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Application of Bethesda System For Cervical Cytology at a Tertiary Care Centre

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Abstract

Background: For early screening of the cervical lesion cervical smear is a sensitive test and most widely used system for describing Pap smear result is The Bethesda System. **Aims and Objectives:** The present study was conducted to look for the epithelial cell abnormalities in cervix using Bethesda system of reporting for cervical cytology, to implicate The Bethesda System of reporting (2014) during assessment of Pap smear, to detect cervical cancer and its pre-cancerous lesions (LSIL-Low grade intraepithelial lesion and HSIL-High grade intraepithelial lesion) at an early stage by Pap smear examination and to create awareness about cervical cancer and importance of Pap smear as a screening test in disease prevention. **Materials and Methods:** The present study was undertaken at a tertiary health care, out of 250 cervical smears, we excluded 10 smears which were inadequate to opine. So, the total of 240 smears got reported according to The Bethesda System 2014. **Observations and Results:** The youngest patient was 18 year old and the oldest was 75 year old female. A total of 4% Pap smears were reported as unsatisfactory. The most common complaint of patients was discharge per vaginum which accounts for a total of 38% followed by abdominal pain is showed in 8% of cases. The most common infection was bacterial vaginosis followed by candida, Trichomonas. The frequency of normal (NILM) cases was 78%. ASCUS was found in 4.58%, LSIL was found in 9.17%, HSIL was found in 1.67% & SCC was found in 3.33% cases. Proportion of LSIL was more in age group 41-50 years i.e. 21.15%. **Conclusion:** Pap test has significant utility worldwide. Bethesda system which is widely used for cervicalcytology is a uniform and standard method for reporting cervical smears. It also gives a descriptive diagnosis that aids the clinicians regarding individualized management of patients. It helps in prevention of invasive cervical cancer by timely detection and treating women having precancerous lesions. The effectiveness is determined by reduction in incidence and mortality.

Keywords: Bethesda System, Cervical Cancer, Infection, Pap Smear, Cervical Cytology

1. Introduction

In 1940, George Papanicolaou introduced cervical cytology into clinical practice[1]. For early screening of the cervical lesions, cervical smear is a sensitive test. The Bethesda System is the most widely used classification system for describing the results of Pap smear[2]. In resource poor setting, it is difficult to screen the cervical cancer due to many barriers in establishing screening programs for detection of cervical cancer[3].

Many prevention as well as early detection, interventions have proven to avert cancer cases and deaths in high and low resource settings. Limited resources for screening are seen in low and middle income countries. There are known means of prevention and for early detection of common cancers amongst females which can be applied in resource appropriate settings[4]. Cervical cancer is a preventable disease having excellent primary as well as secondary prevention strategies[5].

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Using several screening techniques, through early detection, cervical cancers can be prevented. Pap smear test can identify early cervical epithelial changes. It is the primary screening test for detection of precancerous cervical intraepithelial neoplasia and the early stage of invasive cervical cancer[6].

We require an uniform national cytology reporting system for clear guidelines for providing the action to take based on cytology reports. Linkage between the treatment centre and site of screening is inadequate and requires urgent attention, which is required to reduce the mortality of cervical cancer[7].

Primary high risk HPV (hrHPV) testing as additional screening options is used recently. Along with further insights into HPV biology; changes in histopathology terminology; approval and implementation of prophylactic HPV vaccines; and updated guidelines for cervical cancer screening and clinical management[8]. Research related to the biology of cervical cancer, the Bethesda System played a vital role in facilitating and exploring new approaches and strategies for patient management[9].

For cancer incidence and mortality, better organized programs might favour greater impact. For screening of precancerous lesions, the technological developments offer more suitable opportunities for prevention and alternative approaches[10].

The present study was conducted to look for the epithelial cell abnormalities in cervix using Bethesda system of reporting for cervical cytology, to implicate The Bethesda System of reporting (2014) during assessment of Pap smear, to detect cervical cancer and its pre-cancerous lesions (LSIL-Low grade intraepithelial lesion and HSIL-High grade intraepithelial lesion) at an early stage by Pap smear examination and to create awareness about cervical cancer and significance of Pap smear as a screening test in disease prevention.

2. Aims and Objectives

The present study was conducted to look for the epithelial cell abnormalities in cervix using Bethesda system of reporting for cervical cytology, to implicate The Bethesda System of reporting (2014) during assessment of Pap smear, to detect cervical cancer and its pre-cancerous lesions (LSIL-Low grade intraepithelial lesion and HSIL-High grade intraepithelial lesion) at an early stage by Pap smear examination and to create awareness about cervical cancer and importance of Pap smear as a screening test in disease prevention.

3. Material and Methods

A Cross sectional longitudinal study was conducted at Department of Pathology and Department of Obstetrics and Gynaecology of Dr. Vasantrao Pawar Medical College Hospital and Research Centre, Nashik, Maharashtra, India. All cervical smears were received in the Department of Pathology of Medical College and tertiary health care centre. The smears were subjected to detailed cytological examination. In each case, pathological reaction pattern was studied carefully and documented.

3.1 Methodology

A total number of 250 participants were included after satisfying the eligibility criteria with due permission of Department of Obstetrics and Gynaecology. Only those patients who had given valid informed consent were included in the study. Participants along with records of hospitalization underwent a local, general and systemic examination. Clinical history and relevant data of the patients enrolled in the study was taken.

Then the cervical smears were taken whenever possible with valid informed consent.

3.2 Inclusion Criteria

- Married women, 18 years and above.
- Cervical erosion; chronic cervicitis; healed lacerations; cervix that bleeds on touch; suspicious growth/ulcer/polyp on the cervix and abnormal discharges from the cervix.

3.3 Exclusion Criteria

- Pregnant women.
- Unmarried women, females under 18 years of age. Sample size calculation was done by Z^2 [P*(1-P)] l^2 with 189 patients.

3.4 Technique for Smear Collection

- The patient was placed in dorsal lithotomy position.
- A sterile Cusco's bivalve speculum of appropriate size was introduced through vagina; cervix was visualized.
- The speculum was inserted without lubrication.

- The transformation zone is the site of origin for majority of the lesions of cervical neoplasia and should be the focus of cytology smear collection.
- Firstly, a plastic or wooden spatula was used for obtaining a sample of the ectocervix.
- The notched end of the spatula was inserted into the cervix through the vagina.
- It was then rotated 360 degrees around the circumference of the cervical os, retaining the sample on the upper surface of the spatula.
- Direct sampling of grossly visible lesions, including irregular/ discolored/ friable areas should be done.
- The material obtained from the above can be placed on a separate slide, especially if the lesion is distant from other collection areas.

3.5 Standardization of the Pap smear Reporting

The use of the revised Bethesda System has unified various overlapping terminologies and has included specific statements regarding specimen adequacy, general categorization, interpretation and results[11].

3.6 The 2014 Bethesda System for Reporting Cervical Cytology[12]

3.6.1 Specimen Type

Indicate conventional smear (Pap smear) vs liquid based preparation *vs* other.

3.6.2 Specimen Adequacy

- Satisfactory for evaluation (describe presence or absence of endocervical or transformation zone component and any other quality indicators, e.g. partially of obscuring blood, inflammation etc.)
- Unsatisfactory for Evaluation:
  - specimen rejected or not processed
  - specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality

3.6.3 General Categorization

- negative for Intraepithelial Lesion or Malignancy
- Other: see interpretation or result (for example endometrial cells in a women more than or equal to 45 years of age)
- Epithelial Cell Abnormality: See interpretation/Result

3.7 Interpretation/Result

3.7.1 Negative for Intraepithelial Lesion or Malignancy

When there is no cellular evidence of neoplasia, state this in the General Categorization above/or in the Interpretation/Result section of the report whether or not there are organisms or other non-neoplastic findings.

3.7.2 Non-Neoplastic Findings

- Non-neoplastic cellular variation
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- Reactive cellular changes associated with :
  - Inflammation (includes typical repair) Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine Contraceptive Device
- Glandular cells status post hysterectomy

3.7.3 Organisms

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

3.7.4 Other

- Endometrial cells (in a woman >= 45 years of age)

4. Observations and Results

In the present study, we received a total of 250 cervical smears with clinical history. Out of 250 cervical smears, we excluded 10 smears which were inadequate to opine. So, the total of 240 smears got reported according to The Bethesda System 2014.

Maximum number of patients were in the age group of 31-40 years (n= 83, 33.2%) followed by 21-30 years (n=75, 30%), 41-50 years (n=52, 20.8%), 51-60 years

(n=26, 10.4%), 61-70 years (n=6, 2.4%), 11-20 years (n= 5, 2%) and 71-80 years (n=3, 1.2%).

In our study, a total of 10 (4%) Pap smears were reported as unsatisfactory, i.e. 10 smears out of 250 received smears were unsatisfactory
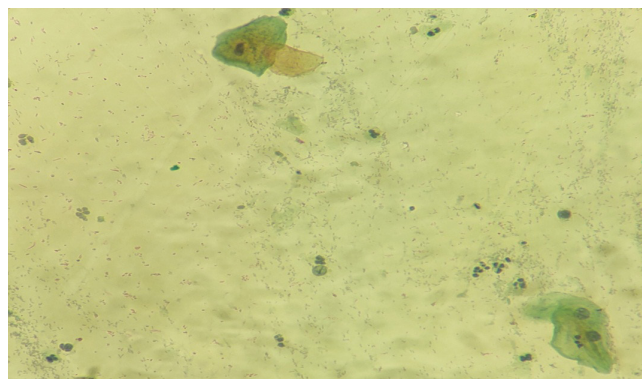
Out of a total 240 cases, 211 (84%) patients presented with complaints.

Patients presented with discharge per vaginum only were 76 (30%) and discharge per vaginum along with itching were 19 (8%). So, the patient's complaining of discharge per vaginum with or without itching accounts for a total of 95 (38%). The second most common complaint of patients were pain in abdomen which was present in 43 (17%) of cases followed by menstrual irregularity and postmenopausal bleeding (n=21, 9%) each, secondary infertility (n=13, 5%), prolapse (n=8, 3%), burning micturition (n=6, 2.1%) and Postcoital bleeding (n= 4, 1.9%) respectively. There were no complaints in 39 (16%) cases.
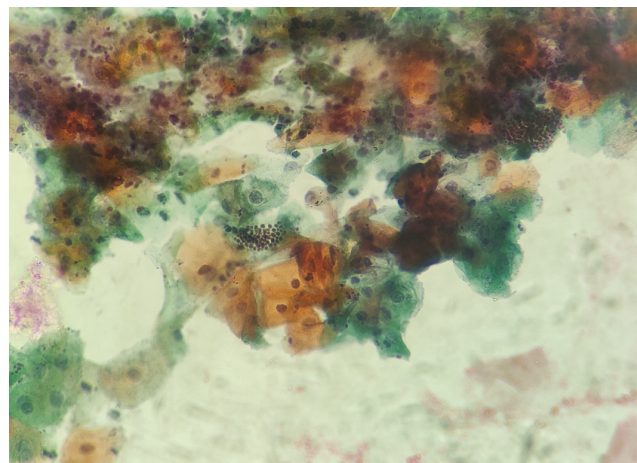
In our study, the most common age group showing inflammatory smear is 31- 40 years (n=70, 34%) followed by 21-30 years (n=60, 29%).

Our study shows that, the frequency of infection by bacterial vaginosis was highest, i.e. 51 followed by candida (n=37) [Photograph 2], Trichomonas (n=22) (Figure 1 and Table 1), [Photograph 1].

In our study, out of 240 satisfactory smears, epithelial lesions were seen in 45 cases. 11 cases of ASCUS (4.58%) were found, out of that, 4 cases were in age 41- 50 years followed by 3 cases in 31-40 years, 51-60 years each and 1 case was in 61-70 years. 22 cases of LSIL (9.17%) were found out of which, 11 cases were in 41-50 years followed by 5 cases in 31-40 and 51-60 years each. One case was in
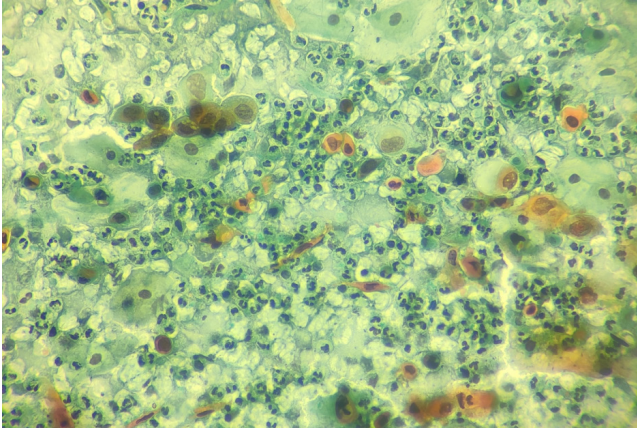

Photograph 2: Candida (400x)
Note clear halos surrounding the yeast forms

61-70 years. 4 cases of HSIL (1.67%) were found, out of which 1 case was found in age groups 31-40, 41-50, 51-60 and 61-70 years each. A total of 8 cases of SCC (3.33%) were found. Out of that, 4 cases were in 51-60 years, 2 cases were from 71-80 years, 1 case was from 41-50 years and 61-70 years each (Figure 2 and Table 2), [Photographs 3, 4, 5]

Our study shows that the frequency of normal (NILM) cases (n=195, 78%) is maximum followed by LSIL (n=22, 8.8%), ASCUS (n=11, 4.4%), SCC (n=8, 3.2%), HSIL (n=4, 1.6%) (Figure 3 and Table 3), [Photographs 3, 4, 5].
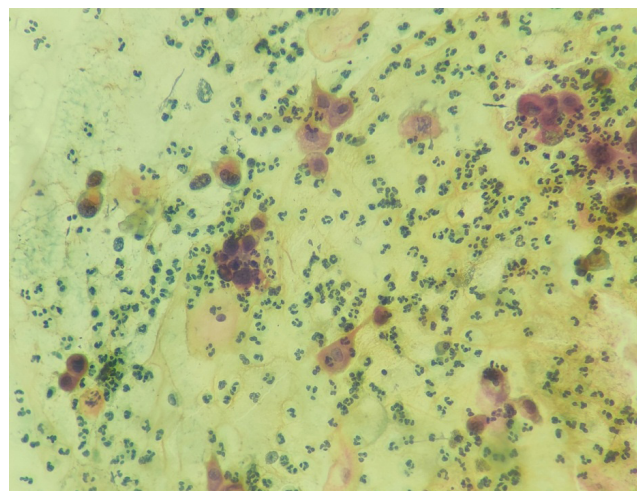

Photograph 3: LSIL (400x)
Nuclei are hyperchromatic with variable sizes (anisonucleosis). Chromatin is uniformly distributed and ranges from coarsely granular to smudgy or densely opaque. Contour of nuclear membranes is variable ranging from smooth to very irregular with notches.

Photograph 1: Trichomonas Vaginalis (400X)
Pear-shaped organism with eccentrically located nucleus and eosinophilic cytoplasmic granules.

Photograph 4: HSIL (400x)

Cells are present singly, in sheets & in syncytial-like aggregates. The cells are smaller. Nuclear to cytoplasmic ratio is higher. Nuclei are hyperchromatic. Chromatin is fine and is evenly distributed.Contour of the nuclear membrane is quite irregular and frequently demonstrates prominent indentations &grooves.
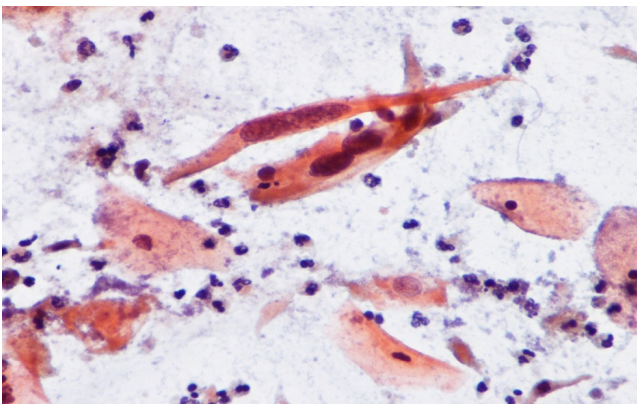
Photograph 5: SCC (Tadpole cells) (400x)

The malignant cells have variable shapes and sizes and show some keratinized "tadpole cells." Nuclei vary from vesicular with irregular nuclear contours and nucleoli to pyknotic in the keratinized cells. The cytoplasm is dense and may be deeplyeosinophilic or cyanophilic.

5. Discussion

In our study, the maximum patients, 33.2% belonged to age group of 31-40 years i.e. 4th decade. In a study by Bal *et al.* (2012)[12] on detection of abnormal cervical cytology in 300 Papanicolaou smears it was seen that the maximum number of patients (45.3%) were in the age group of 31-40 years i.e. 4th decade
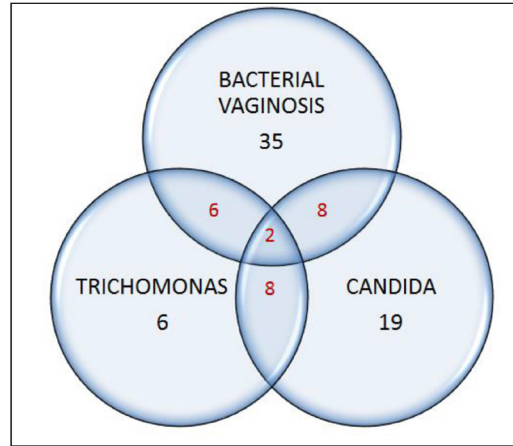

Figure 1. Venn diagram showing multiple infections.

Table 1. Distribution of smears according to infectious etiology

Type of Infection	Frequency
Bacterial Vaginosis (BV)	51
Candida (C)	37
Trichomonas (T)	22
Multiple Infection:	
BV + C BV + T C + T BV + C + T	8 6 8 2

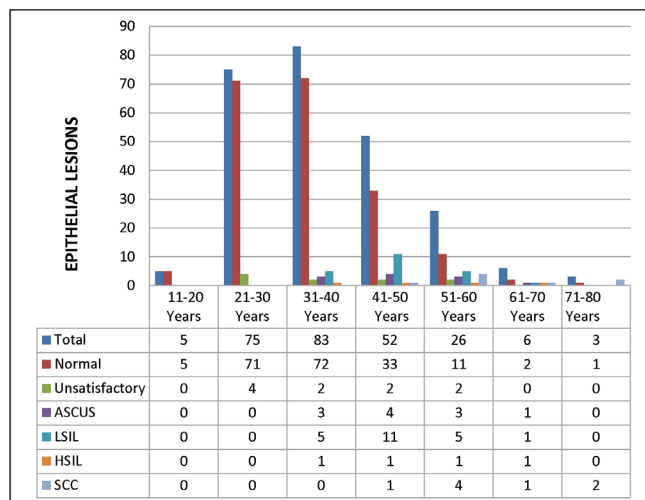|	11-20 Years	21-30 Years	31-40 Years	41-50 Years	51-60 Years	61-70 Years	71-80 Years
Total	5	75	83	52	26	6	3
Normal	5	71	72	33	11	2	1
Unsatisfactory	0	4	2	2	2	0	0
ASCUS	0	0	3	4	3	1	0
LSIL	0	0	5	11	5	1	0
HSIL	0	0	1	1	1	1	0
SCC	0	0	0	1	4	1	2

Figure 2. Bar diagram showing age-wise distribution of cases according to epithelial lesions.

Table 2. Shows age-wise distribution of cases according to epithelial lesions

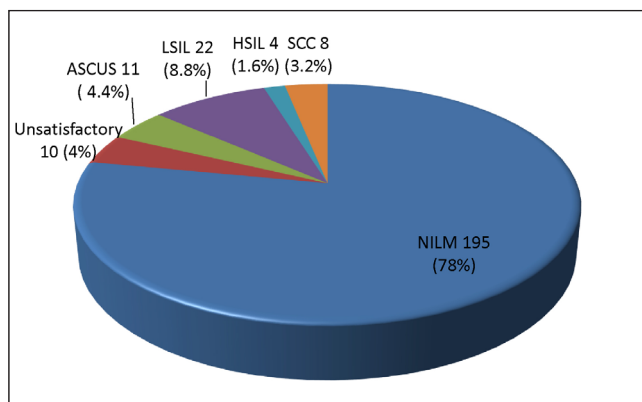| Age Group (In Years)	Total Cases	NILM	Un-Satis-factory	ASCUS	LSIL	HSIL	SCC
11-20	5	5	0	0	0	0	0
21-30	75	71	4	0	0	0	0
31-40	83	72	2	3	5	1	0
41-50	52	33	2	4	11	1	1
51-60	26	11	2	3	5	1	4
61-70	6	2	0	1	1	1	1
71-80	3	1	0	0	0	0	2
Total	**250**	**195**	**10**	**11**	**22**	**4**	**8**

Figure 3. Pie Diagram showing different epithelial lesions.

Table 3. Epithelial lesions in pap smears

Findings	Frequency	Percent (%)
NILM	195	78
Unsatisfactory	10	4
ASCUS	11	4.4
LSIL	22	8.8
HSIL	4	1.6
SCC	8	3.2
Total	**250**	**100**

In our study, average age of patients was 38.1 years with ages ranging from 18-75 years. In a study done by Roghaei et al. (2010)[13] mean age was 42.28 years with ages ranging from 20-67 years.

The most common presenting complaint in our study was discharge per vaginum which is seen in 38% of cases and the second most common complaint was abdominal pain which is present in 17% of cases. In a study done by Sachan et al. (2018)[7] discharge per vaginum which was the most common complaint seen in 36.96% of cases

which is a similar finding with our study. Also the second most common complaint is abdominal pain showing 25.63% of cases. In a study of Vermal et al. (2014)[14] again the most common complaint was discharge per vaginum with much higher incidence of cases i.e. 54.4% of cases and the second most complaint was abdominal pain with 45.6% of cases.

The percentage of unsatisfactory Pap smears was 4% in our study which is comparable to 4.1% as reported by Bal et al. (2012)[12] 3.8% as stated by Sankaranarayana et al. (2004)[16]; 4.8% as studied by Vaghela et al. (2014)[17].

Our study had 4% unsatisfactory smears, which might have been due to dryness of the smears or technical errors or a patient was not co-operative enough to take the smear.

In our study, maximum cases of LSIL i.e. 11 (9.16%) were seen in the 5th decade of life. In study done by Sherwani et al. (2007)[1] 77 (48.1%) cases of LSIL were found in 4th decade of life.

In our study, maximum cases of invasive carcinoma (SCC) 4 (3.33%) were found in 6th decade of life. In study of Aswathy et al. (2015)[18] the peak age of occurrence of cervical cancer in India was 6th decade which showed similar finding with our study.

Our study showed that cases of Negative for Intraepithelial Lesion or Malignancy (NILM) were found in 81.25% of cases. The study by conventional Pap smears showed NILM in 85% cases by Sherwani et al. (2007)[1] 68.8% by Verma et al. (2014)[15] 48.84% by Sachan et al. (2018)[7] 16.7 % by Bal et al. (2012)[12] 74.2 % by Altaf and Mufti (2012)[19] and 50% by Bukhari et al. (2012)[20].

Our study showed that cases of ASCUS were found as 4.58%. The cases of ASCUS were 4.8% in study done by Verma et al. (2014)[15] 2.9% by Sachan et al. (2018)[7] 8.8% by

Sankaranarayana *et al.* (2004)[16] 0.3% by Bal *et al.* (2012)[12] 9.3% by Altaf and Mufti (2012)[18] and 1% Bukhari *et al.* (2012)[19].

Our study showed that cases of LSIL were found as 9.16%. The cases of LSIL were 10.6% in study by Sherwani *et al.* (2007)[1] 5.6% in study done by Verma *et al.* (2014)[15] and 5.09% by Sachan *et al.* (2018)[7].

Our study showed that cases of HSIL were found as 1.66% The cases of HSIL were 0.6% in study by Sherwani *et al.* (2007)[1], 0.8% in study done by Verma *et al.* (2014)[15] 0.48% by Sachan *et al.* (2018)[7] 1.8% by Sankaranarayana *et al.* (2004)[16] 0.7% by Bal *et al.* (2012)[12] 0.9 % by Altaf and Mufti (2012)[19] and 2.2% by Bukhari *et al.* (2012)[20].

Our study showed that cases of Invasive carcinoma (SCC) were found as 3.33% (Figure 3 and Table 3). The cases of invasive carcinoma were 3.7% in study by Sherwani *et al.* (2007)[1] 0.8% in study done by Verma *et al.* (2014)[15] 0.22% by Sankaranarayana *et al.* (2004)[16] 1.3% by Bal *et al.* (2012)[12] 0.06% by Altaf and Mufti (2012)[19] and 1.4% by Bukhari *et al.* (2012)[20].

6. Conclusion

Pap test has significant utility worldwide. Bethesda system which is widely used for cervicalcytology is a uniform and standard method for reporting cervical smears. It also gives a descriptive diagnosis that aids the clinicians regarding individualized management of patients. It helps in prevention of invasive cervical cancer by timely detection and treating women having precancerous lesions. The effectiveness is determined by reduction in incidence and mortality.

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