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Original Research Article

Findings on Liquid-Based Cytology Prepared Smear among Women with Vulvo-Vaginal Itching and Discharge

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Abstract:

Aim: To analyse pathological changes and association between socio-demographic characteristics of itching and discharge, in women presenting vulvo-vaginal itching and discharge.

Method: This was a descriptive cytological hospital based prospective study conducted in Pathology department obtained from outpatient clinic of Obstetrics and Gynaecological department. Study was based on 274 women with complaints of vulvo-vaginal itching and discharge were obtained for cytological examination in a Liquid Based Cytology (LBC) collection vial to look for pathological changes. Pap smears samples (EZIPREP) were taken from all these patients with spatula and endocervical cytobrush and slides prepared. The remnant material on the spatula and cytobrush was centrifuged after being treated in Pap spin collecting liquid. The cell button was used to prepare smears, which were then fixed and stained with Papanicolaou stain. Data analysis was done using appropriate statistical tests.

Result: The age of participants ranged from 20 years to 72 years with mean age being 33.4 years. All patients had vaginal discharge with different consistency. Bacterial vaginosis was the most prevalent infection found in 34 (12.4%) cases. Trichomonas infection was found in 6 (2.19%) cases, candida species in 4 (1.45%) cases, squamous intraepithelial lesion SIL 14 (5.09 %) cases and majority were negative for intraepithelial lesion NILM 216 (78.83%) cases. Bivariate analysis showed that in case of factors associated with discharge, women of 41-50 years of age showed higher risk, but in case of factors associated with itching, women aged >60 years showed higher risk, and when associated with both discharge and itching, women aged between 31-40 years showed higher risk. Our study found that age exhibited mild variation in risk-related symptoms, recommending further research with larger sample sizes.

Conclusion: LBC is likely to continue to be the predominant technique for cervical cytology screening due to societal prerogatives and cytologists' preferences for the method. Bacterial vaginosis is found to be the most predominant cause of vaginal infection and thus serve as a key for researchers to find its underlying cause for an appropriate diagnosis. Common symptoms like discharge and itching have been linked to a variety of conditions, from cancer to bacterial vaginosis, which would not have been found by a simple clinical examination. Thus, Pap smear screening and examination in accordance with accepted standards are required.

Keywords: Cytology, LBC, LSIL, NILM, Vaginal discharge.

Introduction

Cervical cancer is the second most common malignant neoplasm in women worldwide, with an approximate annual incidence of 493,000 new cases and a mortality rate of 274,000 [1]. The successful identification of cervicovaginal malignancies is achieved through the utilisation of cervicovaginal cytology [2]. Although lacking a randomised controlled trial to establish its efficacy, cervical cancer screening utilising the Papanicolaou (Pap) smear is among the limited number of interventions that have received a "A" recommendation from the U.S. Preventive Services Task Force [3].The utilisation of cervical cytology in clinical settings was initially introduced by George Papanicolaou in 1940. In 1945, the Papanicolaou smear was officially endorsed by the American Cancer Society as an efficacious preventive technique for cervical cancer [4]. Utilising a cervical spatula and endocervical brush, a Pap smear specimen is conventionally obtained from the portiovaginalis

region of the cervix and the endocervical canal. The specimen is evenly distributed on a microscope slide and promptly treated with a cytofixative solution. The primary focus of medical practitioners revolves around the utilisation of smear capture techniques and prompt fixation to mitigate the presence of drying artefacts, thereby minimising the occurrence of sampling errors.

Cervical infections are a common occurrence among women in their reproductive years, often associated with clinical manifestations of vaginal discharge [5]. The female genital tract is accessed externally via the vulva, which comprises the labia majora and minora. These structures are characterised by a covering of squamous keratinizing epithelium. During the onset of adolescence, individuals may experience a gradual depigmentation of the labia, resulting in the presence of a delicate keratinized layer on the inner surface of the labia minora [6]. The meatus of the urethra, the terminal portion of the urinary tract, is located approximately 1 centimetre posterior to the clitoral region. The inguinal lymph nodes, known as the primary site of metastasis in malignant vulvar tumours, receive lymphatic drainage from the vulva. Pathogenic microorganisms have the potential to undergo excessive proliferation due to alterations in the intricate equilibrium of the normal vaginal microbiota, leading to the manifestation of vaginal discharge [7]. Due to the presence of abnormal vaginal microbiota, which can generate carcinogenic nitrosamines [8], various risk factors contribute to the development of cervical intraepithelial neoplasia and cervical cancer, including cervical infection. It has been determined that the utilisation of liquid-based preparations facilitates the execution of single-cell analysis, which is highly favoured by automated systems. The aforementioned advancements, in conjunction with enhanced sample equipment, have profoundly revolutionised cervical cytologic screening, a domain that had experienced minimal modifications since the introduction of the Pap smear approximately five decades ago. ThinPrep, the initial liquid-based cytology (LBC) product to obtain clearance from the Food and Drug Administration (FDA), successfully accomplished this milestone in the year 1996 [9]. The AutoCyte Prep, currently recognised as SurePath, obtained official approval from the Food and Drug Administration (FDA) after a span of three years. Despite the relatively recent introduction of Liquid-Based Cytology (LBC) as a diagnostic method, it has gained significant prominence in the field of cervical cancer screening. Current estimates from experts suggest that LBC is responsible for more than 90% of all Pap tests conducted in the United States at present [10].

Vulvovaginal symptoms, including pruritus, irritation, and abnormal vaginal discharge, represent a significant proportion of the chief complaints encountered by gynaecological healthcare professionals. Vaginitis has been observed to have a detrimental effect on patients and is closely linked to significant morbidity [11]. Additionally, it should be noted that candida, a fungal organism, has been identified as the etiological agent responsible for approximately one-third of the cases of vaginitis, as reported in a reputable scientific study [12]. Screening programmes utilising Liquid Based Cytology (LBC) prepared smears have demonstrated significant efficacy in the reduction of cervical cancer incidence and hold potential for further enhancement of screening efforts [13]. In recent times, there has been a growing prevalence of liquid-based cytological methodologies in the medical field. This surge in popularity can be attributed to preliminary research findings, which suggest a correlation between the utilisation of such technologies and a reduction in the frequency of unacceptable cervical smears. The objective of the current investigation was to ascertain the prevalence of various cytopathological abnormalities affecting the cervix among female individuals presenting with vaginal discharge.

Objectives

1. To analyse pathological changes in women presenting with vulvo-vaginal itching and discharge.
2. To look for association between socio-demographic characteristics of itching and discharge in women presenting with vulvo-vaginal itching and discharge.

Materials and method

The study was conducted from December 2021 to December 2022 in Department of Pathology, Tripura Medical College and Dr. BRAM Teaching Hospital, together with Obstetrics and Gynaecology Department on 274 patients with gynaecological complaints of vulvo-vaginal itching and discharge. This retrospective study excluded participants with known cases of cancer and chronic inflammatory diseases. Patients were randomly selected based on their reports of unusual vaginal bleeding [14], persistent vaginal discharge [15], lower abdomen pain, post-coital bleeding [16], and any abnormal findings on per speculum examination. After detailed history and written informed consent, conventional Pap smear taken by ayre's spatula and endocervical cytobrush, slide was prepared and residual material on both spatula and cytobrush was rinsed in 10-15 ml of pap spin collection, a buffered methanol preservative solution in vial. Pap smear slides and pap spin collection fluid along with collected material was sent to laboratory for cytopathology.

In laboratory the pap spin collection fluid along with collected material was centrifuged at 1500 rpm for 10 min then supernatant was discarded, and direct smear was prepared from cell button and fixed in cytofixative with equal part of 95% ethy1 alcohol and ether for at least 20 minutes and then in both type of slides staining was done by standard papanicolaou method and reporting was done according to revised Bethesda system [17]. After reporting patients with suspected malignant or dysplastic changes were subjected to colposcopic guided cervical biopsy. The data was then collected with respect to their age, educational and marital status, and was further analysed. For statistical analysis, data were analyzed using IBM SPSS Statistics for Windows, version 23.0. To compile and describe the information, frequencies and proportions were used. The association between independent and dependent variables was tested using Pearson's chi-square or Fisher's exact test, whichever suitable, and the odds ratio (OR) and its corresponding 95% CI were reported. A multivariable logistic regression model was developed for all variables included in the bivariate analysis, and the adjusted OR and its corresponding 95% CI were reported. P-values < 0.05 were considered statistically significant.

Results

Sample from 274 women were taken for the study. The age of participants ranged from 20 years to 72 years with mean age being 33.4 years. All patients had vaginal discharge with different consistency. Bacterial vaginosis was the most prevalent infection found in 34 (12.4%) cases [Figure 1]. Trichomonas infection was found in 6 (2.19%) cases [Figure 2], candida species in 4 (1.45%) cases [Figure 3], squamous intraepithelial lesion (SIL) with 14 (5.09 %) cases and majority were negative for intraepithelial lesion (NILM) for 216 (78.83%) cases [Figure 4].

The low-grade intraepithelial lesion (LSIL) cells showed large irregular nuclei which were three times the size of intermediate cell nuclei at 40x magnification [Figure 5(a)]. Atypical Squamous Cells, HSIL (ASC-H) with enlarged nuclei which is 2.5 to 3 times the size of normal intermediate cell nuclei was seen at 40x magnification [Figure 5(b)].

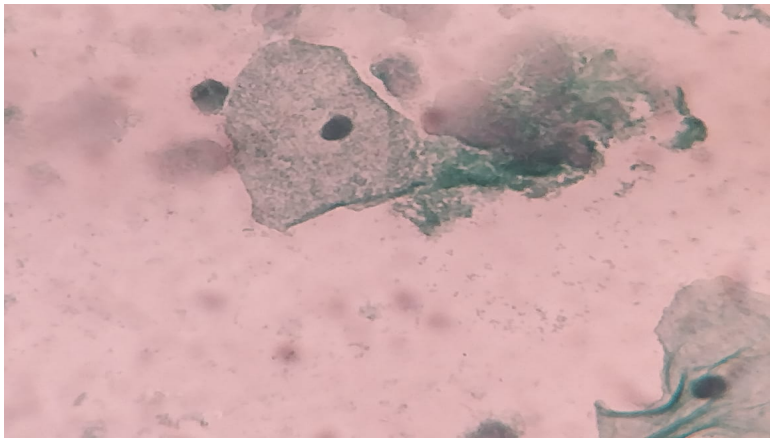


Figure 1: Clue cells (gram-negative bacteria adhering to epithelial cells) exhibited in vaginal smears from women having bacterial vaginosis

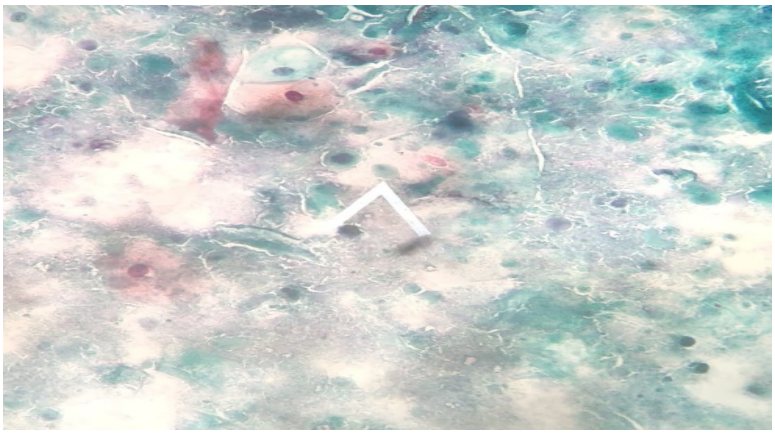


Figure 2: Trichomonas vaginalis- Pear-shaped organism with eccentric oval nuclei (40x)

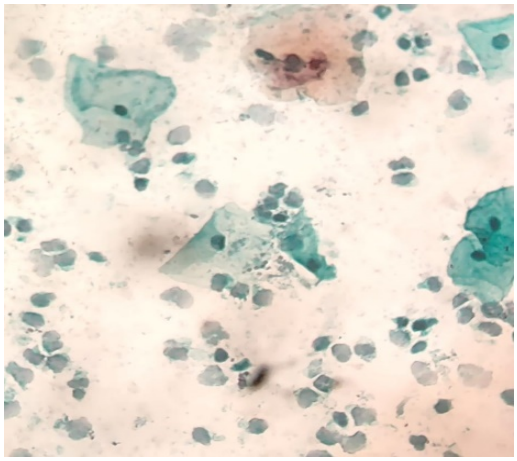
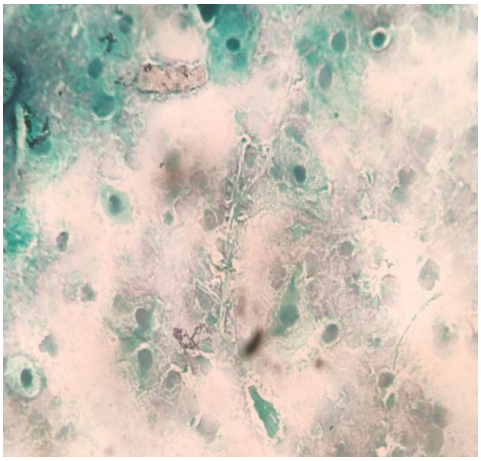
(a)  (b) 

Figure 3: (a) Budding yeast with surrounding Halo-Candida species (40x); (b) Psuedo-hyphae with budding spores – Candida species (40x)

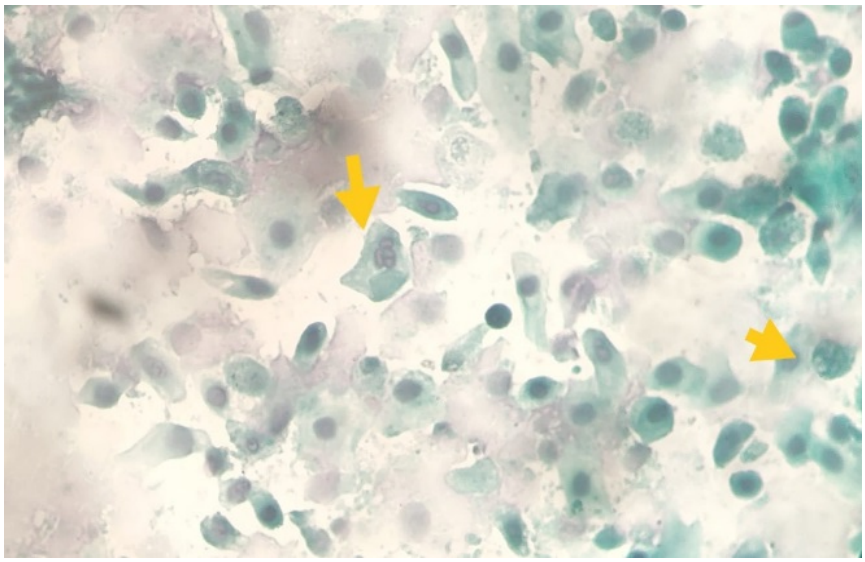


Figure 4: Squamous cells showing nuclear margination, moulding and multinucleation (arrows) suggestive of viral cytopathic changes (HSV infection) at 40x magnification

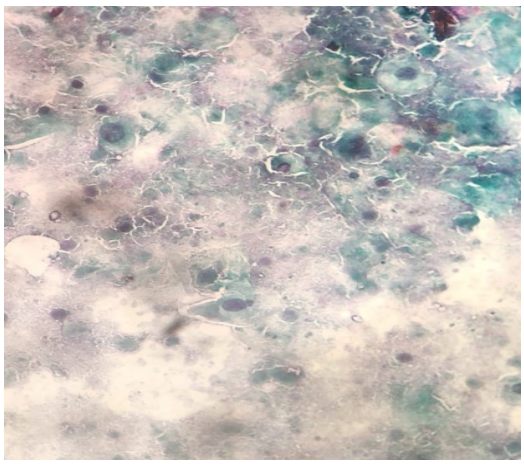
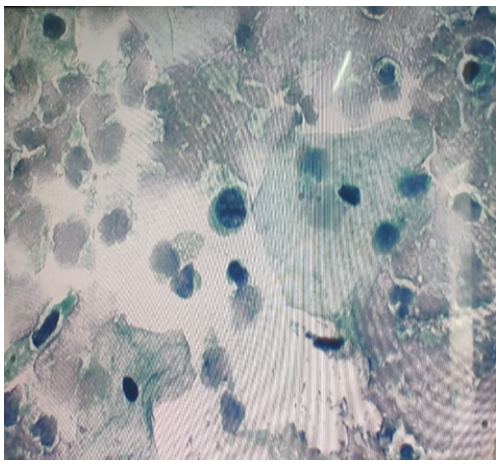
(a)  (b) 

Figure 5: (a) LSIL- Cells with large irregular nuclei (3 times the size of intermediate cell nuclei) (40x). (b) Cells with enlarged nuclei which is 2.5 to 3 times the size of normal intermediate cell nuclei – ASC-H (40x)

Table 1: Factors associated with Discharge

	Bivariate Analysis			Multivariate Analysis		
Factor	**Odd Ratio**	**95% C.I.**	**p-value**	**Odd Ratio**	**95% C.I.**	**p-value**
Age Group (Years)						
21-30	1.0(Ref.)	-	-	1.0(Ref.)	-	-
31-40	1.2	0.6-2.8	0.59	1.2	0.5-2.7	0.73
41-50	1.6	0.7-3.4	0.26	1.5	0.6-3.8	0.42
51-60	0.2	0.1-0.6	<0.01	0.3	0.1-0.9	0.03
>60	0.1	0.03-0.5	<0.01	0.2	0.0-1.1	0.06
Educational Status						
<5	1.0(Ref.)	-	-	1.0(Ref.)	-	-
06-10	0.6	0.4-1.1	0.1	0.8	0.4-1.4	0.38
>11	0.1	0.1-0.3	<0.01	0.4	0.1-1.3	0.11
Marital Status						
Unmarried	1.0(Ref.)	-	-	1.0(Ref.)	-	-
Married	0.89	0.5-1.7	0.69	1.3	0.6-3.3	0.46

In case of factors associated with discharge, bivariate analysis showed that women with age between 41-50 years showed higher risk of discharge with an odd ratio of 1.6. Similar was seen in the case of multivariate analysis [Table 1].

Table 2: Factors associated with Itching

	Bivariate Analysis			Multivariate Analysis		
Factor	**Odd Ratio**	**95% C.I.**	**p-value**	**Odd Ratio**	**95% C.I.**	**p-value**
Age Group (Years)						
21-30	1.0(Ref.)	-	-	1.0(Ref.)	-	-
31-40	3.5	0.7-16.5	0.11	5.3	1.1-27.4	0.04
41-50	4	0.9-18.0	0.07	8.6	1.4-51.9	0.01
51-60	38	7.7-187.3	<0.01	68.5	9.9-474.2	<0.01
>60	47.5	8.6-262.2	<0.01	83	9.9-698.2	<0.01
Educational Status						
<5	1.0(Ref.)	-	-	1.0(Ref.)	-	-
06-10	2.9	1.5-5.6	<0.01	1.8	0.8-4.2	0.16
>11	10.6	4.5-25.1	<0.01	1.8	0.5-6.3	0.37
Marital Status						
Unmarried	1.0(Ref.)	-	-	1.0(Ref.)	-	-
Married	1.8	0.8-4.1	0.14	0.2	0.1-0.8	0.02

In case of factors associated with itching, bivariate analysis showed that women aged >60 years showed higher risk of itching with an odd ratio of 47.5. Similar was seen in the case of multivariate analysis [Table 2].

Table 3: Factors associated with Discharge & Itching

	Bivariate Analysis			Multivariate Analysis		
Factor	**Odd Ratio**	**95% C.I.**	**p-value**	**Odd Ratio**	**95% C.I.**	**p-value**
Age Group (Years)						
21-30	1.0(Ref.)	-	-	1.0(Ref.)	-	-
31-40	0.4	0.2-1.0	0.05	0.3	0.1-0.9	0.03
41-50	0.2	0.1-0.6	<0.01	0.2	0.1-0.4	<0.01
51-60	0.1	0.0-0.6	0.01	0	0.0-0.4	<0.01
>60	0.2	0.0-1.1	0.06	0.1	0.0-0.5	<0.01
Educational Status						
<5	1.0(Ref.)	-	-	1.0(Ref.)	-	-
06-10	0.6	0.3-1.2	0.14	0.8	0.4-1.9	0.67
>11	0.8	0.3-2.3	0.7	3.7	0.7-18.7	0.11
Marital Status						
Unmarried	1.0(Ref.)	-	-	1.0(Ref.)	-	-
Married	0.6	0.3-1.3	0.2	1.8	0.6-5.2	0.27

When associated with both discharge and itching, bivariate analysis showed that women aged between 31-40 years showed higher risk related to discharge and itching with an odd ratio of 0.4. Similar was seen in the case of multivariate analysis [Table 3].

Our study found that age exhibited mild variation in risk-related symptoms, recommending further research with larger sample sizes.

Discussion

The patients presented in our study were the ones complaining of vulvo-vaginal itching and discharge. Regarding the underlying reasons for this high rate, the prevalence of clinically confirmed vaginal discharge among all participants in our study cohort is quite concerning. 12.4% of our study population were found to have bacterial vaginosis infections causing their discharges with respect to Trichomonas and Candida species. A similar study conducted by Abdul-Aziz, Maha, et al. [18], showed the prevalence of 27.2% vaginal infection due to bacterial vaginosis among reproductive-aged women. These variations might result from the traits of the study populations. The relevance of various discharge consistency and their correlation with aberrant cytopathological findings and bacterial vaginitis were highlighted by the current study. The majority of our patients with abnormal cytopathological changes (including squamous intraepithelial lesion (SIL) and NILM) were in the 20–72 years age group. Women with bacterial vaginosis are at high risk of infection. In the present study there were bacterial vaginosis was the most prevalent infection found in 34 (12.4%) cases. Trichomonas infection was found in 6 (2.19%) cases, candida species in 4 (1.45%) cases, squamous intraepithelial lesion SIL 14 (5.09 %) cases and majority were negative for intraepithelial lesion NILM 216 (78.83%) cases. Bivariate analysis showed that in case of factors associated with discharge, women of 41-50 years of age showed higher risk, but in case of factors associated with itching, women aged >60 years showed higher risk, and when associated with both discharge and itching, women aged between 31-40 years showed higher risk.

Changes in the compositions of the vaginal microbiota are associated with correlations between the vaginal pathogenic communities. Vaginal infections in reproductive-aged women are associated with specific sociodemographic characteristics, women's activities, and a history of poor obstetric outcomes. Squamous intraepithelial lesions indicative of aberrant tissue areas with the potential of being cancerous showed a minimum abundance, depicting a lesser chance of having malignancy. Bacterial vaginosis is found to be the most predominant cause of vaginal infection, followed by Trichomonas and candida species. It has been inferred that it contributes to gynaecological issues that might have a significant effect on women. Our study found that age exhibited mild variation in risk-related symptoms, recommending further research with larger sample sizes.

Conclusion

Common symptoms like discharge and itching have been linked to a variety of conditions, from cancer to bacterial vaginosis, which would not have been found by a simple clinical examination. Thus, Pap smear screening and examination in accordance with accepted standards are required. Due to the societal norms and cytologists' preferences for the technique, LBC is expected to remain the most common approach for cervical cytology screening. Enhanced probabilities of a cure are offered by the Pap smear's high incidence of precancerous lesions of cervical cancer detection, low cost, and practicability of application. Despite extensive study and technological advancements, it is still unclear what causes BV to start and what keeps the vaginal polymicrobial environment in place.

Interventions in health education are advised to increase women's knowledge of bacterial vaginosis and its prevention. The study is ought to provide a basis for the researchers to identify the underlying cause of the issue and offer a remedy to it.

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