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Diagnostic Role of Vaginal Ph, Wet Mount and Pap Smear in Women with Vaginal Discharge Attending Gynecological OPD

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Abstract: ***Background: Vaginal discharge is one of the common reasons in women of reproductive age group seeking gynecological consultation. Of all the vaginal infections known, Bacterial vaginosis accounts for 40%-50% of cases, Monilial infection 20%-25% of cases and Trichomonas infection 15%-20% of cases. Aim: To assess the diagnostic role of Pap smear, vaginal pH and wet mount in women with vaginal discharge attending Gynecological OPD at a Tertiary Care Hospital. Methods: Hospital based prospective study in 100 women attending Gynecological OPD, Sri Venkateswara Medical College, Tirupati. Results: Among 100 women, majority of women with pathology were in the age group of 26-35 years (71%), with parity >2 (65%) with BMI >30 (39%). Most of them belong to lower socioeconomic status (86%), 82% were married, 42% have substance abuse, 39% have symptoms in partner, 4% have HIV and 19% have PID changes. In the present study, 95 members have been diagnosed to be having pathology. Most common disease diagnosed was Bacterial Vaginosis (48%), Candidiasis (26%) and Trichomonas vaginalis (11%). Conclusion: Our study showed that Bacterial vaginosis is the most common infection leading to vaginal discharge followed by Candidiasis and Trichomonas vaginalis and considerable number of mixed infections. In low resource settings, results of vaginal pH, wet mount and the presence of characteristic vaginal discharge is of much diagnostic value.***

Keywords: Vaginal discharge, Vaginal pH, Wet mount, Pap smear, Bacterial vaginosis, Vulvovaginal Candidiasis, Trichomonas vaginalis

1. Introduction and Need for Study

Vaginal discharge is one of the common reasons in women of reproductive age group seeking gynecological consultation. It is the second most common clinical problem after menstrual disorders. Globally, it is estimated that one in ten women will present with vaginal discharge in a course of one year and approximately, ten million office visits each year are attributed to vaginal discharge complaints. It occurs in 1-14% of all women of reproductive age throughout the world and its prevalence in India is estimated to be 30% (Thulkar et al., 2010).[1] Most common documented causes of symptomatic vaginal discharge includes Bacterial vaginosis (BV), followed by Vulvovaginal candidiasis (VVC) and Trichomoniasis (Rekha and Jyothi, 2010).[2] Not all women with vaginal symptoms have vaginitis; approximately 40% of women with vaginal symptoms will have some type of vaginitis.[3] Many of the causes of vaginitis have a disturbed vaginal microbial ecosystem associated with them. Identifying the infectious source of vaginal discharge can be challenging, because of mixed infections.

Of all the vaginal infections known, Bacterial vaginosis accounts for 40%-50% of cases, Monilial infection 20%-25% of cases and Trichomonas infection 15%-20% of cases. The others are rare, though the incidence of Chlamydia infection is increasing. Most of the genital tract infections are sexually transmitted.[4]

The most common pathology with vaginal discharge is Bacterial vaginosis. Bacterial vaginosis is not an infectious process in the classic sense, typical inflammatory characteristics such as presence of increased leukocytes in vaginal discharge is absent. Microbiological characteristics include quantitative and qualitative decline in Lactobacillus colonisation together with an increase in anaerobic bacteria such as Garderella vaginitis, Mobiluncus spp, and Mycoplasma hominis. Vulvovaginal Candidiasis is a condition caused by monilial spp, characterized by the presence of itching and curdy white vaginal discharge, where local signs of inflammation are present as a consequence of invasion of the epithelium by the pathogen. Classic local signs of inflammation also occur with Trichomoniasis characterized by greenish yellow malodorous vaginal discharge and vulvovaginal erythema, cervical petechiea which is typically named as “strawberry cervix”. Aerobic vaginitis presents with signs of inflammation and profuse mucopurulent discharge and increased number of polymorphonuclear leucocytes.

Aim

To assess the diagnostic role of Pap smear, vaginal pH and wet mount in women with vaginal discharge attending Gynecological OPD at a Tertiary Care Hospital.

Objectives

To evaluate the diagnostic value of Pap smear, vaginal pH and wet mount.

2. Material and Methods

Source of Data: Women attending Gynecological OPD, Sri Venkateswara Medical College, Tirupati.

Study Design: Hospital based prospective study.

Study place: Gynecological OPD, Government Maternity Hospital.

Study Period: From approval of Ethical Committee to a period of one month.

Sample size: 100.

Inclusion criteria:

1) All women aged 20 to 45 years presenting to Gynecological OPD with vaginal discharge.

Exclusion criteria:

1) All women who are Postmenopausal.
2) All women who are unmarried.
3) All pregnant women.

3. Methodology

- The Institute Ethics Committee clearance obtained and informed consent is taken from the recruited women.
- A detailed history elicited regarding Socio-demographic factors like age, education, occupation, marital status, socioeconomic status, residence and living conditions, personal history like diet, sleep, stress levels, smoking, alcohol intake, family history. A detailed past obstetric history elicited.
- Detailed general examination including general condition, height, weight, BMI, thyroid, breast, spine, and gait are recorded. Vitals are recorded and systemic examination of patient including CVS and RS is done.
- A thorough gynecological examination is done and abnormalities in the vulva, vagina and cervix are noted. The amount, odor, color and consistency of vaginal discharge are noted.
- The vaginal pH is measured directly using pH indicator strips against the lateral vaginal wall.
- A sterile cotton swab is used to collect the vaginal discharge from the posterior vaginal fornix under direct vision and the specimen thus obtained is subjected to a series of laboratory tests.
- The Papanicolaou smears (Pap smear) taken.
- A detailed bimanual examination is done to exclude any pathology.

For each patient, two samples of vaginal discharge from the posterior fornix are collected with sterile cotton swabs. The first swab is subjected immediately to wet mount for clue cells, motile trichomonads and polymorphonuclear leucocytes under 100x and 400x magnifications. With the second sample, a 10% potassium hydroxide (Add 10 grams of Potassium Hydroxide pellets to 100ml of Distilled water to make to 100 ml of 10% KOH w/v solution) mount is prepared and whiffed for the presence of fishy odor (Amine test) and also for the presence of budding yeast cells.

Table 1: Clinical and Pathological Features of Various Vaginal Pathologies[5]

S.No	Entity	Bacterial Vaginosis	Vulvovaginal Candidiasis	Trichomoniasis	Aerobic Vaginitis
1	Medical history	Increased discharge, fishy odor, no dyspareunia	Itching, pain, dyspareunia, dysuria	Dyspareunia, dysuria, malodorous discharge	Dyspareunia, burning sensation (introitus, vagina)
2	Clinical findings	No signs of inflammation	Vaginal erythema or edema; eczematous, vesicular, follicular vulva	Vulvovaginal erythema, cervical petechiae (“strawberry” cervix)	Vaginal inflammation
3	Vaginal discharge	Grayish, thin, homogenous	Curdy white, usually odorless	Greenish-yellow, malodorous	Greenish-yellow, malodorous
4	Vaginal pH	Higher than 4.5	Lower than 4.5	Higher than 4.5	Higher than 4.5
5	Wet mount	Mixed bacterial flora, clue cells, isolated leukocytes	Blastospores, pseudohyphae, lactobacilli, possibly elevated leukocyte count	Trichomonads, clue cells, elevated leukocyte count	Parabasal cells, elevated leukocyte count, no lactobacilli
6	KOH (whiff) test	Positive	Negative	Often positive	Negative

Table 2: Sizes of microbial and cellular components seen in wet mount.[5]

- Trichomonad : ≤25µm
- Nucleus of squamous epithelial cells : 15µm
- Leucocyte (Granulocyte) : 10-15µm
- Erythrocyte : 6-8µm
- Blastospores : 5-7µm

Table 3: Wet mount and “whiff” test.[5]

Note: Microscopic examination of wet mount should be done within 10 minutes of preparation; if there is a suspicion of trichomonads use unstained wet mount.	
Unstained wet mount:	1 drop pf 0.9% NaCl is placed on a slide; a spatula is used to take a sample of the discharge from the vaginal vault; the sample is carefully mixed with the 0.9% NaCl on the glass slide; this is then carefully covered with a cover slide (avoid “smearing” and trapped air).
Stained wet mount:	Instead of the unstained NaCl solution, place 1 drop of 0.9% NaCl stained with methylene blue on the slide. Preparation of the solution: draw up 0.2 ml of 0.5% methylene blue and 1.8 ml of 0.9% NaCl in a 2 ml syringe. The solution can be used for several days after preparation if the syringe is kept closed.
Whiff test:	A second discharge sample (prepared in the same way as the unstained wet mount) is incubated with one drop of 10% KOH solution (from the pharmacy) without a cover slip. The fishy amine odor which this could trigger is extremely volatile. A sniff test should therefore be done immediately.

The diagnoses of different vaginal diseases are as follow:

1) Trichomoniasis:
   - Wet mount: Pear shaped organisms approximately the same size as that of a lymphocyte (10-20 µm) or that of a small neutrophil with characteristic jerky movements
   - Pap smear: Blue or grey pear shaped organisms with bright red granules; other features include moderate to marked inflammatory response and a proteinaceous blue grey background
2) Bacterial vaginosis based on Amsel's criteria in which the presence of three out of four criteria
   - Excessive homogenous uniformly adherent vaginal discharge
   - Vaginal pH more than 4.5
   - Positive amine test (Whiff test) – vaginal discharge collected from the posterior fornix of vagina was swabbed onto a glass slide followed by the addition of 10% of potassium hydroxide (KOH).Presence of a fishy odor was taken as a positive Whiff test
   - Presence of clue cells on microscopic examination.
3) Candidiasis was based on positive microscopy characterized by the presence of budding yeast cells.
4) Mucopurulent vaginitis defined as the presence of 30 or more polymorphonuclear leukocytes per oil immersion field in cervical mucus.

Statistical analysis

The data collected was tabulated in Microsoft Excel Worksheet and computer based analysis was performed. For comparison of means, unpaired *t* - test. For comparison of proportions, Chi -square test was used.

Ethical issues

Before collection of data all the subjects are briefed about the purpose of study and written informed consent will be obtained All investigations will be done free of cost ,no financial burden will be imposed on the patient.

4. Results

Table 4: Socio-demographic and risk behaviour characteristics

S.No	Parameters	Number of women with pathology n	Percentage %
1	**Age (Years)**		
	≤25	12	12
	26–35	71	71
	36-45	17	17
2	**Mean Parity**		
	Nulliparous	8	8
	1-2	27	27
	>2	65	65
3	BMI		
	18-24	27	27
	25-30	34	34
	>30	39	39
4	**Socioeconomic Status**		
	Upper	2	2
	Middle	12	12
	Lower	86	86
5	**Marital Status**		
	Married	82	82
	Widow	14	14
	Others	4	4
6	**Risk Behavior**		
	Substance abuse	42	42
	Symptoms in partner	39	39
7	STI		
	HIV	4	4
	PID	19	19

Table 5: Symptoms in Women Included in our Study

S.No	*Symptoms*	*Percentage of Patients (%)*
1	Discharge characteristics	
	Premenstrual flare	8
	Profuse	35
	Foul smelling	29
2	Lower abdominal pain	70
3	Vulvar itching	63
4	Dysuria	52
5	Dyspareunia	28
6	Post coital bleeding	1
7	Painful ulcers over genitalia	1

Table 6: Examination Findings in Women Included in Our Study

S.No	Signs	Percentage of Patients (%)
1	Discharge characteristics	
	Profuse	25
	Malodour	32
	Mucopurulent/purulent	35
2	Vulvar erythema	10
3	Vulvar excoriation	12
4	Vaginal erythema	18
5	Cervical congestion/ erosion	29
6	Adnexal tenderness	21

Table 7: Spectrum of Vaginal Discharge in Our Study

S.No	Diagnosis	Percentage of Patients %
1	Infectious causes	
	Bacterial vaginosis	48
	Candidiasis	26
	Trichomoniasis	11
	Mixed infections	10
2	Non infectious causes	
	Malignancies	3
	Physiological vaginal discharge	2
	Unclassified vaginal discharge	0
4	TOTAL	100

Table 8: Clinico - Investigative Criteria For Diagnosis

S. No	Parameter	Bacterial Vaginosis	Candidiasis	Trichomoniosis	Mixed Infections
1	Vaginal discharge	40	33	12	10
2	Pap smear				
	Normal	0	0	0	0
	Inflammatory	40	33	12	10
	ASCU/LSI	0	0	0	0

	L/ HSIL				
	Others	0	0	0	0
3	Vaginal pH				
	Normal	12	4	0	0
	Acidic	0	0	0	0
	Alkaline	28	29	12	10
4	Wet mount				
	Positive	28	29	12	10
	Negative	12	4	0	0

Table 9: Diagnostic Value

S.No	Parameter	Sensitivity	Specificity	PPV	NPV
1	Vaginal discharge	90	85	95%	80%
2	Pap smear	-	-	-	-
3	Vaginal pH	83	100	96.7%	23.1%
4	Wet mount	88	99	97%	24%

5. Discussion

Vaginal discharge is the most common medical problem in women which leads to significant discomfort and morbidity. Among 100 women who were studied in this present study, Majority of women with pathology were in the age group of 26-35 years (71%), with parity >2 (65%) with BMI >30 (39%). 86% of them belong to lower socioeconomic status, 82% were married, 42% have substance abuse, 39% have symptoms in partner, 4% have HIV and 19% have PID changes.

According to Indira Guntoory et.al, the prevalence of vaginal discharge was found to be 28.9%. It is more prevalent in younger age group, illiterate, women belonging to low socio- economic status and those who were married less than 18 years of age.[6] According to Ebtisam Hashem Zaher et.al, the prevalence of vaginal discharge is more common in the age group of 25-36 years and most of them were marries (83%) which is concurrent with the present study.[7] Study done by Shresta A et.al have shown that 50.3% patients belong to class V socioeconomic status and 2.6% belong to class-I status.[8] According to Ishita Gosh et.al, sensitivity, specificity, PPV and NPV of vaginal discharge as an indicator of STI/RTI were 85.5%, 99%, 99.3% and 80% respectively.[9]

In the present study, 70% had complaints of Lower abdominal pain, 35% had profuse discharge, 29% had foul smelling vaginal discharge, 63% presented with vulvar itching, 52% complained of dysuria and 28% had dyspareunia. 1% had post coital bleeding as their chief complaint and 1% had painful ulcers over genitalia. On examination, 35% had mucopurulent discharge, 29% had cervical congestion, 18% had vaginal erythema and 21% had significant adnexal tenderness. According to Indira Guntoory et.al, lower abdominal pain, dysuria and backache were the most prevalent co-morbidities with vaginal discharge.[6]

n the present study, 95 members have been diagnosed to be having pathology. 2% had physiological vaginal discharge and 3% with evidences of malignancies. Most common disease diagnosed was Bacterial Vaginosis (48%), Candidiasis (26%) and Trichomonas vaginalis (11%).
10% had mixed infections.

Prevalence of BV, VVC and TV in different studies[10]

S. No	Study group	Year	Bacterial vaginosis BV %	Vulvovaginal Candidiasis VVC %	Trichomonas vaginalis TV %
1	Vishwanath et al	2000	26.0	-	10.0
2	Bhalla et al	2007	32.8	16.9	2.8
3	Patel et al	2006	17.8	8.5	-
4	Dan et al	2003	23.5	35.5	8.1
5	Puri et al	2003	45.0	31.0	2.0
6	Fonck et al	2000	9.0	50.0	23.0
7	Zaki et al	2010	39.1	1.0	30.0
8	Sood N et al	2014	21.5	15.5	1.5

Among 48 cases who were found positive for Bacterial vaginosis, 40 cases had typical thin homogenous vaginal discharge with fishy odour .Nearly all had an inflammatory result in pap smear. 28 cases had alkaline pH and 12 had normal. Wet mount examination was positive in 28 cases and negative in 12 cases. 33% of cases showed curdy white discharge who all had an inflammatory result in pap smear, 29 cases had an alkaline pH. 29 had hypae and spore like structures seen on wet mount.12 cases showed typical features of trichomonar vaginalis and were seen positive on wet mount.

6. Conclusion

Our study showed that Bacterial vaginosis is the most common infection leading to vaginal discharge followed by Candidiasis and Trichomonas vaginalis and considerable number of mixed infection. In low resource settings, results of vaginal pH, wet mount and the presence of characteristic vaginal discharge is of much diagnostic value.

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