

Evaluating Spectrum of Epithelial Cell Abnormalities in Cervical Cytology Smears with a Focus on Atypical Squamous Cells of Undetermined Significance (ASCUS): A Study in Eastern India

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Abstract

Background: Cervical cancer being one of the major causes of death in women living in developing countries like India, this study evaluates the spectrum of epithelial cell abnormality while primarily focusing on the prevalence of ASCUS and their correlation with predisposing factors.

Materials and Methods: Descriptive type of study was conducted over 456 patients. Patients underwent per speculum examination followed by cervical smear examination. Cervical lesions were categorized according to Revised Bethesda system, 2014. HPV detection & sequencing was done as required.

Results and conclusion: ASCUS was most prevalent in the sexually active age group (7.01%). The incidence of intraepithelial lesions among multiparous women was high (50.8%). Early coital age, smoking, poor economic conditions, multiple sexual partners, contraceptive usage and HPV infection were associated with higher chances of squamous cell abnormality.

Cervicitis, cervical erosion, and hypertrophic cervix need careful investigation for the presence of ASCUS due to its high prevalence in sexually active females in Eastern India. Henceforth, addressing these factors and spreading awareness about early diagnosis, necessary treatment, HPV vaccination along with the maintenance of proper sexual and menstrual hygiene becomes a cornerstone in the reduction of the overall burden of atypical cervical cytology and finally, cervical cancer.

Keywords: ASCUS, Cervical Intraepithelial Neoplasia, Uterine Cervical Dysplasia

Introduction

According to epidemiological data, cervical cancer has become one of the major causes of death

in women living in developing countries.^[1] It has become one of the leading causes of morbidity and mortality in India and other developing countries with estimated new cases emerging every year

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being around 1,26,000.^[2,3] In 2007, according to the national cancer registry of India, the number of new cases of cancer cervix was 90,708 approximately, with a five-year survival rate of around 48%.^[3] The cervix is easily accessible and the cervical cells have the propensity to exfoliate, aiding in early diagnosis of the spectrum of epithelial lesions using cervical smear examination.^[4,5]

The 1988 Bethesda system for reporting cervical/vaginal cytologic diagnoses introduced the term "atypical squamous cells" (ASC) for those cellular abnormalities that are markedly greater than those to be designated as reactive/inflammatory cellular changes yet deficient quantitatively or qualitatively to ascertain a definitive diagnosis of a squamous intraepithelial lesion (SIL).^[1] Hence these lesions were termed as atypical squamous cells-undetermined significance (ASCUS) including cells with cytologic atypia that are indicative of a squamous intraepithelial lesion, yet insufficient for a definitive diagnosis and atypical squamous cells- cannot exclude high grade squamous intraepithelial lesion (ASC-H), which include cells with cytologic atypia indicative of HSIL, yet insufficient as per criteria for definitive diagnosis.

This study aims to evaluate cervical cytology smears as per the Bethesda system of reporting cervical cytology, to determine the prevalence of various epithelial cell abnormalities in cervical cytology in the Eastern Indian population. The study also aims to assess the association between the various epithelial cell abnormalities specially ASCUS and various clinicopathological features like age group, sexual activity, HPV infection, socioeconomic status and findings of per speculum examination.

Materials and Methods

A descriptive type of study was carried out for a period of 3 years (February 2019 to March 2022). All procedures performed in the current study were approved by Institutional Ethics Committee. A total of 456 patients were taken into account. All these patients underwent cervical smear examination as directed by their respective treating clinicians in the department of Gynaecology. Unstained cervical smears were then sent to the department of Pathology along with a requisition form encompassing patient particulars and relevant information. It was recorded

only after taking informed signed consent from the patient or guardian of the patient. The following information was obtained from the requisition forms or the patient's medical records:

Patient's name and age, socio-economic status, history of early coitus, multiple sexual partners, and per speculum observations.

Pathological evaluation:

After performing per speculum examination, the cervical smears were collected in the Gynaecology department. These smears were fixed in ethanol for processing and staining by the conventional method using Papanicolaou's technique. The 2014 Revised Bethesda system of reporting was used for the gradation of cytopathological findings observed in the cervical smears and were finally categorized into Negative for Intraepithelial lesion or Malignancy (NILM), Atypical squamous cell of undetermined significance (ASCUS), Atypical squamous cells cannot exclude HSIL (ASC-H), Low-grade squamous intraepithelial Lesion (LSIL), High-grade squamous intraepithelial lesion (HSIL) and Squamous cell carcinoma (SCC). Stained smears were evaluated according to the morphological criteria of amphophilia, perinuclear halo, dyskeratosis, nuclear criteria (binucleation, multinucleation), increase in the nucleus/cytoplasm ratio, anisokaryosis, hyperchromasia, nuclear atypia, and karyorrhexis.^[6]

HPV detection & sequencing was done after DNA extraction using the Amplitude Liquid Media Extraction Kit from cases having atypical cervical cytology. HPV types found were recorded. These results were correlated with history along with per speculum findings.

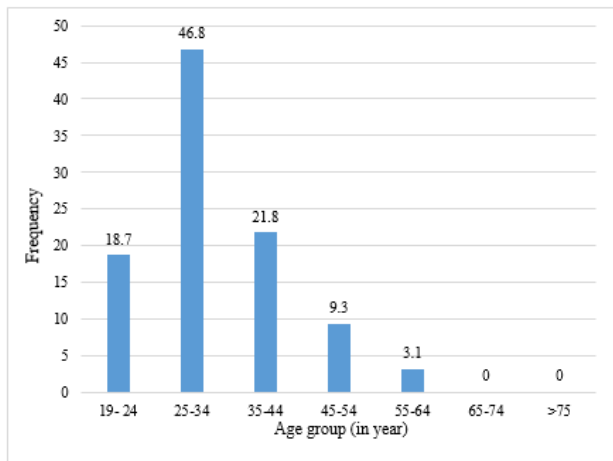
Statistical analysis:

Appropriate statistical analysis was done as and when required. Data were analyzed using SPSS version 22.0 (Statistical Product for Services Solutions).

Results

A thorough cytological assessment of 456 patients revealed that NILM was the most common finding while ASC-US was the most prevalent epithelial abnormality in the sexually active age group.

The prevalence of ASCUS in our study is 7.01%. LSIL was also associated with sexually active women (0.21%). ASC-H on the other hand was more prevalent in postmenopausal women. SCC is prevalent in the age group 41-50 years. [Table 1] [Figure- 1]



ASCUS incidence is raised in younger and sexually active women between 19- 44 years, after which it reduces significantly.

Most of the cervical smears with epithelial abnormalities had per speculum finding of cervical erosion (86.8%). Suspicious looking cervix with aceto-

white patches and erosion was found only in 3 cases (7.89%). [Table 2]

Early coital age was associated with higher chances of the squamous intraepithelial lesion (p-value< 0.05). [Table 3]

Atypical intraepithelial squamous lesion was found predominantly in women with low socioeconomic status (p-value <0.05) [Table 4] and those having more than one sexual partner (p-value <0.05). [Table 5]

On the other hand, multiple sexual partners are a vital risk factor in the pathogenesis of atypical squamous lesion (p-value <0.05) [Table 5]

HPV infection is often associated with cervical epithelial abnormalities [Table 6]. HPV 16 /18 were among the most common types found.

ASCUS has atypical features comprising orangophilia, and nucleomegaly of squamous cells in an inflammatory background. ASC-H comprises orangophilia, nucleomegaly of squamous cells in addition to the presence of parabasal cells in an inflammatory background.

Table 1: Age wise frequency distribution table.

Age Group (years)	Squamous epithelial findings					
	NILM*	ASCUS#	ASC-H•	LSIL ^Δ	HSIL ⁺	SCC**
19-24	106	6	0	0	0	0
25-34	152	15	0	0	0	0
35-44	79	7	0	1	0	0
45-54	50	3	1	0	0	1
55-64	26	1	1	0	0	0
65-74	3	0	0	0	1	0
>75	2	0	0	0	1	0
Prevalance	91.6	7.01	0.43	0.21	0.43	0.21

- * NILM, Negative for intraepithelial lesion or malignancy;
- # ASCUS, Atypical squamous cell of undetermined significance;
- ASC-H, Atypical squamous cells cannot exclude HSIL;
- Δ LSIL, Low- grade squamous intraepithelial Lesion;
- + HSIL High- grade squamous intraepithelial lesion and
- ** SCC, Squamous cell carcinoma.

Table 2: Frequency distribution according to per speculum finding.

Per speculum findings	Lesion in pap smear	No of cases
Normal	ASCUS	2
Cervical erosion with ectropian	ASCUS	30
	ASC-H	1
	LSIL	1
	HSIL	1
Aceto-white patches with erosion	ASC-H	1
	SCC	1
	HSIL	1

Table 3: Association table between early coital age and atypical squamous lesion.

Atypical cytological findings	Early age at coitus (< 18 years)	Appropriate age (>18 years)	Chi -square value	p value
Present	15	23	18.56	.000
Absent	55	363		

Table 4: Association table between socioeconomic status and atypical squamous lesion.

Atypical cytological findings	Low-socio economic status N= 250	High and middle socioeconomic status N= 206	Chi -square value	p value
PRESENT	35	3	23.2	.000
ABSENT	215	203		

Table 5: Association between number of sexual partners and atypical squamous lesion.

Atypical cytological finding	0 - 1	≥2	Chi -square value	P value
Present	9	29	154.3	.000
Absent	390	28		

Table 6: Distribution of Human Papilloma Virus (HPV) types in atypical cervical cytology smears

Atypical cytological smears (n = 38)	Total number of cases	Type of HPV infection (Number of cases infected)
ASC- US	32	HPV 16 (9 cases) HPV 18 (5 cases) HPV 33 (1 case) Rest 17 cases were not associated with HPV infection
ASC-H	2	HPV 16 (2 cases)
LSIL	1	HPV 18 (1 case)
HSIL	2	HPV 16 (1 case) HPV 18 (1 case)
SCC	1	HPV 16 (1 case)

Discussion

In developing countries, the commonest form of cancer in women is cervical cancer. WHO ascribes an 80% death rate to cervical cancer in these countries due to poor hygienic conditions and lack of awareness.^[7] India contributes to one-quarter of the worldwide cervical cancer burden.^[8] The Bethesda classification, 1988 describes ASCUS as a median lesion between the inflammatory conditions and LSIL of the cervix.^[9] Jahic et al. defined it as "Atypical squamous cells of undetermined significance (ASCUS) is a term that refers to inflammatory, reactive and reparative processes which are atypical and of higher level and insufficient to be classified as cervical intraepithelial lesions (CIN)." ^[9] ASCUS may be caused due to an infection (HPV) or may be due to low hormonal levels (post-menopausal) or following a benign growth.

In our study, the incidence of ASCUS was found to be 7.01 %, which is in concordance with the data cited in the literature by Srivastava AN et al.^[10] They found 8.8 % incidence of ASCUS. Yet another research reported an ASCUS incidence of 4.9% in the cervical cytology of women attending Pravara Rural Hospital, Loni.^[11] Analysis shows sexually active women below 44 years were more prone to develop epithelial abnormality. Srivastava M et al. showed a roughly similar age group with a predominance of epithelial abnormality.^[11] We found most of the abnormal cytology cases between the age group of 19 to 54 years but a study by Sachan et al. found the same to be between 40 to 60 years.^[12] Women between 25 to 34 years were reported to have maximum cases of ASCUS whereas in the study by Wendel et al. the mean age of presentation for ASCUS was 35.7 years.^[13] Gupta et al. showed ASC-H, LSIL, HSIL, and SCC were seen mostly above 40 years which was almost in approximation with our study. He also reported 3.23% cases with epithelial abnormality, while in our study 8.3 % of all cases were diagnosed to have the same.^[14]

Srivastava A et al in their research noted that epithelial abnormalities mainly including HSIL and carcinoma cervix increase with early age of consummation which matches our monograph.^[15] Our study revealed that high-grade squamous intraepithelial lesions and squamous cell carcinoma

were found more or less in parallel age groups. Cervical erosion was the most common finding in our study. This observation was also found by Bamanikar et al.^[16] Also a study by Duttagupta et al. associated the presence the cervical erosion, hypertrophy, and ulcerative growth with most cases of cervical carcinoma.^[17] Epithelial abnormalities were predominantly seen in patients belonging to low socioeconomic status probably due to poor hygiene status and lack of follow-up. HPV 16 was the prevalent genotype (46.80 %) associated with ASCUS followed by HPV 18 and other prevalent types which were similar to other studies.^[18,19]

So, to conclude, cervical dysplasia and invasive cervical cancer are preventable. Low income, followed by early marriage and early age of coitus had a higher correlation with abnormal cervical smears. Patients having cervicitis, cervical erosion, and hypertrophic cervix should be carefully investigated for the presence of ASCUS, due to its high prevalence in sexually active females in Eastern India. HPV testing should also be done accordingly. Henceforth, addressing these factors and spreading awareness about early diagnosis, necessary treatment, HPV vaccination along with the maintenance of proper sexual and menstrual hygiene becomes a cornerstone in the reduction of the overall burden of atypical cervical cytology and finally, cervical cancer.

Ethics Statement

This study was approved by the Institutional Review Board. Informed consent was sought from each participating patient.

Conflicts of Interest: No potential conflicts of interest.

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