, age at marriage and history of opportunistic infections. More number of women came under para 1 and 2, i.e; 74% (148/200). Women who were below the poverty line were 86.5% (173/200) where as women above the poverty line were 13.5% (27/200). Illiteracy, poor personal hygiene, illnourishment and vitamin deficiencies were also observed in low socio economic group. More number of women belonged to the rural areas, 69.5% (139 out of 200) and urban women were about 30.5% (61 out of 200). In our study age at marriage was noted. Women who were married before 19yrs of age were 79% (158/200) .We come across 69 women with either previous or present history of opportunistic infections (34.5%) like oral thrush, skin rash, genital warts, ulcers and pulmonary tuberculosis.





Pap Smear With Non specific Inflammatory changes in cervix

Pap Smear With Non Specific Inflammatory Changes In Cervix In High Power Field



Smear With Ascus Showing Squamous Cells With Dysplastic Changes



Pap Smear With Agc, Endocervical Cells Show Atypical Changes



Pap Smear In Hsil- Lp View



Pap Smear Show Metaplasic Squamous Cells



Pap Smear With Trichomonas Infection



Pap Smear In Candidiasis



Pap Smear In Lsil

V. Conclusion

The study revealed maximum number of inflammatory smears (81.3%), followed by smears showing squamous intra epithelial lesion (13.82%). Early diagnosis of these lesions can reduce the morbidity of HIV seropositive patients. Thus cytological screening should gain much popularity and should be accessible to all. PAP smear can be recommended as a part of routine medical examination in gynaecological practice. Papanicolaou smear screening is an adequate tool for detecting cytologic changes in women with Human Immunodeficiency Virus. Cervical cancer is a preventable disease if women receive regular screening and timely followup and treatment after abnormal papanicolaou smear result.

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