Study of cytodiagnosis of discharge per vaginum with specific reference to human papilloma virus and herpes simplex virus at tertiary care center, indore

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ABSTRACT

Background: Cervical cancers are the second most frequent type of female cancer. Human papillomavirus (HPV) infection is the most important risk factor for cervical cancer. **Objective:** The aim is to detect cytomorphological changes of HPV and herpes simplex virus (HSV) to early diagnose cervical cancer to prevent it at early stage. **Materials and Methods:** The study includes total 1542 patients presenting with the complaints of vaginal discharge and/or associated symptoms. Samples were taken, and prepared smear was stained with papanicolaou (PAP) stain and reporting was done according to The Bethesda system - 2014. The study was prospective study and statically analyzed. **Results:** Totally, 1542 PAP smears were analyzed. Out of which, normal smears 364 (23.61%), inadequate smears 58 (3.76%), inflammatory smear 1093 cases (70.88%), and epithelial cell abnormality comprises 27 cases (1.75%). Out of 27 cases, atypical squamous cells of undetermined significance was the most common epithelial cell abnormality (63%). Cytomorphological features of HPV infection are seen in 2 cases of low-grade squamous intraepithelial lesion, 2 cases of high-grade squamous intraepithelial lesion, and 5 cases of the malignant lesion while inflammatory smear suspicious of HSV comprises 2 cases (0.13%). **Conclusions:** The incidence of cervical cancer can be decreased if PAP smear screening is done at an early age and in the females having high-risk factors.

KEY WORDS: Papanicolaou Smear; Epithelial Cell Abnormality; Cytodiagnosis; Discharge Per Vaginum

INTRODUCTION

Cervical cancers are the second most frequent type of female cancer, responsible for about 5% of cancer deaths in females' worldwide.[1] Human papillomavirus (HPV) infection is the most important risk factor for cervical cancer.[2] and strongly associated with it (up to 100%).[3,4]

Cervical cancer is one of the leading cancers in women with an estimated 500,000 new cases every year, of which 80% occur in developing countries.[5] In India, it is estimated that the number of cases is over 140,000[6] most frequent among females between 15 and 44 years.

The incidence of cervical cancer and cervical intraepithelial neoplasia was found to be low relative to the high frequency of HPV infection showing that not all the HPV infected women develop cervical lesion, and not all the pre-neoplastic lesion get converted into invasive cervical cancer.[7] This shows that most of the HPV infections are transient, and viral persistence is required for the progression of the disease.[7]

Herpes simplex virus-2 (HSV-2) is contributed to be a risk factor for cervical cancer. There is interaction between HSV-2 and HPV-16, or HPV-18 occurs during the development of cervical carcinoma.[8]

The papanicolaou (PAP) smear screening, a simple, non-invasive, and a cost-effective method,[9] for the detection of precursor lesion of cervical carcinoma has significantly reduced the mortality and morbidity of cervical carcinoma.[10] The 2014 Bethesda System has been introduced recently, which is used for reporting cervical smear in our study.

MATERIALS AND METHODS

The present study included cervical smears of patients attending outpatient Department of Obstetrics and Gynaecology and further sent to Department of Pathology, at M.G.M. Medical College and M.Y. Hospital, Indore, Madhya Pradesh, India, during March 2015 to February 2016, for cytomorphological analysis. Totally, 1542 patients were screened.

Inclusion Criteria

- Age range of 19-80 years
- Presenting with the complaints of vaginal discharge.

Exclusion Criteria

Menstruating females

Proper history was recorded. Smears were taken with Ayres wooden spatula. The cellular material collected was smeared on a clean glass slide, properly labeled, fixed in 95% ethyl alcohol immediately and subsequently stained by PAP stain. After staining, slides were mounted with digital picture exchange, screened under light microscope, and reported according to 2014 Bethesda system.

RESULTS

Analysis of PAP smear is described in Table 1. Different types of epithelial cells abnormalities are presented in Figure 1. Out of 27 cases of epithelial cell abnormality are of atypical squamous cells of undetermined significance (ASCUS) (17), atypical glandular cells of undetermined significance (AGCUS) (1), low-grade squamous intraepithelial lesion (LSIL) (2), high-grade squamous intraepithelial lesion (HSIL) (2) and invasive carcinoma cervix (5). Cytomorphological features of HPV infection are seen in 2 cases of LSIL, 2 cases of HSIL, and 5 cases of malignant lesion (Table 2).

The majority of patients belong to age group 21-40 years, i.e., 1101 (71.40%). Maximum cases of LSIL were detected in 41-50 years of age group and that of HSIL were detected in 31-60 years of age group whereas maximum cases of carcinoma cervix were detected in 51-60 years of age group. Maximum number of atypical epithelial cells of uncertain significance was found in age group 21-40 years (76.5%) (Table 3).

Maximum patients 1159 (75.17%) have complaints of white discharge per vaginum. About 50% of patient of LSIL presented with white discharge and rest presented with serosanguinous discharge. While 50% patient of HSIL presented with serosanguinous discharge and the rest presented with post-coital bleeding. Nearly 80% cases of carcinoma cervix presented with the complaint

Table 1: Analysis of PAP smear

PAP smear	*n* (%)
Total smears	1542 (100.00)
Adequacy of smears	
Satisfactory for evaluation	1484 (96.23)
Unsatisfactory for evaluation	58 (03.77)
General categorization	
Normal smears	364 (23.61)
Benign cellular changes	
Acute inflammatory smear	775 (50.26)
Non specific inflammatory lesion	223 (14.47)
Inflammatory smear suspicious of HSV	02 (0.13)
Bacterial vaginosis	65 (04.21)
Trichomonas vaginalis	16 (01.03)
Candidiasis	1 (0.06)
Squamous metaplasia	4 (0.26)
Reactive changes	1 (0.06)
Atrophy	6 (0.38)
Epithelial cells abnormality	27 (1.75)
AGCUS	1 (0.06)
ASCUS	17 (1.11)
LSIL	2 (0.13)
HSIL	2 (0.13)
Malignancy	5 (0.33)

HSV: Herpes simplex virus, AGCUS: Atypical glandular cells of undetermined significance, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, PAP: Papanicolaou
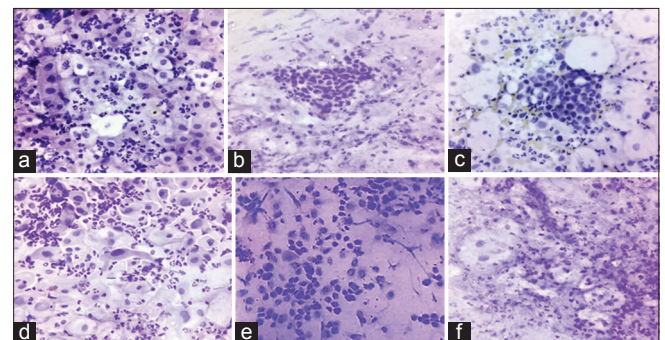
Figure 1: (a) Atypical cells of undetermined significance, (b) low-grade squamous intraepithelial lesion, (c) high-grade squamous intraepithelial lesion, (d) squamous cell carcinoma, (e) changes of herpes simplex virus, and (f) human papillomavirus – koilocytic changes

of bleeding per vaginum and others presented with post-coital bleeding. The 41.1% of patient of ASCUS presented with post-coital bleeding and 58.8% patient presented with serosanguinous discharge. Patient of AGCUS presented with post-coital bleeding. However, many patients presented with more than one complaint (Table 4). The majority of patients belongs to the parity 3 and above (Table 5).

DISCUSSION

In our study (Tables 1 and 2), ASCUS (1.11%), AGCUS (0.06%), LSIL (0.13%), HSIL (0.13%) and squamous cell carcinoma (SCC) (0.33%), and inflammatory smear suspicious of HSV comprises 2 cases (0.13%). A study by Tailor et al.[11] shows ASCUS 0.77%, ASC-H 0.35%, HSIL 0.35%, SCC 0.14%, and AGCUS 0.28%. A study by Banik et al.[12] revealed the following scenario: 0.18% ASCUS, 0.12% AGC, 6.36% LSIL, 1.18% HSIL, and 0.35% malignancy. Al Eyd et al.[13] studied that the overall frequency of cervical intraepithelial abnormalities was

3.3%, out of which 1.8% had ASCUS, 1.2% had LSIL, and 0.3% had HSIL. Edelman et al.[14] studied PAP smears from 29,295 females over a period of 1 year, and the PAP smear abnormalities were as follows: 9.9% ASCUS, 2.5% LSIL, 0.6% HSIL, and 0.2% invasive cancer. Mulay et al.[15] 0.64% ASC-US, 0.31% AGCUS, 0.21% LSIL, 0.16% HSIL, and 0.06% invasive cancer.

In our study, ASCUS (1.11%) is found to be the most common epithelial cell abnormality. As the women included in our study were routinely screened, they presented with an early form of cytological interpretation in the cervical smear supporting our results. Similar results were obtained in other studies also.[13,16]

Cytomorphological of HPV infection are seen in 2 cases of LSIL, 2 cases of HSIL, and 5 cases of malignant lesion. Yim and Park[17] showed that the strong association led to the suggestion that HPV is not only the main cause of cervical cancer cases but is also a necessary cause. Fusté et al.[18] and Insinga et al.[19] also found that HPV is associated with more than 99% of all cervical cancer cases which favor the result of our study. HPV causes almost 100% of cases of cervical cancer and limitation of study methodologies is most important reason behind an underestimation of HPV prevalence in cervical cancer.[20]

In Table 3, bulk of patient 1101 (71.40%) belongs to age group 21-40 years. LSIL was found in 2 cases (0.13%). It was mainly in the age group 41-50 years. HSIL was found in 2 cases (0.13%). It was mainly in the age group 31-60 years. Invasive carcinoma cervix was found in 5 cases (0.33%). It was mainly in the age group 51-60 years. Similar results are also found in other studies. Bamanikar et al.[21] found maximum cases in

Table 2: Distribution of epithelial cells abnormalities

Epithelial cell abnormality	*n*=27 (%)
ASCUS	17 (62.96)
AGCUS	1 (3.70)
LSIL	2 (7.41)
HSIL	2 (7.41)
SCC	5 (18.52)

ASCUS: Atypical squamous cells of undetermined significance, AGCUS: Atypical glandular cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

Table 3: Age distribution in relation of ASCUS, ASGUS, LSIL, HSIL and SCC

Age (years)	AGCUS *n*=1 (%)	ASCUS *n*=17 (%)	LSIL *n*=2 (%)	HSIL *n*=2 (%)	SCC *n*=5 (%)	Total
15-20	-	-	-	-	-	0
21-30	-	7 (41.2)	-	-	-	7
31-40	-	6 (35.3)	-	1 (50)	1 (20)	8
41-50	1 (100)	3 (17.7)	2 (100)	-	1 (20)	7
51-60	-	1 (5.8)	-	1 (50)	3 (60)	5
61 and above	-	-	-	-	-	0

AGCUS: Atypical glandular cells of undetermined significance, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

Table 4: Distribution of ASCUS, ASGUS, LSIL, HSIL and SCC according to type of discharge

Discharge	ASCUS *n*=17 (%)	AGCUS *n*=1 (%)	LSIL *n*=2 (%)	HSIL *n*=2 (%)	SCC *n*=5 (%)	Total
White discharge	-	-	1 (50)	-	-	1
Bleeding per vaginum	-	-	-	-	4 (80)	4
Post-coital bleeding	7 (41.1)	1 (100)	-	1 (50)	1 (20)	10
Sero-sanguinous discharge	10 (58.8)	-	1 (50)	1 (50)	-	12

AGCUS: Atypical glandular cells of undetermined significance, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

Table 5: Distribution of cases of ASCUS, ASGUS, LSIL, HSIL, SCC according to parity

Parity	ASCUS *n*=17 (%)	AGCUS *n*=1 (%)	LSIL *n*=2 (%)	HSIL *n*=2 (%)	SCC *n*=5 (%)	Total
0	-	-	-	-	-	0
1	1 (5.88)	-	-	-	-	1
2	2 (11.76)	-	-	-	-	2
3	7 (41.18)	1 (100)	-	1 (50)	1 (20)	10
4	4 (23.53)	-	1 (50)	-	2 (40)	7
>5	3 (17.65)	-	1 (50)	1 (50)	2 (40)	7

AGCUS: Atypical glandular cells of undetermined significance, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

the age range of 20-75 years. Tailor et al.[11] found maximum cases in the age range of 25-70 years. The difference in the age incidence of intraepithelial lesions and carcinoma cervix could be due to wide variation in selection criteria.

In Table 4, white discharge was most common (75.17%) followed by lower abdominal pain (40.9%), serosanguinous discharge (7.1%), foul smelling discharge (7.0%), yellow discharge (1.04%), post-coital bleeding (0.65%), and bleeding per vaginum (0.26%). Similar results are also found in other studies. Aikat et al.[22] found symptomatic cases (50%), Juneja et al.[6] found bleeding P/V (65%), Alexander and Khanapur[23] found white discharge (72%) as most common presenting complaint.

In our study (Table 5), maximum number of patients with epithelial cell abnormality, belong to parity 3 and above. Shrivastav et al.[24] abnormal cytology was detected in 3.6% of the women in study group, i.e., with parity >3. Juneja et al.,[25] Mishra and Sinha[26] and Alexander and Khanapur[23] (2015) also found similar results. Early sexual life and early age of first intercourse are more important contributing factors than the number of pregnancies. Statistical analysis reveals that there is a significant difference between parity <2 and higher parity with $P < 0.05$.

Epithelial cell abnormalities are at a higher rate in poor (88.88%) socioeconomic status. Similar results were also found by Coppleson and Brown[27] and Roy et al.[28] Statistical analysis reveals that there is a significant difference between socioeconomic status with $P < 0.05$.

The variability in results from the studies by different workers is due to multiple risk factors associated with causation of cervical cancer such as age at marriage, age at first delivery, age distribution of women screened, and methods utilized in obtaining cytologic smears. The low incidence of dysplastic smears and cancer yield in the present study may be because screening has been done on the females of 15 years and above.

CONCLUSION

Cervical cytology is method for early detection of intraepithelial lesions and malignancy and therefore should be established as a routine diagnostic aid. The patient attending the Gynecology Outpatient Department was targeted in this study, so as to screening all the females, even with minor symptoms such as discharge per vaginum and to detect the cytomorphological changes caused by HPV at an earlier stage. The study reveals that the cases of cervical cancer can be detected at early stage if there are proper education and awareness in the society.

REFERENCES

1. WHO/ICO Information Centre on HPV and Cervical Cancer. Available from: http://www.who.int/hpv center. [Last cited on 2009 May 05].
2. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007;370(9590):890-907.
3. Van Den Brule AJ, Walboomers JM, Du Maine M, Kenemans P, Meijer CJ. Difference in prevalence of human papillomavirus genotypes in cytomorphologically normal cervical smears is associated with a history of cervical intraepithelial neoplasia. Int J Cancer. 1991;48(3):404-8.
4. Resnick RM, Cornelissen MT, Wright DK, Eichinger GH, Fox HS, ter Schegget J, et al. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. J Natl Cancer Inst. 1990;82(18):1477-84.
5. Tristen C, Bergström S. Cervical cancer in developing countries. A threat to reproductive health. Lakartidningen. 1996;93(39):3374-6.
6. Juneja A, Sehgal A, Sharma S, Pandey A. Cervical cancer screening in India: Strategies revisited. Indian J Med Sci. 2007;61(1):34-47.
7. Hildesheim A, Schiffman MH, Gravitt PE, Glass AG, Greer CE, Zhang T, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis. 1994;169(2):235-40.
8. Hildesheim A, Mann V, Brinton LA, Szklo M, Reeves WC, Rawls WA. Herpes simplex virus Type 2: A possible interaction with human papillomavirus types 16/18 in the development of invasive cervical cancer. Int J Cancer. 1991;49:335-40.
9. Roohi M, Sahi SC. Incidence of cervical intraepithelial neaplasia in Faisalabad. Pak J Med Res. 1997;32:164-5.
10. Wright TC Jr, Cox JT, Massad LS, Carlson J, Twiggs LB, Wilkinson EJ; American Society for Colposcopy and Cervical Pathology. 2001 consensus guidelines for the management of

women with cervical intraepithelial neoplasia. J Low Genit Tract Dis. 2003;7(3):154-67.

11. Tailor HJ, Patel RD, Patel PR, Bhagat VM. Study of cervical pap smears in a tertiary care hospital of South Gujarat, India. Int J Res Med Sci. 2016;4(1):286-8.

12. Banik U, Bhattacharjee P, Ahamad SU, Rahman Z. Pattern of epithelial cell abnormality in pap smear: A clinicopathological and demographic correlation. Cytojournal. 2011;8:8.

13. Al Eyd GJ, Shaik RB. Rate of opportunistic pap smear screening and patterns of epithelial cell abnormalities in pap smears in Ajman, United Arab Emirates. Sultan Qaboos Univ Med J. 2012;12(4):473-8.

14. Edelman M, Fox AS, Alderman EM, Neal W, Shapiro A, Silver EJ, et al. Cervical Papanicolaou smear abnormalities in inner city Bronx adolescents: Prevalence, progression, and immune modifiers. Cancer. 1999;87(4):184-9.

15. Mulay K, Swain M, Patra S, Gowrishankar S. A comparative study of cervical smears in an urban Hospital in India and a population-based screening program in Mauritius. Indian J Pathol Microbiol. 2009;52(1):34-7.

16. Patel MM, Pandya AN, Modi J. Cervical pap smear study and its utility in cancer screening, to specify the strategy for cervical cancer control. Natl J Community Med. 2011;2(1):3.

17. Yim EK, Park JS. Biomarkers in cervical cancer. Biomark Insights. 2007;1:215-25.

18. Fusté P, Santamaría X, Carreras R. New therapeutic strategies for human papillomavirus related anogenital lesions in HIV patients: Highly active antiretroviral therapy and HPV vaccines. Med Clin (Barc). 2008;131(1):30-4.

19. Insinga RP, Liaw KL, Johnson LG, Madeleine MM. A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. Cancer Epidemiol Biomarkers Prev. 2008;17(7):1611-22.

20. WHO/ICO HPV Information Centre. Human Papillomavirus and Related Cancers. Summary Report Update. June 22; 2010.

21. Bamanikar SA, Baravkar DS, Chandanwale SS, Dapkekar P. Study of cervical pap smears in a tertiary hospital. Indian Med Gaz. 2014;148(7):250-4.

22. Aikat M, Gupta S, Aikat BK. Critical evaluation of cervical cytology. Indian J Med Res. 1974;62:655-61.

23. Alexander AM, Khanapur BR. Study on profile of patients undergoing pap smear at a tertiary centre in Jabalpur. Natl J Med Dent Res. 2015;3(4):206-12.

24. Shrivastav P, Jairaj P, Balasubramaniam N, Krishnaswami H. Selective screening for cancer of the cervix uteri in South Indian women. Int J Gynaecol Obstet. 1986;24(5):337-42.

25. Juneja A, Murthy NS, Sharma S, Shukla DK, Roy M, Das DK. Selective cervical cytology screening: Discriminant analysis approach. Neoplasma. 1993;40(6):401-4.

26. Mishra NK, Sinha TK. Cytologic screening for the detection of cancer in the uterine cervix - A survey in Patna (India). Cancer Lett. 1990;52(1):21-7.

27. Coppleson LW, Brown B. Observations on a model of the biology of carcinoma of the cervix: A poor fit between observation and theory. Am J Obstet Gynecol. 1975;122(1):127-36.

28. Roy A, Dutta K, Majumdar J, Basu S, De S, De N, et al. Cervical cytology screening in Calcutta and adjoining areas with special reference to carcinoma of the uterine cervix. Indian J Public Health. 1990;34(2):98-106.