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Characterization of vaginal discharge among women complaining of genital tract infection

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Abstract

High vaginal swabs were collected from 360 women attending Al –Batnan medical center were examined microbiologically and the causative agents were isolated from 310 patients (86%). Bacterial vaginitis is the most common cause of abnormal vaginal discharge and constituted 79.5% of total cases, with (*Staphylococcus aureus* 21.8%, *Escherichia coli* 14.2% and *Klebsiella* species 13.6%) followed by candidal infections 13.6%. A comparatively Bacterial vaginosis (due to *Gardenerlla vaginalis*) (2.2%) was least seen as a cause of vaginal discharge.

pH was > 5 in 83.3% of women complaining of abnormal vaginal discharge, due to bacterial infection but was, < 5 with candidal infections. 34 swabs (10% of cases) showed positive amine test. Pus cells were detected in 85% of cases. The appearance of the discharge varied according to the causative agents and pH; yellow-green purulent discharge was associated with pH over 4.5and *Trichomonas vaginalis*, white discharge with pH below 4.5 and *Candida albican* and grey, offensive, thin discharge with pH over 4.5 in those caused by, *G. vaginalis* infections. Abnormal vaginal discharge was the commonest symptom found in the age group of 26 to 30 years.

Key-Words: Vaginal discharge, Bacterial vaginitis, Bacterial vaginosis, Clue cells, Amine' test, Candidal infection

Introduction

The female genital tract (FGT) provides a satisfactory environment for many pathogenic microorganisms and multiple infections are therefore common (Mukhlis, 1997). It is colonized by a variety of species of commensal bacteria causing no harm except under abnormal conditions**. (**Finegold *et al.*, 1986).

At birth, vagina of the newborn is colonized initially by anaerobic and aerobic bacteria acquired during passage through birth canal. The epithelium of the vagina at this time is rich in glycogen as a result of the influence of placental and maternal estrogens. This resulted in a low pH (3.7-6.3). Several weeks after birth the epithelium becomes thin, atrophic and largely devoid of glycogen, the pH rises to 6-8 (remaining so until puberty) and the predominant flora are Gram-positive cocci and bacilli (Gunning, 1992). At puberty the estrogen increases the proliferation of vaginal epithelial cells and glycogen deposit in it. .*Lactobacillus* proliferates and cause enzymatic breakdown of cellular glycogen, resulting in lactic acid and H2O2 which lower the pH to 3.5-4.5. This indicates normal vaginal environment, which inhibits the growth of pathogenic organisms (Caillouett *et al*., 1997).

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After the menopause, lactobacilli again diminish in numbers and mixed flora return (Jawetz *et al*.,1987). The normal vaginal flora often includes group β-hemolytic *Streptococci*, anaerobic *Streptococci (Peptostreptococci), Bacteroids* species, *Clostridia, Gardnerella vaginalis, Ureaplasma urealyticum*, and sometimes *Listeria* or *Mobiluncus* species but the cervical canal and fallopian tubes are sterile. (Read, Duerden, Porter, and Turk, 1983).

Vaginal discharge may be physiological or pathological. Physiological discharge comprises secretions of the Bartholin's gland and the endocervix with cells shed from the vaginal walls. These secretions are affected by hormonal changes during the menstrual cycle. Cervical ectropions, the intra uterine contraceptive device and the combined oral contraceptive may increase physiological discharge. There is a natural increase in vaginal discharge at the time of puberty, ovulation, premenstrual and during pregnancy, this consider under heading of leucorrhoea. The pre-pubertal and postmenopausal vagina, as they are not well estrogenised is more prone to infection (Kumar, 1994)

Pathological discharge in women of reproductive age, is usually caused by infection and causative organisms may or may not be sexually transmitted. Pathological discharges are due to infections, usually vaginitis

(*Candida albicans*, *Trichomonas vaginilis* and anaerobic vaginosis) or cervicitis (*Chlamydia trachomatis*, *Neisseria gonorrhoeae* and Herpes simplex). Pathological discharge may also due to non-infective disease such as genital tract tumors or fistula and chemical vaginitis as result of the use of perfumed soaps, bath additives, spermicides or antiseptic douche and foreign bodies in vagina (Hudson *et al*.,1998).

Vaginal discharge is a common, but neglected, health problem in women in their reproductive age. It is therefore important to know the exact prevalence of vaginal discharge in women with genital tract infections. The present study carried out to find the characteristics of vaginal discharge, prevalence of pathogenic agents causing vaginal discharge and the prevalence of pus cells in relation to pathogenic agents.

Experimental

This study was conducted at Al Batnan Medical Center, Tobruk, over the period from October, 2008 to October, 2009. The study included 310 inpatients and outpatients who referred to the gynecological clinic of Al-Batnan hospital complaining of abnormal vaginal discharge. The age of patients ranged between 18-50 years (mean age 30.8 years).

Vaginal swabs were taken by the gynecologist. Two high vaginal swabs were obtained from each patient. Both were taken in the same fashion, one of the swabs was kept in a sterile Amies transport medium (a modified Stuart's transport medium used to preserve the viability of anaerobes such as, *Neisseria gonorrhoeae* and other pathogens, obtained from Oxoid Ltd manufacturers) for culture to identify the causative organisms. The other swab was used for microscopical examination and Gram's staining (Gunning, 1992). Vaginal swabs (except a wet preparation that was examined immediately) were delivered to the Lab within 24 hours of collections.

Amine test (Amine odor with KOH - Whiff test) was performed by mixing a drop of 10% KOH with an equal volume of the vaginal discharge since the fishy amine-like odor that constitutes a positive result is transient, the mixing was done under the nose and the mixture was immediately sniffed (Al-Chowdhury, 1986). Vaginal pH was measured by dipping a short piece of the pH strip into the vaginal discharge, and then was compared to the colors on the measuring scale. (Miteb, 2000).

Wet mounted film was used for detecting *Trichomonas vaginalis*, yeast, pus cells, and epithelial cells. Vaginal swab was obtained, spread on a glass slide, air dried and Gram stained. Gram stained film was prepared to detect yeast, bacteria, pus cells and clue cells.

High vaginal swabs were cultured on the following culture media, blood agar, Chocolate agar, MacConkey agar. Sabouraud agar and Modified Thayer Martin medium .The inoculated culture media were incubated at 37°C for 24-48 hrs Chocolate agar and modified Thayer-Martin medium were incubated in candle Jar to provide an increased CO2 tension 5-10% for optimum growth of *Gonococci* and *Gardnerella vaginalis*.The isolated organisms were diagnosed by: colonial morphology, cultural characteristics, and biochemical tests using API system.

Results and Discussion

Vaginal discharge is a common health problem among women in the reproductive age. Whether asymptomatic or symptomatic, it is usually neglected by women making the diagnosis more difficult.

The incidence of pathogens in vaginal discharge varies in different regions of the world (Kumar, 1994). Although precise data are difficult to obtain, the prevalence of genital infection can lead to considerable morbidity and mortality among neonates and their mothers (Sharief, 1998).

High vaginal swabs were collected from 360 patients with symptomatic genital tract infection and abnormal vaginal discharge during the period from October, 2008 to October, 2009. The age of patients ranged between 18-50 years. Swabs were examined microbiologically for causative pathogens (*Yeast, bacteria, and Trichomonas vaginalis*). The results of this study showed that potential pathogenic agents were isolated from 310 cases.

Laboratory investigations of 310 high vaginal swabs, (Table 1) revealed that bacterial vaginitis (75.8%) was the most common cause of abnormal vaginal discharge followed by candidiasis (11.9%), *Trichomonas vaginalis* (9%) and combined infections (3%). *Gardnerella vaginalis* infection was found in 2% of patients.

Candida is normally present in 25-50% of healthy females, it is not an Sexually transmitted disease but is commonly associated with STDs. Candidal vaginal discharge was burning, itching, erythema, thick cruddy adherent discharge, odorless; (Everett, 1980).This result coincides with Jyoti. & Kusum (2004) who found that candidiasis was the second most common cause, and it was found in 7.5 percent of women. It was diagnosed by wet mount of vaginal discharge with 10% KOH solution .In contrast (Gupta *et al*.,2002) in their study of 139 women reported that bacterial vaginosis was the commonest cause of genital tract infections as it was found in 44.6% of women. It was followed by candidiasis (9.3%) and Trichomoniasis (7.9%).

The combination of two pathogens had been investigated in the present study (Table 2). The most common combined pathogen was *Staphylococcus aureus –Candida* spp which accounted for 40% of specimen followed by the combination between *Candida* spp- *Trichomonas vaginalis* and of *Candida* spp – *Gardenerella vaginalis* with 30% each.

In fifty specimens of total examined specimen there were no pathogenic organisms could be isolated in spite of the sign of infection in the speculum examination. These cases might have been caused by *Chlamydia*, *Mycoplasma*, or viral agents, for which methods of isolation were not available in the present study.

Gram negative bacteria were isolated from167 individuals (59.2%), whereas Gram positive bacteria were isolated from 68 (24.1%) individuals (Table 3). *Staphylococcus aureus* appear the most common Gram positive bacteria and the predominant organisms isolated from examined specimens, it was isolated from 21(.28%), in contrast to *Streptococcus* spp which was isolated from only 8 individuals (2.8%).

The results of the present study reveal higher prevalence of Gram negative bacteria which including *Escherichia coli* which were recorded in 39 cases (13.8%), *Klebsiella* spp in 38 cases (13.4%), and *Enterobacter* spp in 33 cases (11.7%) in addition to *Pseudomonas aeruginosa* which was recorded in 27 cases (9.57%). Other Gram negative bacteria of lower prevalence they were *Proteus* spp recorded in 5% of the cases, *Garderenella* (2%) in addition to *Neisseria*, *Acinetobacter*, and *Citrobacter* with 1% each. Bacterial vaginosis "clue cell vaginitis" " (vaginal epithelial cells studded with bacteria) is now recognized as a polymicrobial superficial vaginal infection involving the loss of the normal lactobacilli and an overgrowth of anaerobes. Factors influencing the change in the microbiologic flora include hormonal changes (during menstruation: relapse of bacterial vaginosis around the first 7 days of menstruation and resolved bacterial vaginosis during midcycle), pregnancy or antibiotic administration (Deborah, 2007). However, *Gardnerella vaginalis* has been reported in from 16-42% of women with no signs of symptoms of bacterial vaginosis

In our study, *G. vaginalis* infection was found in 2 % of patients only: this in contrast to Jyoti & Kusum (2004) who found that bacterial vaginosis was the most common cause of genital tract infection. It was prevalent in 19% of women and accounted for 63.3 percent of lower genital tract infections. It was diagnosed with Gram's staining and identification of clue cell. In addition to other study which has reported that incidence of bacterial vaginosis ranged from 9 to 44 % in sexually active women complaining of vaginal discharge (Khaira *et al*.,2003).

Vagina has its own defense mechanisms to prevent infection. Vaginal pH is maintained at 4.5 by vaginal flora during the reproductive age. Any change in vaginal flora changes the surrounding pH and may lead to vaginitis (Dutta, 2001).

Characteristic of vaginal discharge of patients differed according to the pathogenic agents as shown in Table 4 and sometimes its properties give impression about the causative agents that would be isolated and identified. The pH of vaginal secretions was greater than 5 in a large proportion of the examined cases which included women with aerobic bacterial infection and *T. vaginalis* but women with *Candida* infection, the pH is slightly near the normal value but women with Candida infection, the pH was slightly below the normal value. These results are in agreement with Caillouette *et al*., (l997), who demonstrated that pH value in patients with aerobic bacterial infection is higher than that obtained from patients with either normal flora or yeast infection .In the present study, positive whiff test with KOH (Amine test) was positive in 34 cases most of them having bacterial vaginosis.

Microscopic examination of patient's vaginal discharge revealed that pus cells were present in 85. 1% cases, meanwhile, 14.89% showed absence of pus cells (Table 5) and the prevalence of pus cells in vaginal discharge was significantly related to the type of microorganism (X^2 =61.865, DF=3, P<0.05) .There was variations in number of pus cells present in examined vaginal discharge (as shown, in Table 6), it was ranged, between few pus cells H.p.f. (<5 cells) to field full with pus cells.

37 % of high vaginal swabs were presented with <5 pus cells H.p.f., 34%, with 5-10 pus cells H.p.f. (+), 13% with 10-15 pus cells H.p.f., 10% with >20 pus cell H.p.f. and 6% exhibited full field with pus cells. The result revealed significant relationship between the frequency of pus cells observed in wet preparation of the high vaginal smears and the species of microorganisms (X^2 =165, DF=52, P <0.05.).

Copious pus cells, were present in *G. vaginalis* (17%) followed by *Klebsiella* with 9%, *Proteus spa* (7.5%), *S. aureus* (7%), and *E. coli* (6%). Such findings were coincides with results of Miteb (2000). In the present study, the presence of copious pus cell with *G. vaginalis* might be related to the presence of other microorganisms like *Chlamydia*, *Mycoplasma* or viral infections, these agents require sophisticated techniques for isolation which were not available at the time of this study.

Abnormal vaginal discharge was most prevalent in the age groups of 23- 33 years. In the present work the highest prevalence of sexually transmitted infection (32%) has been found in the age group of 29 - 33 years. This is in agreement with Saxena and Yadaf, (2001) that attributed this to higher sexual activities in this age group. Absence of pus cell in about 15% of cases in spite of the signs and symptoms of infection this might be related to the variation in the immunological response .Alternatively, the cause of vaginal discharge might be chemical, physiological discharge, herpes, syphilis, (Miteb, 2000).

Conclusion

Out of 310 cases with pathogenic abnormal vaginal discharge, bacterial infection was detected in235 (75.5%). This might mean that bacterial vaginitis is the most common etiology of abnormal vaginal discharge followed by Candidial infections which was 37 cases (13.12%) of abnormal vaginal discharge. Combined infections which was isolated from 10 patients (2.6%).Pus cells were present in (85%) of cases.

Table 1: Distribution of female genital tract infection leading to vaginal discharge according to pathogen

Causative agent	Number	%
Bacterial infection	235	75.80
Candidal infection	37	11.90
Trichomonal infection	28	9.08
Total single infection	300	96.78
Combined infection	10	3.22
Total positive cases	310	100
No growth	50	
Total		360

Table 2: Number and Percentage of every pair pathogens in combined infection associated with vaginal discharge

Pathogen Combination	Number of Cases	%
Candida spp- Staphylococcus aureus	4	40
Candida spp- Trichomonas vaginalis	3	30
Candida spp-Gardnerella vaginalis	3	30
Total	10	100

Table 3: Numbers and percentages of pathogenic agents isolated from vaginal discharge

Pathogenic microorganism	No	%
Staphylococcus aureus	60	21.28
Streptococcus spp	8	2.84
Escherichia coli	39	13.83
Klebsiella spp	38	13.48
Enterobacter spp	33	11.70
Pseudomonas aeruginosa	27	9.57
Proteus spp	15	5.32
Gardnerella vaginalis	6	2.13
Acinetobacter calcoaceticus	3	1.06
Niesseria gonorrhoeae	3	1.06
Citrobacter freundii	3	1.06
Candida spp	37	13.12
Combined culture	10	3.55
Total	282	100

Table 4: Characteristics of vaginal discharge according to pathogenic agents

Pathogenic agent causes	pH		Odor		Appearance
	Value	% of cases	Amine test	No. of +ve cases	
Bacteria Aerobic bacteria Bacterial vaginosis (nonspecific infection)	 > 5 > 5 5.5	 75.80 73.87 1.93	 - - +	2296	*variable grey
Candida spp.	<5	13.13	-	**37**	White crude
Trichomonas vaginalis	>5	9.08	+	**28**	Yellow-green
Mixed (combined) pathogens	> 5.5	3.55	+/-	**10**	variable

* Variable, White, Yellow, Green, Bloody,…etc.

Table 5: Prevalence of pus cells in relation to microbial pathogen in wet mount preparations vaginal discharge smears

Pathogens	Pus Cells absent		Pus cells Present		Total
	No	%	No	%	
Staphylococcus aureus	6	10	54	90	60
Streptococcus spp	-	-	8	100	8
E. coli	4	10.25	35	89.75	39
Klebsiella spp	6	15.78	32	84.22	38
Enterobacter spp	2	6.o6	31	93.94	33
Proteus spp	2	13.33	13	86.67	15
Citrobacter freundii	-	-	3	100	3
Pseudomonas aeruginosa	4	14.81	23	85.19	27
Gardnerella vaginalis	-	-	6	100	6
Neisseria gonorrhoeae	-	-	3	100	3
Acinetobacter calcoaceticus	-	-	3	100	3
Candida spp	12	32.43	25	67.57	37
Mixed pathogens	6	60	4	40	10
Total	42	14.89	240	85.11	282

Table 6: The frequency of pus cells in relation to type of pathogen isolated from women complaining of vaginal discharge

Pathogens		< 5		*+		*++		*+++		*++++		Total
		No	%	No	%	No	%	No	%	No	%	
S. aureus		22	41	15	28	6	11	7	13	4	7	54
Streptococcus spp		2	25	2	25	2	25	2	25	-	-	8
E.coli		10	28	15	43	6	17	2	6	2	6	35
Klebsiella spp		15	47	11	35	1	3	2	6	3	9	32
Enterobacter spp		12	39	14	45	2	6.5	2	6.5	1	3	31
Proteus spp		8	62	3	23	1	7.5	-	-	1	7.5	13
C.freundii		-	-	1	33	2	67	-		-		3
P.aerugenosa		11	48	6	26	4	17	2	9	-	-	23
G.vaginalis		-	-	2	33	2	33	1	17	1	17	6
N.gonorrhoeae		-		-		-		3	100	-	-	3
A.calcoaceticus		-		-		-		3	100	-	-	3
Candida spp		10	40	10	40	5	20	-	-	-	-	25
Mixed pathogen		-	-	4	100	-	-	-	-	-	-	4
Total	No	90		81		31		24		14		240
	%	37		34		13		10		6		

X^2 =206, DF=52 P < o.o5

*+ : 5-10/H.p.f *++:10-15/H.p.f *+++:>20/H.p.f *++++:full

Table 7: The prevalence of pathogens causing vaginal discharge in different age groups

Pathogens	No	Age groups(years)												Mean Age
		18-22		23-28		29-33		34-39		40-45		46-50		
		N0	%	No	%	No	%	No	%	No	%	No	%	
Candida	37	3	8	11	30	10	27	8	22	3	8	2	5	30.1
Bacteria	235	34	14	39	17	83	35	27	12	32	14	20	9	31.2
T.vaginalis	28	6	21	12	42	5	18	3	11	1	4	1	4	27
Combined	10	0	-	6	60	2	20	2	20	0	-	0	-	30.9
Total	310	43	14	68	22	100	32	40	13	36	12	23	7	30.8

$X^2 = 24.99$, DF= 15, P<0.05

References

1. Al-Chowdhury, M. N. H. (1986). Diagnosis of *Gardnerella vaginalis* associated vaginitis: a practical guide. Saud Med.
2. Cailloutte, J.C., Sharp, C.F., Zimmerman, G.J., and Joy, (1997). Vaginal pH as a marker for bacterial pathogens and menopausal status. Am. J. Obestet.Gynecol. 176 (6): 1270-1277.
3. Deborah B. Nelson, (2007). Bacterial Vaginosis and Pregnancy: Clinical,Center for Epidemiology and Biostatistics University of Pennsylvania School of Medicine Implicationsand Current Research, http://www.med.upenn.edu/crrwh/Nelson.html
4. Dutta, D .C (2001). Text Book of Gynecology. 3rd edition, New Central Book Agency, Kolkata, 503 - 505.
5. Everett, V. J. (1980). Vaginal discharge. Med. Dig.; 6 (8): 17-24.
6. Finegold, S. M. and Baron, E. J (1986). Formula and preparation of culture media and reagents. pp.: 859-900 In Baily & Scott's diagnostic microbiology 6th ed., C. V. Mosby Co., USA.
7. Gunning J. (1992). Vaginal and vulvar infection. In: Essentials of Obstetrics and Gynecology. [N. F. and Moore, J. G., Eds.]. 2nd ed., pp.: 377-381. Saunders Company Press, USA.
8. Gupta V, Chatterjee B, Prasad D. (2002) Clinical spectrum and microbial etiology of reproductive tract infections in rural women in the hills of North India. J Obst & Gynaec India, 52: 130-34.
9. Hudson, M. M.; Tidy, J. A; McCullouch, T. A. and Rogstad, K. E. (1998). When is bacterial vaginosis not bacterial vaginosis? A case of cervical carcinoma presenting as a recurrent vaginal anaerobic infection. Genitourin. Med. 73 (4): 306-307.
10. Jamil, J. J.; Al-Najar, L.; Shaio, J. K. and Abdul-Kadir, D. (1986). Vaginal infections diagnosis and antibiotic sensitivity. Iraqi Medical Journal; 34: 47-56.
11. Jawetz, E.; Melnick, J. and Adelberg, E. (1987). Normal flora of the vagina. In: Review of medical microbiology, 17th ed., pp.: 316, Appleton & Lange, California, USA.
12. Jyoti Pawanarkar & Kusum Chopra .(2004). Prevalence of lower reproductive tract infection in infertile women. Health and Population-Prespectives and Issues: 27 (2) 75,
13. Khaira HK, Puri KJ, Madan. A. (2003) Presence of bacterial vaginosis in sexually active females. J Obst & Gynaec India, 53: 178-80.
14. Kumar, A. V. (1994). Gynecology and Obstetrics. In: infectious diseases diagnosis and management in clinical practice, 1st ed., pp. 319-339. CBS Publish India
15. Miteb, M. M.(2000). Isolation and identification of common pathogens among women complaining of vaginal discharge. MSc Thesis. Kufa University. Iraq
16. Muklis, F. A. (1997). The prevalence of *Neisseria gonorrhoea*e in women with genital tract infection, and ifertility due infection . J. Comm. Med. Iraq; 10: 29 – 36.
17. Read, M. S.; Duerden, B. I.; Porter, I. A. and Turk, D. C. (1983). The female genital tract. In:
18. A Short Text book of Medical Microbiology. 5th ed., pp. 380. Hodder and Stoughton-London.
19. Saxena U. & Yadav S. (2001). STD prevalence in sexually active women attending the STD Clinic of a tertiary level general hospital. J Obst & Gynaec India, 51: 134-37.
20. Sharief, M. (1998). Genital tract infection among women using various contraceptive methods in Basrha, Iraq. J. Eastern Mediterranean Health; 4(3):487 – 492.
21. Thakur A, Bhalla P, Agarwal D .S. (1986) Incidence of *Gardenerella vaginalis* in non-specific vaginitis. J M R, 8: 567-74.
22. Thomason, J. L; Gelbart, S. M; Monagle, L. M; James, J. A .and Brochuizen, F. F. (1990). Is pH test as accurate as the electronic measurement of the pH of vaginal secretions? Am. J. Obstet. Gynecol .162 (5): 1213-1214.