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Prevalence of the Genital Tract Bacterial Infections after Vaginal Reconstructive Surgery

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Abstract: Due to frequent childbirth, heavy lifting and the structure of the lives of rural women in Shahrekord region, Iran, cystocele and rectocele are of the main medical problems of the women in this area and for its correction, vaginal reconstructive surgery is needed which causes infection. The purpose of this study was to identify the bacteria causing infection after vaginal reconstructive surgery and performing antibiogram to help these patients for faster recovery. Patients enrolled this study were 92 who had undergone previous vaginal reconstructive surgery and now had infection. After examination, the group of patients taking antibiotics (n = 26) were excluded and the remaining 66 completed the study questionnaire. A gynecologist performed sampling; related tests (aerobic and anaerobic culture using an anaerobic culture gas pack jar and type A which provides absolute anaerobic conditions) were performed; antimicrobial susceptibility testing using Disk Diffusion Method was carried out; and the results were recorded. All the positive samples were polymicrobial. *Gardnerella vaginalis* in 20 cases (31%), *peptostreptococci* and anaerobic cocci in 9 cases (13.6%), *staphylococcus aureus* in 8 cases (9.1%), bacteroides and fusobacterium in 7 cases (10.6%), streptococcus group B in 4 cases (6%), yeast cells in 11 cases (16.6%) and *Trichomonas vaginalis* in wet mount of 4 (6%) existed. Anaerobic bacteria showed 85% sensitivity to clindamycin, 82% to chloramphenicol, 85% sensitivity to ceftizoxime and 45% to penicillin. Facultative anaerobic bacteria showed a sensitivity rate of 90% to ceftizoxime, chloramphenicol and cephalothin. According to our findings, the rate of vaginal bacterial infection in women with vaginal reconstructive surgery has increased; from which, infections with anaerobic bacteria origins have increased dramatically. We recommend antibiotic prophylaxis prior to genital reconstructive surgeries.

Key words: Bacterial vaginosis, reconstructive surgery, *Gardnerella vaginalis*, bactericide

INTRODUCTION

Infectious diseases have existed among human societies for centuries and are a serious threat to human health. Their prevalence differs in human societies based on social, demographical and geographical factors (DuGas and Knor, 2011). Female genitalia infections particularly vaginitis is one of the common problems and the most important reason for referring to health centers (Ryan, 1999; Hay, 2009).

Approximately, 95% of vaginitis are caused by infections of one of the three organisms of *Candida albicans*, *Trichomonas vaginalis* and *Gardnerella vaginalis* (Andrist, 2001). Other factors include *lactobacillus*, *staphylococcus epidermidis*, diphtheroids and aerobic and anaerobic *streptococci* (DuGas and Knor, 2011).

Patients with bacterial infections may have a variety of symptoms or may be asymptomatic (Franklin and Monif, 2000). About half of the patients complain of vaginal odor (Cunningham *et al.*, 2001). Vaginal infections are caused by reduced or absent *lactobacilli*, increased anaerobic gram-negative bacteria, *Gardnerella vaginalis*, mobiluncus and other species, anaerobic gram-positive bacteria and *Mycoplasma hominis* (Hillier *et al.*, 2007; Fredricks *et al.*, 2005).

Although, the recovery rate of bacterial infections with metronidazole or clindamycin after a week is about 94%, but the effect of these drugs after a period of 12 months is about 50% (Bradshaw *et al.*, 2006). Hydrogen peroxide (H₂O₂) is produced by lactobacilli in the vagina, decreases bacterial vaginosis and significantly speeds healing (Cherpes *et al.*, 2008; Hawes *et al.*, 1996; Austin *et al.*, 2005). H₂O₂ reduce the risk of bacterial

infections and women without it have bacterial vaginosis after treatment. Exogenous introduction of probiotic of lactobacilli can help in restoring the normal vaginal microflora (Reid *et al.*, 2005; Gardiner *et al.*, 2002; Antonio and Hillier, 2003).

Bacterial vaginosis complications include preterm birth, pelvic inflammatory disease, endometritis after surgery and premature rupture of membranes with chorioamnionitis. After-cesarean endometritis is 5 times more in women with bacterial vaginosis; in addition, the wound infection after cesarean can be caused more commonly (Ness *et al.*, 2005; Larsson *et al.*, 2005; Falagas *et al.*, 2007; Price and Jackson, 2009). Studies also showed that in these women, after hysterectomy, the infection increases (Nieboer *et al.*, 2009; Makinen *et al.*, 2001). To reduce bacteria, antibiotics are used to reduce postoperative complications (Ledger, 2006; Lofgren *et al.*, 2004; Kjolhede *et al.*, 2009).

Due to frequent childbirth, heavy lifting and the structure of the lives of rural women in Shehrekord region, Iran, cystocele and rectocele are common, causing sexual problems, urinary problems and feeling heaviness in the vagina. In high-degrees, cystocele and rectocele would need to undergo reconstructive surgery and during the postoperative care, vaginal infections are more prevalent. Due to not recognizing the bacterial source, the treatment is done according to clinical findings that tend to less response to the prescribed medications and infection recurrence.

The present study aimed to identify the bacteria causing vaginal infections in women undergone vaginal reconstructive surgery by performing antibiogram to take an important step in helping these patients for a faster recovery, cost and unnecessary use of antibiotics significantly.

MATERIALS AND METHODS

This descriptive-sectional study was performed on 92 women who had vaginal reconstructive surgery and then, genital area infection in Hajar hospital, Shahrekord, Iran. After re-examination, the group of patients received antibiotic (n = 26) were excluded and the remaining 6 patients entered the study. Data collecting tool was a questionnaire and information recording sheet (Include: age, number of childbirth,..). The subjects grouped in six age groups (from ages 25 to 50 years and over).

Sampling took place by a gynecologist using a disposable sterile speculum. The vaginal mucosa regarding the type of secretions and the quantity was determined and vaginal pH was measured by paper pH meter (nitrazine paper manufactured by Merck, Germany)

and recorded. Samples were obtained by two sterile swabs; one of the samples placed in a sterilized physiological serum and the other in a sterile thioglycollate broth. The samples transferred to the laboratory of faculty of Medicine as soon as possible.

Whiff test (amine test) was conducted on samples containing physiologic serum; after smear centrifugation, the wet prep and smear were directly prepared for gram staining and the results were recorded. Samples in the thioglycollate broth were incubated to culture on blood agar, Brain Heart Infusion Agar (BHIA), Ison medium and Eosin Methylene Blue (EMB) agar in two sets, one for aerobic and the other for anaerobic culture. To ensure optimum anaerobic conditions, indicators with anaerobic mediums containing methylene blue, glucose and caustic soda can be used. Methyl blue is blue in the oxidized form and after being revived, it is colorless. In anaerobic conditions, the reagent color remains colorless and in case of oxygen leak, it will gradually gain its primary color and it shows that the system is not completely anaerobic. In this study, due to the proportional growth of anaerobic bacteria, this indicator was not needed.

After 24 to 48 h, the mediums were evaluated in terms of bacterial growth and microbiological tests such as Whiff test and the medium suggested by Ison *et al* were done to identify the *Gardnerella vaginalis*. *Gardnerella vaginalis* colonies were observed in Ison medium with 1-2 mm size, creamy color, round and convex shape and thin halo of beta hemolysis. In gram staining of the colonies, gram-positive coccobacilli were observed. With catalase, oxidase tests and hippurate hydrolysis, *Gardnerella vaginalis* was confirmed.

For other bacteria, colony morphology (size and shape), made pigments, growth rate, hemolysis and available bacteriological tests including gram staining, catalase, coagulase, indole, urease, optochin disk, bacitracin and IMViC (Indole, Methyl red, Voges-Proskauer in citrate) tests were used.

RESULTS

In this study, 66 women who had previous vaginal reconstructive surgery were studied in the age groups of 25 to over 50-years-old. Most of the cases with vaginal infection after surgery were in the age groups of 40-45 years (22%) and the least were in age group over 50 years (8%) (Table 1). Table 2 shows the clinical findings of the patients at the time of examination.

After the microbiological test, it was indicated that all of the positive samples were polymicrobial. In the present study, patients who had undergone vaginal reconstructive surgery and currently had genital infection were studied. From 97 (after excluding those who had

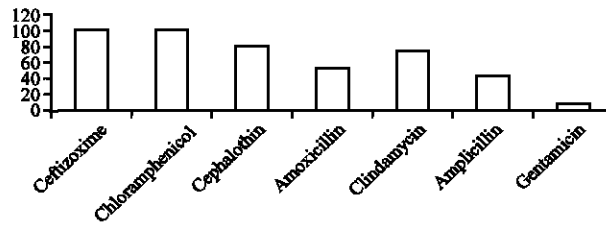


Fig. 1: Resistance percentage of *Gardnerella vaginalis* microorganisms isolated during proestrus against different tested antibiotics

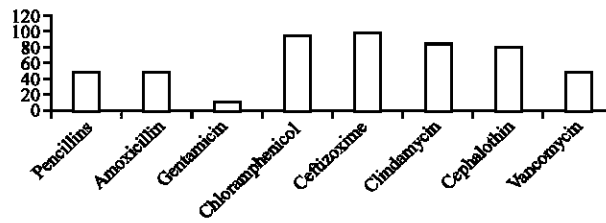


Fig. 2: Spectral sensitivity of anaerobic bacteria (*Fusobacterium* and *Bacteroides*) against antibiotics

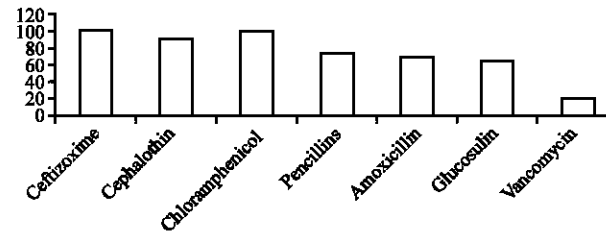


Fig. 3: Spectral sensitivity of coagulase positive staphylococci against antibiotics

Table 1: Frequency distribution of age

Patient's age	No. of patients	Age groups percentage
25-30	19	20
30-35	16	16
35-40	18	19
40-45	21	22
45-50	15	15
50 <	8	8

Table 2: Clinical findings of the patients at the time of their examination

Type of secretion	No. of patients
Cheesy secretions	9
Malodorous discharge	18
Fishy odor discharge	23
No discharge	11
Others	5

Table 3: Distribution of frequency bacteria and other organisms isolated from the patients

Isolated bacterial species	No. of patients	Percentage
<i>Gardnerella vaginalis</i>	20	31.0
<i>Bacteroides</i> and <i>Fusobacterium</i>	7	10.6
Coagulase-positive staphylococci	8	12.9
Peptostreptococci and anaerobic cocci	9	13.6
Group B streptococcus	4	6.0
Yeast cells	11	16.6
<i>Trichomonas vaginalis</i>	6	4.0

taken antibiotics prior to sampling), 66 patients had positive cultures. Regarding the frequency of bacteria isolated from patients, *Gardnerella vaginalis* (31%), *peptostreptococcus* and anaerobic cocci (13.6%), *Staphylococcus aureus* (9.12%), *Bacteroides* and *Fusobacterium* (10.6%), group B streptococcus (6%) and yeast cells (16.6%) were reported. In wet mount, 4 cases (6%) were found with *Trichomonas vaginalis* (Table 3).

In the antibiotics sensitivity test, anaerobic bacteria had 85% sensitivity to clindamycin, 82% to chloramphenicol, 85% to ceftizoxime and 45% to penicillin. Facultative anaerobic bacteria had a sensitivity rate of more than 90% to ceftizoxime, chloramphenicol and cephalothin (Fig. 1-3).

DISCUSSION

In the present study, patients who had undergone vaginal reconstructive surgery and currently had genital infection were studied. From 97 (after excluding those who had taken antibiotics prior to sampling), 66 patients had

positive cultures. Regarding the frequency of bacteria isolated from patients, *Gardnerella vaginalis* (31%), *peptostreptococcus* and anaerobic cocci (13.6%), *Staphylococcus aureus* (9.12%), Bacteroides and *Fusobacterium* (10.6%), group B streptococcus (6%) and yeast cells (16.6%) were reported. In wet mount, 4 cases (6%) were found with *Trichomonas vaginalis*.

In normal state, in the female genital tract of women in reproductive age, bacteria including *Gardnerella vaginalis* and mobiluncus and *Mycoplasma hominis* exist. The increasing population and overgrowth of these bacteria are called bacterial vaginosis. These bacteria do not cause permanent and lasting damages (Brown *et al.*, 1989). In a study in Kerman, Iran, bacterial vaginosis was reported in 37.7% which was related to those with lower social class and low-education levels (Ashraf-Ganjoei, 2005). These statistics are similar with the percentage of *Gardnerella vaginalis* isolated bacteria in the present study. However, about other bacteria, since the anaerobic bacteria was not cultured relevant information was not available (Borjian *et al.*, 2002).

Borjian *et al.* (2002) conducted a study in Buroujen, Iran and reported bacterial vaginosis in 18.9%. This amount was inconsistent with the amount of bacterial vaginosis reported in the present study (31%). The reason for this difference might be that the patients in the present study underwent surgery and it is natural that surgery causes increase in the infections including colonized bacterial infection in tissues and other miscellaneous bacteria such as anaerobics. However, some studies have indicated that after genital surgery, infections of anaerobic bacteria increase compared to aerobic bacterial infections (Guaschino *et al.*, 2002).

In the present study, the amount of anaerobic bacterial infections had significantly increased. In a study conducted on the women's ward of a hospital in United States, the international system of health also recommended that clindamycin, cephalosporins and penicillins might be used as prophylaxis before surgery. However, it is not yet clear exactly what drugs should be used widely as prophylaxis or not. In Italy, this type of prophylaxis has had little effect on bacterial vaginosis; however, a routine prophylaxis regime with topical clindamycin for 7 days prior to genital surgery is recommended (Borjian *et al.*, 2002).

Various nationalities for bacterial vaginosis based on direct smear and gram staining in pregnant women prior to membrane rupture and reported 21% bacterial vaginosis (Core La *et al.*, 2000). In Ghana on the presence of potential pathogens in the female genital tract showed that nearly two-thirds (46.2%) of women had potentially pathogenic bacteria in their lower genital tract. Besides, simultaneous infection of bacterial and *Candida albicans*

was reported 17.2% (Lassey *et al.*, 2004). The effects of various antibiotics on the anaerobic bacteria and found that *Fusobacterium*, *Porphyromonas* and *Prevotella* (previously known as *Bacteroides melaninogenicus*) were significantly resistant to tetracycline, erythromycin and penicillin (Arzese *et al.*, 2000). In the present study, fusobacterium species were 45% sensitive to tetracycline and penicillin antibiotics (in vitro). Bacteroides were 45% sensitive to penicillin and 70% were sensitive towards tetracycline. According to the results, it appears that the anaerobic bacteria resistant compared to the mentioned antibiotics are less in the discussed region. Patients who have previously undergone gynecologic surgery compared with before the surgery were three times more likely to suffer from urinary tract infections (Culligan *et al.*, 2003).

Since the patients had previously undergone genital surgery, significant growth of some bacteria such as staphylococci and anaerobic bacteria was observed that is lower in other patients.

Recent data indicate that screening and treatment for bacterial vaginosis prior to hysterectomy surgery and other surgeries will reduce postoperative anaerobic infections (Koumans *et al.*, 2001). It seems logical that before genital surgery operation, prophylactic therapy for prevention of postoperative infections be performed.

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REFERENCES

- Andrist, L.C., 2001. Vaginal health and infections. J. Obstetric Gynecol. Neonatal Nurs., 30: 306-315.
- Antonio, M.A.D. and S.L. Hillier, 2003. DNA fingerprinting of *Lactobacillus crispatus* strain CTV-05 by repetitive element sequence-based PCR analysis in a pilot study of vaginal colonization. J. Clin. Microbiol., 41: 1881-1887.
- Arzese, A.R., L. Tomasetig and G.A. Botta, 2000. Detection of tetQ and ermF antibiotic resistance genes in *Prevotella* and *Porphyromonas* isolates from clinical specimens and resident microbiota of humans. J. Antimicrob. Chemother., 45: 577-582.
- Ashraf-Ganjoei, T., 2005. Risk factors for bacterial vaginosis in women attending a hospital in Kerman, Islamic Republic of Iran. Eastern Mediterranean Health J., 11: 410-415.

- Austin, M.N., R.H. Beigi, L.A. Meyn and S.L. Hillier, 2005. Microbiologic response to treatment of bacterial vaginosis with topical clindamycin or metronidazole. *J. Clin. Microbiol.*, 43: 4492-4497.
- Borjian, S., H. Shojaei, M. Shabamian and F. Deris, 2002. Diagnosis of gardenella associated vaginosis in Borujen women's outpatient clinic, 2000. *Shahrekord Univ. Med. Sci. J.*, 3: 38-44.
- Bradshaw, C.S., A.N. Morton, J. Hocking, S.M. Garland and M.B. Morris *et al.*, 2006. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *J. Infect. Dis.*, 193: 1478-1486.
- Brown, R., J.G. Collee, I.R. Poxton and A.G. Proser, 1989. *Bacteroides Fusobacterium and Related Organisms*. In: Mackie and McCartney *Practical Medical Microbiology*, Mackie, T.J., J.E. McCartney and J.G. Collee (Eds.). 13th Edn., Churchill Livingstone Co., Edinburg, London, pp: 553-571.
- Cherpes, T.L., S.L. Hillier, L.A. Meyn, J.L. Busch and M.A. Krohn, 2008. A delicate balance: Risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race and positive herpes simplex virus type 2 serology. *Sex Transm. Dis.*, 35: 78-83.
- Core La, B.Q., J.M. Mastrobattista, K. Bishop and E.R. Newton, 2000. Gram-stain diagnosis of bacterial vaginosis after rupture of membranes. *Am. J. Perinatol.*, 17: 315-318.
- Culligan, P., M. Heit, L. Blackwell, M. Murphy, C.A. Graham and J. Snyder, 2003. Bacterial colony counts during vaginal surgery. *Infect. Dis. Obstet. Gynecol.*, 11: 161-165.
- Cunningham, F.G., N.F. Gant, K.J. Leveno, L.C. Gilstrap, J.C. Hauth and K.D. Wenstrom, 2001. *Williams Obstetrics*. 21st Edn., McGraw-Hill, New York, ISBN-13: 9780838596470, pp: 229.
- DuGas, B.W. and E.R. Knor, 2011. *Nursing Foundation*. 1st Edn., Golban Medical Publication, Tehran.
- Falagas, M., G.I. Betsi and S. Athanasiou, 2007. Probiotics for the treatment of women with bacterial vaginosis. *Clin. Microbiol. Infect.*, 13: 657-664.
- Franklin, T.L. and G.R. Monif, 2000. *Trichomonas vaginalis* and bacterial vaginosis. Coexistence in vaginal wet mount preparations from pregnant women. *J. Reprod. Med.*, 45: 131-134.
- Fredricks, D.N., T.L. Fiedler and J.M. Marrazzo, 2005. Molecular identification of bacteria associated with bacterial vaginosis. *N. Engl. J. Med.*, 353: 1899-1911.
- Gardiner, G.E., C. Heinemann, A.W. Bruce, D. Beuerman and G. Reid, 2002. Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA. *Clin. Diagn. Lab. Immunol.*, 9: 92-96.
- Guaschino, S., D. De Santo and F. De Seta, 2002. New perspectives in antibiotic prophylaxis for obstetric and gynaecological surgery. *J. Hospital Infect.*, 50: S13-S16.
- Hawes, S.E., S.L. Hillier, J. Benedetti, C.E. Stevens, L.A. Koutsky, P. Wolner-Hanssen and K.K. Holmes, 1996. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J. Infect. Dis.*, 174: 1058-1063.
- Hay, P., 2009. Recurrent bacterial vaginosis. *J. Womens Health*, 18: 1163-1167.
- Hillier, S., J. Marrazzo and K.K. Holmes, 2007. Bacterial Vaginosis. In: *Sexually Transmitted Diseases*, Holmes, K., P. Sparling, W. Stamm, P. Piot, J. Wasserheit, L. Corey and M. Cohen (Eds.). 4th Edn., McGraw-Hill, New York, pp: 737-768.
- Kjoholhede, P., S. Halili and M. Lofgren, 2009. The influence of preoperative vaginal cleansing on postoperative infectious morbidity in abdominal total hysterectomy for benign indications. *Acta Obstet. Gynecol. Scand.*, 88: 408-416.
- Koumans, E.H., J.S. Kendrick and CDC Bacterial Vaginosis Working Group, 2001. Preventing adverse sequelae of bacterial vaginosis: A public health program and research agenda. *Sex Transm. Dis.*, 28: 292-297.
- Larsson, P.G., M. Bergstrom, U. Forsum, B. Jacobsson, A. Strand and P. Wolner-Hanssen, 2005. Bacterial vaginosis. Transmission, role in genital tract infection and pregnancy outcome: An enigma. *APMIS*, 113: 233-245.
- Lassey, A.T., K.R. Adanu, M.J. Newman and J.A. Opintah, 2004. Potential pathogens in the lower genital tract at manual vacuum aspiration for incomplete abortion in Korle Bu Teaching Hospital, Ghana. *East Afr. Med. J.*, 81: 398-401.
- Ledger, W.J., 2006. Prophylactic antibiotics in obstetrics-gynecology: A current asset, a future liability? *Expert Rev. Anti-Infect. Ther.*, 4: 957-964.
- Lofgren, M., I.S. Poromaa, J.H. Stjerndahl and B. Renstrom, 2004. Postoperative infections and antibiotic prophylaxis for hysterectomy in Sweden: A study by the Swedish National Register for Gynecologic Surgery. *Acta Obstet. Gynecol.*, 83: 1202-1207.

- Makinen, J., J. Johansson, C. Tomas, E. Tomas and P.K. Heinonen *et al.*, 2001. Morbidity of 10 110 hysterectomies by type of approach. *Hum. Reprod.*, 16: 1473-1478.
- Ness, R.B., K.E. Kip, S.L. Hillier, D.E. Soper and C.A. Stamm *et al.*, 2005. A cluster analysis of bacterial vaginosis-associated microflora and pelvic inflammatory disease. *Am. J. Epidemiol.*, 162: 585-590.
- Nieboer, T.E., N. Johnson, A. Lethaby, E. Tavender and E. Curr *et al.*, 2009. Surgical approach to hysterectomy for benign gynaecological disease. *Cochrane Database Syst. Rev.*
- Price, N. and S.R. Jackson, 2009. Advances in laparoscopic techniques in pelvic reconstructive surgery for prolapse and incontinence. *Maturitas*, 62: 276-280.
- Reid, G., D. Charbonneau, J. Erb, B. Kochanowski, D. Beuerman, R. Poehner and A.W. Bruce, 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: Randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunol. Med. Microbiol.*, 35: 131-134.
- Ryan, K.J., 1999. *Kistner's Gynecology and Women's Health*. 7th Edn., Mosby, New York, ISBN: 9780323002011, Pages: 660.