INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-4: Oct - Dec -2011 IJABPT *ISSN 0976-4550*

SIGNIFICANCE OF DIRECT SMEAR EXAMINATION FOR THE DIAGNOSIS OF FEMALE GENITAL TRACT INFECTIONS.

Annie Rajaratnam, Thomas. S. Kuruvilla and *Beena Antony.

*Department of Microbiology, Father Muller Medical College, Mangalore

Department of Obstetrics & Gynaecology, Father Muller Medical College, Mangalore.

E- Mail ID: beenafmmc@gmail.com

ABSTRACT: The present study is conducted to demonstrate the value of direct smear examinations (Gram stain and wet mount) of vaginal discharge from infected female patients. The diagnosis of bacterial vaginosis and other infections were carried out by direct gram stained smear and wet mount preparations of vaginal discharge in 1004 cases of various infections affecting these patients. Gram stain was a useful tool to pick up various infections of the genital tract and wet mount was helpful in detecting Trichomonas vaginalis. In short , direct smear examination will facilitate clinician in the diagnosis of female genital tract infections with minimum requirements

Key words: Bacterial vaginosis, Gram stain, Vaginal discharge, Wet mount.

INTRODUCTION

Female genital tract infections are of polymicrobial aetiology including bacteria, viruses, fungi and parasites. These infections include vulvovaginitis, vulvovaginal pyogenic infections, abscesses of Bartholin's and Skene's glands, infected labial inclusion cysts, labial abscesses & furunculosis, endometritis, salpingitis, pelvic inflammatory disease, tubo-ovarian and pelvic abscesses. An abnormal vaginal discharge associated with these symptoms may be of different colours like green, yellow and brown with or without foul smelling odour, pruritis, irritation, dysuria etc depending on the type of infection. (Nwadioha S et.al., 2011). Apart from anaerobic bacteria like Bacteroides fragilis group, Mobiluncus, Gardnerella vaginalis, aerobes like Neisseria gonorrhoeae, Escherichia coli and other organisms like Trichomonas vaginalis, Candida species, Herpes simplex virus, Chlamydia, Ureaplasma urealyticum also contribute to the pathogenicity. A healthy vagina normally is predominated by species of Lactobacilli like *Lactobacillus crispatus* and *Lactobacillus jensenii*. These prevent colonisation of vagina by other pathogens and multiplying them to cause symptoms. A change in normal bacterial flora including the reduction of lactobacillus, which may be due to the use of antibiotics or pH imbalance, allows more resistant bacteria to gain a foothold and multiply. Certain physical factors that contribute to development of infection have to be considered, such as constantly wet vulva due to tight clothing, chemicals coming in contact with the vagina via scented tampons, antibiotics, birth control pills, or a diet favoring refined sugar and yeast. The sexual history of the patient is also important in assessing the role of various organisms in bringing about a change in the vaginal flora. (Eishibly S et.al., 1996). Various standardized techniques and scoring systems were proposed for its diagnosis as described by Amsel, Speigel, Nugent et al (Morgan J D et.al., 1996) with moderate to excellent ranges of reliability. The present study is investigated to assess the value of direct smear examination of vaginal fluid by Gram stain and wet mount examination to diagnose vaginitis without employing cumbersome culture techniques.

MATERIALS AND METHODS

Our study was conducted at a tertiary care centre in Mangalore spanned over a period from January 2008 to June 2010. Relevant history of the patients pertaining to our study was taken and the specimens were obtained by a gynaecologist from a target group of women in the reproductive age group who attended the out patient department of our institute. Subjects on oral antimicrobial therapy or any form of vaginal medication were excluded from the study. A control group of 100 healthy women in the reproductive age group, attending gynaecology out patient clinic for family planning consultation.

Collection of the samples

A total of 1004 vaginal samples were collected during this period by the gynaecologist. The posterior and lateral vaginal fornices and cervix were sampled by high vaginal and cervical swabs after exposing the cervix with a sterile, unlubricated Cusco's speculum. Quality of samples from collection to transport were maintained and determined throughout the study. Five vaginal epithelial cells were to be seen per high power field before the smear was considered adequate for assessment.

Method of processing

Samples were processed within two hours of the time of collection. The characteristics of the discharge such as quantity, colour and smell were recorded and the pH of the discharge was determined directly with a pH indicator paper. An immediate fishy odour on addition of 1-2 drops of 10% KOH was considered as a positive amine test . The vaginal flora was graded by using the Nugent's scoring system:

(i) Vaginal pH > 4.7

(ii) Thin homogenous discharge.

(iii) Presence of **" Clue cells "**.

(iv) Positive amine test.

The subject was considered as a case of non bacterial vaginosis if three of these criteria were not met with. Further wet mount and Gram staining were done for the presence of pus cells, Lactobacilli, Mobiluncus, yeasts, Trichomonas & anaerobes. The swab was rolled over a clean glass slide, fixed with heat and stained by Gram's technique, using dilute carbol fuchsin as the counter stain. Each Gram stained smear was evaluated under oil immersion (1,000x) and its significance in female genital tract infection was assessed.

RESULTS

All the 1004 patients in the study were divided into various range of age groups from ≤20 to ≥ 50. Table 1 shows the distribution with an emphasis on the reproductive age group.

TABLE 1. AGE WISE DISTRIBUTION OF THE CASES STUDIED

AGE GROUP	NO OF PATIENTS	PERCENTAGE
≤ 20	68	6.7
21-30	441	43.92
31-40	378	37.64
41-50	90	8.96
≥ 50	27	2.68
Total	1004	

Table 2 shows the various organisms and infections detected by Gram stain & direct wet mount of the samples obtained from the female genital tract, in the test and control group. The maximum number of cases were of bacterial vaginosis (BV) 543 (54.08 %) followed by Candidiasis in 166 (16.53%) whereas 3% of the control group showed signs of BV by direct Gram stain. In 9% of the cases of the control group, yeast like cells ie. Candida was seen without pseudomycelium. Vaginal lactobacillosis was observed in 75 (7.47%) cases in the test group but none in the control group. 155 cases of the test group revealed Gram positive bacilli resembling Lactobacilli and numerous vaginal epithelial cells indicating normal vaginal flora. Trichomonas was demonstrated in the direct smear examination in 15 (1.49%) cases in the test group and one case in the control group.

Table 3 assigns Nugent's criteria in 543 (54.08 %) cases of BV in the present study. These smears were predominated by gram variable bacilli morphologically resembling Gardnerella vaginalis and anaerobic bacteria as well as many clue cells (vaginal epithelial cells studded with gram variable bacilli). The lactobacillus morphotype was consistently absent, or if present, only in low numbers (1 +, 2+), in all patients diagnosed clinically as having BV. Heavy counts of lactobacilli (3 +, 4 +) when recorded in the cases indicated normal flora.

TABLE 2. PATTERN OF VAGINAL INFECTION OBSERVED IN THE PRESENT STUDY BY DIRECT SMEAR EXAMINATION

MICRO ORGANISM	TOTAL	CONTROL
Bacterial vaginosis (Gram variable bacilli & clue cells with or without pus cells)	543 (54.08 %)	3
Candida species (Yeast cells with or without pseudomycelium)	166 (16.53 %)	9
Trichomonas vaginalis	15 (1.49 %)	1
Gonococci	7 (0.69 %)	---
Vaginal Lactobacillus	75 (7.47%)	-----
Miscellaneous Organisms	43 (4.28%)	14
Normal Flora (Gram positive bacilli + Vaginal epithelial cells)	155 (15.43%)	73
TOTAL	1004	100

TABLE 3. NUGENT SCORING FROM CASES OF BACTERIAL VAGINOSIS

NUGENTS SCORE	WITH BACTERIAL VAGINOSIS
> 7	215
4-6	306
0-3	22
TOTAL	543

DISCUSSION

Vaginitis is a clinical condition associated with inflammation of vagina due to Candida, Trichomonas, bacteria and certain viruses. The main categories of vaginal infections are bacterial vaginosis or Non specific vaginitis caused by G.vaginalis and anaerobes, Trichomonas vaginalis vaginitis and moniliasis (Candida vaginitis).

Bacterial vaginosis is more likely to occur in sexually active women of reproductive age group. It is a disorder of the chemical and biological balance of the normal flora. Pregnant women and women with sexually transmitted infections are especially at risk and sometimes women after menopause may also get infected.
(Eishibly. S et.al., 1996) . Review of literature has revealed that women with bacterial vaginosis are at increased risk of developing bartholinitis, skenitis and pelvic inflammatory disease. A recent study from north India has estimated that the incidence of poor pregnancy outcome was higher in bacterial vaginosis with urinary tract infection, hence they recommended antenatal screening of patients for the presence of bacterial vaginosis and other vaginal infections. (Lata et.al., 2010).
Gardnerella vaginalis, a marker for bacterial vaginosis is usually seen in combination with bacteria morphologically resembling anaerobes like Mobiluncus, Prevotella, Porphyromonas group and Bacteroides fragilis. In the present study, incidence of BV was found in 543 cases out of 1004 (54.08 %). Various Indian studies reported the prevalence of BV from 22-56%. (22.6% by Pandit et.al., 1993, 36.5% by Duggal et.al., 1992 and 56% by Babu et al.,1987). The inverse relationship between the presence and concentration of Gardnerella and Lactobacillus morphotypes in the Gram-stained smears that we observed in this study is in agreement with Amsel et.al. 1983 . In our study lactobacilli were well detected by Grams stain compared to wet mounts.
The expertise and time required to diagnose these infections by culture and other sophisticated newer methods may not be very practical. Gram stain coupled with wet mount preparations of vaginal secretions can together enhance the overall sensitivity of the diseases in this context. All cases with a clinical diagnosis of BV had the same diagnosis made from the Gram-stained smear alone, thus indicating a high positive correlation between the two. This finding correlates well with a study by Rotimi V.O et al.,1991. An increased prevalence of Gram negative bacilli, Gram variable coccobacilli suggestive of a polymicrobial etiology was observed in the smears obtained from patients with BV. This finding is in accordance with the reports of other workers.
(Spiegel et.al.,1980)

Trichomoniasis is a sexually transmitted disease caused by Trichomonas vaginalis and commonly affects the urethra and the vagina in women. Clinical features include inflammation of the cervix, urethra and vagina which produce an itching or burning sensation. There may also be a yellow-green, itchy, frothy foul smelling vaginal discharge and rarely with lower abdominal pain.(Feo LG et.al., 1952). The prevalence of Trichomonas in the present study was very low 15 (1.49 %). This may be due to the unavoidable delay in the transit and processing of the specimen . The characteristic motility of Trichomonas was demonstrated in fresh samples by wet mount. This method was found to be better than Gram stain. Gonococci was also seen in low numbers ie. 7 (0.69%). Very low finding of this organism may be owed to indiscriminate and wide use of antibiotics. (Anderson et.al., 2000).

Yeast vaginitis is very uncommon before menarche and after menopause in the absence of taking any estrogen. Vaginal pH is lower in yeast infections than other types of vaginitis usually in the range of 3.8-4.2 but almost always less than 4.5. Interpretation of Candida in vaginal smears is always a matter of debate as it may be present as normal flora. The presence of pseudomycelium may be useful in differentiating the pathogenic and commensal status. In our study out of 166 cases of Candidiasis in the test group, 108 exhibited pseudomycelium in Gram stain and the rest 58 had either predominant yeast like cells or clinical correlation. About 25-30% of reproductive age women have some yeast present in the vagina. Wet preparations are also accurate if both hyphae and yeast like cells are seen. The most common yeast like organism is Candida albicans but other species such as C. glabrata, C. tropicalis, C. guilliermondii and C. parapsilosis also produce viginities.(Speigel et.al., 1983). In the present study, Candida was seen in 166 (16.53 %) cases and 9% in the control group. Few studies from African countries, Candida species was reported as the predominant organism peaked with 42.0% (Wadioha S N et.al., 2011) and 52.5% (NWokedi E E et.al.,2003).

A clinical entity which did not acquire much attention among the clinicians as well as the microbiologists is vaginal lactobacillosis, an infective stage. It is a cyclic condition characterized by vaginal discharge and discomfort occurring seven to ten days before menses and associated with the occurrence of long serpiginous rod like organisms, visible in wet mount preparations and in Gram stain. Only very few studies have been reported in regard to the prevalence rates of vaginal lactobacillosis.(Horowitz B.J et.al., 1994). In our study, 75 (7.47%) cases of vaginal lactobacillosis were encountered.

Women presenting with cytolytic vaginosis complain of a thick or thin white cheesy vaginal discharge, pruritis, dyspareunia, vulvar dysuria, and a cyclic increase in symptoms that are more pronounced in the luteal phase. This condition too shows large number of lactobacilli and an increased evidence of cytolysis with a paucity of white blood cells. (Cibley LG 1991, Secor RM 2001 and Hutti HM et.al., 2000)

Gram stain had been employed and evaluated by certain investigators for the diagnosis of vaginitis (Spiegel et.,1983, Morgan J D et al., 1996). In the present study we employed both Gram stain and wet mount. Trichomonas vaginalis was appreciated well in the wet mount due to the characteristic motility. For other forms of vaginitis Gram stain was found to be more satisfactory. In short, vaginitis remains serious question, social psychological as well as medical. As culture requires time and expertise, Gram stain and wet mounts studies of vaginal secretions can have a great significance providing rapid cost effective diagnosis in trivial female genital tract infections both in primary and tertiary health care settings.

REFERENCES

1. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK (1983). Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. Am J Med: 74: 14-22.
2. Anderson M R, Klink k, Cohrsen A (2000).Evaluation of vaginal complaints.JAMA:291: 1368.
3. Babu H M, Subbannayya K, Lakshmi V.(1987). A bacteriological study of vagina in non specific vaginitis. Indian J Pathol Microbiol:30:127-134
4. Cibley LJ (1991). Cytolytic vaginosis. Am J Obstet Gynecol:165:1245-1249.
5. Duggal S, Aggarwal, Arora et.al.(1992). Bacteriology of NSV with special reference to Gardnerella vaginalis. Ind J Med Microbiol:10:185-188
6. Eishibly S, Ingegerd Kallings, Dan Heliberg,Per-Anders Mardh(1996). Sexual risk behaviour in women carriers of Mycoplasma hominis. Br J Obstet Gynaecol: 103:1124-1128.
7. Feo LG, Dellette BR.Leptotrichia (Leptothrix) vaginalis (1952). Am J Obstet Gynecol:64:382-386.
8. Horowitz B. J,Per-Anders Mardh,Elisabeth Nagy and Elliot L.Rank. Vaginal lactobcillosis. Am J Obstet Gynecol:170(3):857-861.
9. Hutti MH, Hoffman C.(2000). Cytolytic vaginosis: An overlooked cause of cyclic vaginal itching and burning. J Am Academy of Nurse Practitioners:12:55-57.
10. Lata I, Pradeep Y, Sujatha, Jain A. (2010).Estimation of the incidence of BV and other vaginal infections and its consequences on maternal / fetal outcome in pregnant women attending an antenatal clinic in a tertiary care hospital in North India. Ind J Com Med: 35(2):285-289.
11. Morgan J D, Aboud C J (1996). Comparison of gram stained smears prepared from blind vaginal swabs with those obtained at speculum examination for assessment of vaginal flora. Br J Obstet Gynaecol:103:1105-1108.

12. Nwokedi E E,Anyiam NN. (2003).A study of high vaginal swabs in Kano teaching hospital; A prelimnary report. Highland Med Res J:1:57-61.
13. Pandit D V, Bhatt R R, Karnad JM, Deodhar LP.(1993).Microbial screening of females with vaginitis. Ind J Obstet Gynaecol:43:244-252.
14. Rotimi V. 0., Z. yakubu, 0. 0. Abudu and T. 0. Banjo(1991). Direct Gram's stain of vaginal discharge as a means of diagnosing bacterial vaginosis. J Med. Microbiol:35:103-106.
15. Secor RM (2001).Bacterial vaginosis common, subtle, and more serious than ever. Clin Rev:11:59-68.
16. Spiegel CA, Amsel R, Eschenbach D, Schoenknecht F, Holmes KK (1980). Anaerobic bacteria in nonspecific vaginitis. N Eng J Med: 303: 601-607.
17. Spiegel CA, Eschenbach DA, Amsel R, Holmes KK. (1983) Curved anaerobic bacteria in bacterial (nonspecific) vaginosis and their response to antimicrobial therapy. J Infect Dis:148:817-822.
18. Wadioha N S, Egah D, Nowkedi E, Onwvezobe I. (2011).A study of female genital swab in primary health care centres in Jos, Nigeria. Asian Pac J Trop Dis: 52-54.