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PREVALENCE OF COMMON TYPES OF VAGINAL INFECTIONS AMONG WOMEN ATTENDING GYNAECOLOGY CLINICS IN EGYPT

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ABSTRACT

The aim of exploratory study was; to assess the common types of vaginal infection. Four hundred women had abnormal vaginal discharge attending Gynecology Clinics at Elmainal University Hospital were recruited. Data were collected through an interviewing questionnaire schedule, physical and vaginal examination, laboratory investigation, and follow up sheet. The study results indicated that the mean age of the sample was 31.44 ± 6.14 years old, 42.5% of the sample were not educated, 54.3%were lives in urban, 95% were married, 82% were house wives, Ninety percent of them had regular menses, the mean interval of menstruation were 27.67 ± 4.16 days, the mean duration of menstruation were 4.9 ± 1.97days, 76.9% of the sample were multigravida. While 72.2% of them multiparous. Thirty nine point four of the sample had history of chronic illness. 36.7% only of the sample used cotton underwear.39.6% of the sample used vaginal douche. Vaginal swabs were examined including wet preparation, Whiff test and gram stain using a scoring system. Also 5-Bromo 4-Chloro-Indolyl-α-D-N-Acetylneuraminic Acid in a spot test was applied to a sample of the study. Gram stain done for women complaining of vaginal discharge revealed that 40.9% were found suffering from Vulvovaginal candidiasis, 10.2% from bacterial Vaginosis, 6% from Trichomonas vaginalis, 17.1% having an intermediate stage between the normal vaginal flora and Bacterial vaginosis as graded by the gram stain scoring system and 2.1% women were suffering from co-infection. The BCIN Spot Test confirmed the diagnosis of 60% of cases diagnosed by gram stain scoring system and further evaluation of the test is recommended in a larger scale study which was not easy to fulfill as the test was too costly. More over 94.9% of the Vaginosis cases were positive by Amesl`s Criteria..

Keywords: Vaginal Infection, Prevalence, Gram Stain, Spot Test

INTRODUCTION

The most prevalent causes of vaginitis are bacterial vaginosis, vulvovaginal candidiasis and trichomonal vaginitis (Seltzer and Pearse, 2002). Bacterial vaginosis (BV) is the commonest cause of infectious vaginitis in women of reproductive age (Nygren *et al*., 2008). Microscopic examination remains the initial diagnostic test in the processing of vaginal specimens in the clinical microbiology laboratory (Murray 1999; Church, Melnyk & Ungar, 2000). However, the preparation and analysis of a Gram stain is a highly complex procedure that requires extensive experience for a correct result to be reported (Health Care Financing Administration, 1993).

It is postulated that the BV-related flora ascends to the uterus and causes the release of inflammatory mediators that initiate labour (Howe et al 1999 and Wiggins et al., 2000). One potential causal mechanism is the sialidase activity produced by BV-related microorganisms (Cauci *et al*.,1998 and Wiggins *et al*., 2000).

Given the association between sialidases, BV and the difficulty in diagnosing BV in a clinical setting, a colorimetric test for sialidase using 5-bromo-4- chloro-3-indolyl- α -D-N-acetylneuraminic acid (BCIN) was used as a substrate (Wiggins *et al*., 2000).The objective of the study was to asses common types of vaginal infection among women during childbearing period as well as evaluation of the use of 5-Bromo 4-Chloro-Indolyl-α-D-N- Acetylneuraminic Acid in a spot test to identify sialidase activity in vaginal swabs from women with bacterial vaginosis in comparison with interpretation of Gram-stained vaginal smears as a golden standard using a scoring system which allows gradations in severity and which uses the morphotypes that are most reliably identified for diagnosing bacterial vaginosis.

Ethical Consideration

An official permission was obtained from hospital administrators. Also, all participants and health care providers were informed about the purpose of the study. Benefits were discussed; women were informed that the study posed no risks or hazards on their health. The investigator emphasized that the participation in the study was voluntary and that participants have the right to withdraw at any time without given any reason and without affecting their care or health. Measures were taken to insure confidentiality through coding the data, and participants were informed that data collected will be used only for the purpose of the study, and oral consent was obtained.

METHODOLOGY

Four hundred women attending the outpatient gynaecology Clinics in Kasr El Aini Cairo University in Egypt, suffering from abnormal vaginal discharge during the child bearing period and for whom a vaginal speculum examination was necessary, were studied. Data related to socio-demographic characteristics as well as medical history of chronic disease such as diabetes, anaemia, renal disease and allergy were studied. Also

past and current obstetrical history including gravidity, parity, abortion as well as detailed history of last pregnancy were correlated. Moreover, assessment of current condition, which covers all the symptoms signs of vaginal infection and risk factors including disease, medication, contraception, personal hygiene, as well as sexual factors were done. Two high vaginal swabs were taken simultaneously after speculum insertion from the vaginal fornices. The first swabs were subjected to the following ; pH estimation, wet preparation for detection of true clue cells, hyphae , yeast cells and trichomonas vaginalis and Whiff test for detection of an amine (fishy) odour by adding a drop of the discharge to a drop of 10% potassium hydroxide (KOH) solution. The second swab was rolled along a glass slide and allowed to air dry for the gram stain that was examined and interpreted according to table 1 (Nugent, Krohn & Hillier, 1991). Data for the normal and intermediate grades were pooled for the purposes of the statistical analysis.

Amsel Criteria which includes vaginal pH>4.5, presence of clue cells, positive Whiff test and characteristic vaginal discharge was used to assist presence of vaginosis (Amsel *et al*., 1983).

Table 1: Diagnosis of BV using a Gram stained smear of vaginal secretions

Score	Lactobacillus mophotypes	Gardnerella and Bacteroides spp	Curved gram variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	2+ or 3+
3	1+	3+	
4	0	4+	

Total score = Lactobacillus morphotypes+ Gardnerella / Bacteroides morphotytpes +Mobiluncus morphotypes. A score of 0 to 3 is considered normal, 4-6 isintermediate and 7 to 10 indicates BV.

A third swab was collected from 33 cases only to be used for the sialidase spot test (Wiggins et al., 2000); that was placed immediately into a buffer composed of 2 ml of 25 mM Tris–HCl–Tween 20 (pH 7.0). The swabs in buffer were refrigerated at 4°C until the end of the clinical study.

The substrate 5-Bromo 4-Chloro-Indolyl-α-D-N-Acetylneuraminic Acid (BCIN) (Rose Scientific, Edmonton, Alberta, Canada) was chosen as it gave a

strong colorimetric response on filter paper to sialidase.

The specificity of the substrate was validated by BV-associated organisms and did not show any nonspecific effect of pH variation (Wiggins *et al*., 2000).

The substrate, 5-Bromo 4-Chloro-Indolyl-α-D-N-Acetylneuraminic Acid (BCIN), was prepared at 0.63 mM in 150 mM sodium acetate–25 mM CaCl–1 mM NaCl (pH 5.5) and was used to dampen a 10-cm diameter piece of Whatman no. 1 filter paper. The swabs were removed from the buffer and were applied to the substrate inoculated filter paper. The filter paper was placed in a covered petri dish, and the petri dish was incubated for 30 min at 37°C. A blue spot became

visible in between 2 and 30 min, indicating that sialidase activity was present. In the absence of sialidase activity the paper remained colorless even after a period of 2 h.

RESULTS AND DISCUSSION

This study included four hundred women who were attending the out patient gynaecology Clinics in Kasr El Aini Cairo University in Egypt. Nineteen patients had dry swabs that were found unsuitable for microscopic examination when stained with gram stain, thus were excluded from the study.

The age of the patients range was 18-43 years with a mean of 31.44 ±1.4 years. As regards the residence 207 (54.3 %) were from urban areas while 174 (45.7%) were from rural areas. As the hospital, site of the study, is an Educational University hospital presenting free or low charge services, 78% of the sample had an income less than 500 LE with a mean 453.35±138.

Examination of vaginal discharge

Ph examination, Whiff test, wet film examination and gram stain done for 381 women attending the out patient gynaecology clinic complaining of vaginal discharge revealed that ;156 women (40.9%) were found suffering from vulvovaginal candidiasis, 39 (10.2%) from bacterial vaginosis, 23 (6%) from Trichomonas vaginalis (table 2), 65 (17.1%) having an intermediate stage between the normal vaginal flora and Bacterial vaginosis as graded by the gram stain scoring system (Nugent et al, 1991) and 8 women (2.1%) were suffering from co- infection; (4 cases (1.049%) had concomitant vaginosis with candidiasis , while 3 cases(0.8%) had candidiasis with trichomoniasis and one case (0.3%) had bacterial vaginosis and trichomoniasis)

Table 2: Incidence of different causes of vaginal discharge

Causes	Candidiasis	Bacterial Vaginosis	Trichomonas
Number	156/381	39/381	23/381
Percentage	40.9%	10.2%	6%

Correlation between different vaginal infection and Demographic factors

As regards the age, the highest incidence of candidiasis was in an age range of 20-23 years and 30-40 years (47.4% in both groups). While 60.9% of cases with trichomonas infection were in the group age of 20-30 years (Table 3).

More over according to the education level, 52.2% of cases with trichomonas infection were illiterate patients (Table 3).

Table 3: Age, educational level and economic level in women with different vaginal infections.

	Candidiasis (n=156)		Bacterial Vaginosis (n=39)		Trichomonas (n=23)	
	Number	%	Number	%	Number	%
Age (years)						
<20 years	2	1.3	0	0	0	0
20-30 years	74	47.4	16	41	14	60.9
31-40years	74	47.4	20	51.3	6	26.1
>40years	6	3.8	3	7.7	3	13
Education						
Illiterate	58	37.2	13	33.3	12	52.2
literate	21	13.5	7	17.9	1	4.3
Primary	15	9.6	3	7.7	4	17.4
Preparatory	19	12.2	3	7.7	3	13
Diploma	35	22.4	12	30.8	3	13
Above middle education	4	2.6	1	2.6	0	0
High education	4	2.6	0	0	0	0
Federal poverty level Monthly Income(LE)	456.92±146.6	74.4	467.94±123.8	74.4	(454.3±134.76)	73.9

Gynaecological and Obstetric correlation
The age at menarche had a range of 11-17 years with a mean 13.47±1.41 years. Regarding menstrual regularity, 90% of the sample had a regular menstruation, and the inter-menestrual period ranged between 15 and 45 days with a mean of 27.67±4.16 days, the duration of menstruation range was 1-5 days with a mean of 4.94±1.97 days. However, 37.5 % of the sample suffered from menorrhagia. The age of marriage ranged from 12-35 years with a mean of 19.92±3.3 years, on the other hand duration of marriage ranged from 3 months to 27 years with a mean of 11.28±6.54 years. Moreover as regards the obstetric history, 12.3% of the patients were never pregnant, while 87.7% were pregnant before with a mean number of pregnancies of 3.27±2.36. Also 15% were nulli Para, while 85% of them had live births with a mean of 2.46±1.77, and 29.9% of them mentioned that they had abortions.

Table 4: Gynecological history in women with different vaginal infections

	Candidiasis (*n*=156)		Bacterial Vaginosis (*n*=39)		Trichomonas (*n*=23)	
	Mean	/Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Interval between menses	28.11	±4.07	27.32	±4.62	28.33	±4.81
Duration of menses	4.89	±1.98	5	±1.94	5.52	±1.75
Rhythm	Number	%	Number	%	number	%
regular	140	89.7	38	97.4	21	91.7
irregular	16	10.3	1	2.6	2	8.7
Amount						
Scanty	12	7.7	1	2.6	0	0
Mild	15	9.6	5	12.8	3	13
Moderate	75	48.1	16	41	10	43.5
Menorrahgia	54	34.6	17	43.6	10	43.5
Interval (Mean±SD)	28.11 ± 4.07		27.32 ± 4.62		28.33 ± 4.81	
Duration (Mean±SD)	4.89 ±1.98		5.03±1.94		5.52 ± 1.57	
Age of marriage	12-35years	19.89±3.7	14-35	20.49±4.43	13-27	19.52±3.97
Duration of marriage	1y-25y	11.25±6.48	1y-23y	11.82±5.49	2-25	11.87±7.55

According to the rhythm of menses, 89.7% of cases with candidiasis and 91.7% of cases with trichomonas, had a regular rhythm (Table 4).

Table 5: Distribution of the sample according to their gravidity among different vaginal infections.

	Candidiasis (n=156)		Bacterial Vaginosis (n=39)		Trichomonas (n=23)	
	Number	%	Number	%	Number	%
Nulligravida	22	14.1	4	10.3	1	4.3
Primigravida	16	10.3	35	89.7	22	95.7
Multigravida	118	75.6	0	0	0	0

Table 6: Distribution of the sample according to their parity among different vaginal infections.

	Candidiasis (n=156)		Bacterial Vaginosis (n=39)		Trichomonas (n=23)	
	Number	%	Number	%	Number	%
Nulligravida	22	14.1	4	10.3	1	4.3
Primigravida	16	10.3	35	89.7	22	95.7
Multigravida	118	75.6	0	0	0	0

As regards gravidity, 95.7% of cases suffering from trichomonas vaginalis were primigravida (Table 5), while as regards parity, 95.7% were primipara (Table 6).

Medical Condition in association with vaginal infections

Table 7:Medical diseases associated with different vaginal infections.

	Candidiasis (n=156)		Bacterial Vaginosis (n=39)		Trichomonas (n=23)	
	Number	%	Number	%	Number	%
Anaemia	55	35.3	13	33.3	4	17.4
Rheumatoid	17	10.9	1	2.6	2	8.7
Urinary tract infection	14	9	0	0	3	13
Diabetes	6	3.8	3	7.7	4	17.4
Uterine Prolapse	5	3.3	1	2.6	0	0

Moreover, as regard association with medical condition 35.3% of candidiasis and 33.3% of vaginosis cases were associated with anemia (Table 7).

History of medication intake with different vaginal infections.

Also candidiasis, Bacterial vaginosis and trichomonas cases were associated with high incidence of antibiotic intake and contraceptive intra- uterine device usage (Table 8, 9).

Table 8: Incidence of intake of different medications with different vaginal infections.

	Candidiasis (n=156)		Bacterial Vaginosis (n=39)		Trichomonas (n=23)	
	Number	%	Number	%	Number	%
Antibiotic Intake	103	66	21	53.8	17	73.9
Antirheumatic	16	10.3	1	2.6	2	8.7
Corticosteroid	5	3.2	2	5.1	2	8.7
antihistamic	5	3.2	0	0	0	0

Table 9: Incidence of usage of family planning methods with different vaginal infections.

	Candidiasis (n=156)		Bacterial Vaginosis (n=39)		Trichomonas (n=23)	
	Number	%	Number	%	Number	%
Contraceptive IUD	66	42.3	20	51.3	11	47.8
Contraceptive Pills	21	13.5	4	10.3	1	4.3
Contraceptive Injections	9	5.8	1	2.6	1	4.3
Contraceptive Implants	1	0.6	0	0	0	0
Tubal Legation	0	0	1	2.6	0	0
Male Condom	3	1.9	0	0	0	0

Different tests diagnosing Bacterial vaginosis

Gram stain examination showed the highest incidence of detection in comparison to Whiff test and pH examination (Table 10)

Table 10: Different tests diagnosing Bact--erial vagi -nosis (n=39)

	Bacterial Vaginosis (n=39)		
		Number	%
pH≥4.5	Positive	39	100
	Negative	0	0
Whiff test	Positive	33	84.6%
	Negative	6	15.4%
Gram Examination	Positive	39	100
	Negative	0	0

Table 11: The results of Gram staining and estimation of sialidase by the BCIN spot test in 33 cases.

		Gram stain Examination (n=33)		
		Score0-3 Normal	Score4-6 intermediate	Score7-10 BV
BCIN Spot	Positive (14)	1	4	9
	Negative (19)	5	8	6

The BCIN Spot Test confirmed the diagnosis of 9 out of 15 cases (60%) diagnosed by gram stain scoring system and further evaluation of the test is recommended in a larger scale study which was not easy to fulfil as the test was too costly (Table 11).

More over 94.9% of the vaginosis cases (39) were positive by Amesl`s Criteria which was defined as follows: the women suffering from BV had at least 3 of the 4 Amsel criteria (Amsel *et al*., 1983).

Also, Whiff test was positive in 34 of 39cases (84.6%) confirming BV.

In our work (40.9%) of women studied were found suffering from vulvovaginal candidiasis. It was previously mentioned that candidiasis also called yeast infection, thrush, and moniliasis is the most common vaginal infection after bacterial vaginosis (Seltzer and Pearse, 2000).

Bacterial vaginosis was revealed in 10.2% of cases in our study. It was mentioned in different studies that BV is a common vaginal infection with a prevalence of 9-37 % (Goldenberg *et al*,1996 and Hiller *et al*., 1988). Others also revealed a prevalence of 38.7% of BV in a population (Gutman et al, 2005).

Our study revealed that (6%) were suffering from Trichomonas vaginalis. Trichomoiasis is the third most common cause of vaginal infection and also one of sexually transmitted diseases that is caused by the flagellated parasite (Berek, 2002). Prolonged, untreated, or inadequately treated Trichomoniasis can result in an increased risk of cancer (Greenberg, Bruess & Haffner, 2002).

Moreover, in our study, (17.1%) had an intermediate stage between the normal vaginal flora and Bacterial

vaginosis as graded by the gram stain scoring system (Nugent, Krohn & Hillier, 1991). The intermediate category and its relationship to both BV and "normal" vaginal flora needs further elucidation. This category does not fulfil the definition of BV, and it is unclear whether the intermediate grade is an indication that vaginal colonization with BV-related microorganisms undergoes a stepwise progression from normal flora through intermediate flora to BV-related flora (Hay, Taylor-Robinson & Lamont, 1992 and Wiggens et al., 2000). It was also found by others that either the flora of 62% of women with intermediate flora "reverted" to normal flora or that the women developed BV, indicating the instability of this type of colonization (Hillier et al., 1992). In our study gram stain using the scoring system as described by Nugent et al., 1991 graded the flora as normal, intermediate, and BV.

Gram stain of vaginal discharge is considered a reliable means of diagnosing bacterial vaginosis and offers the added ability to qualify and classify bacterial load. In that respect, Gram stain has been the primary means used to diagnose bacterial vaginosis (Nygren et al., 2008). Thus, gram stain is believed by many to be the gold standard for diagnosing bacterial vaginosis. However, interpreting the Gram stain requires experience, and it is often difficult to get timely results for the clinical diagnosis of bacterial vaginosis (Gutman et al., 2005).

The BCIN Spot Test which reveals presence of sialidase in the vaginal secretions, confirmed the diagnosis of 9 out of 15 cases (60%) of vaginosis diagnosed by gram stain scoring system and further evaluation of the test is recommended in a larger scale study which was not easy to fulfill as the test was too costly.It was also mentioned by others that elevated levels of sialidase activity were detected in 84% of vaginal fluid specimens from women with bacterial vaginosis and none in vaginal fluids without bacterial vaginosis (p<0.001) (Briselden et al., 1992) .

Bacterial vaginosis may be more common among women with low socioeconomic status (French, McGregor & Parker, 2006; Tolosa et al., 2006). It was revealed by others that the enzyme sialidase, causes adverse obstetric and gynaecological outcomes when it is produced by BV associated flora. Sialidase activity may act to remove sialic acids from cervical mucins and diminish the viscosity inherent in the mucus rendering the mechanical and bacteriostatic properties of mucin less effective as barrier mechanisms. Second, exposure of cryptic structures in oligosaccharides can promote bacterial adhesion to the secreted mucus and the underlying epithelial glycocalyx. This may create conditions for bacterial invasion of the upper reproductive tract. Identification of sialidase activity

by the spot test in patients with BV may therefore be beneficial for two reasons. First, it can be undertaken in the same room as the patient, as it does not require the use of light microscopy for Gram staining. Second, as sialidase activity is not always present in patients with BV, its presence may be an indicator that the condition is more detrimental to the host in these cases. This possibility requires further investigation.

In Egypt, Suallam et al., (2001), found that the overall prevalence of reproductive tract infections was 52% and 52.8% respectively of gynecological morbidities in rural Egyptian community. Worldwide, klufio, Amoa, Delamare & Kariiga (1995); Al Quaiz (2000); Huong, Kurzhals & Rasch (2004); Geva et al., (2006), reported that the prevalence of vaginal infection was 57%, 56.6%, 54.4%, and 48%, respectively. As well, Braban et al., (2009) mentioned that, the prevalence of vaginal infection among women in Nigeria was 43.8%. High incidence of vaginal infection among women during reproductive age on the different countries, also the mean age of the current study was 31.44 ± 6.14 years.

In a similar study, Aggarwal et al., (1999), who found in their study of 2324 married rural Indian women during childbearing period, 32% of them have Vaginitis. As well, Braham, Baghchesereie, & Torabi (2009) found in their study of 500 nonpregnant women from Iran that 27.6% of them had vaginal infection. These differences in results might be related to that the sample was recruited in the current study with symptomatic conditions.

Concerning the prevalence of Vulvo-vaginal Candidiasis (VVC), the result of the current study revealed that about two fifth of them had VVC. Ahmed, Mohamed & Shamy (2000) and Omar (2001), detected that the prevalence of VVC in Egypt was 44%, and 39%, respectively. At the same time, Sweet & Gibbs (1995); Huong et al., (2004); Paulo et al., (2007); Braban et al., (2009); reported that the prevalence of VVC, worldwide was 39%, 43%, 44.1% 45.4%, and 39.9% respectively.

In Egypt, Abdel-Hakiim, Ahmed & Abdel- Azeem (2002), who reported that the prevalence of BV was 36% among women attending the gynecology clinic at Bab El-Sharia University Hospital, Cairo. As well Ahmed, Mohamed & Shamy (2000) found in their study of 100 women from Assiut University Hospital, that the prevalence of BV was 26%. The high incidence in the previous two studies, could be not diagnosed by Gram staining score "Nugent's criteria", so, the prevalence of BV was higher than result of the current study because it included intermediate and BV together.

The overall prevalence of TV among African American women was 13.3%, among white women 1.3%, and among Mexican American women 1.8% (CDC, 2007). Differences in results of the previous researches might be related to different sample size, different cultures, and different health awareness, also the low incidence in the current study might be due to most of the sample were housewife and had single partner "behavior abstinence".

CONCLUSION

The study concluded that less than three fifths of the women (58.8%) had vaginal infection, Vulvovaginal Candidiasis was affecting 41%, Bacterial vaginosis was affecting 10.2%, 6% had Trichomonas Vaginalis infection, and six women (1.6%) had mixed infection. Considering the health educational program, women who received health teaching and follow up reported relieved symptoms of vaginitis and the cure rate was generally high with a highly statistically significant difference (negative vaginal swab for vaginal infection).

RECOMMENDATION

Based on the results of this study, the following may be recommended:

All nurses in gynaecology clinics should be trained to have adequate health information to conduct health education classes to women having vaginal infection to continue course of treatment, prevent recurrence and complications of vaginal infection. Importance of vaginal swab examination for women having abnormal vaginal discharge before prescribing medication; because accurate diagnosis improves treatment and reduces incidence of recurrent vaginitis. Teaching women during childbearing period about predisposing factors of vaginal infection and importance of medical consultation for symptoms of vaginal infection. Developing a case registration system to detect for the extent of vaginal infection among Egyptian women. Providing greater access to condom at affordable prices and helping people use it correctly.

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