

ASSOCIATION BETWEEN TRICHOMONIASIS AND BACTERIAL VAGINOSIS: EXAMINATION OF 600 CERVICOVAGINAL SMEARS

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SUMMARY

To determine whether there is a relationship between trichomoniasis and bacterial vaginosis (BV), cervicovaginal smears obtained from 600 women were stained with the Papanicolaou technique and examined cytologically. Thirty-six (6%) of 600 women were diagnosed as having *Trichomonas vaginalis* [TV(+)].

Sixteen (44.4%) of 36 TV(+) cases were observed as having BV [BV(+)]. Thirty-one of 564 TV(–) vaginal smears were also positive for BV (5.5%). There was a statistically significant correlation between trichomoniasis and bacterial vaginosis ($p < 0.05$).

It is possible that TV might create an anaerobic environment, thereby changing the vaginal flora. This flora becomes a more suitable milieu for growing of anaerobic microorganisms. It can be postulated that TV might be the most important protozoan that contributes to the overgrowth of BV-related microorganisms.

Key words: *Trichomonas vaginalis*, bacterial vaginosis, cervicovaginal smears

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INTRODUCTION

TV is the causative agent of trichomoniasis and a strict anaerobic protozoan due to lacking of mitochondria (1–7). Trichomonads predispose to an anaerobic vaginal flora for growing of the anaerobic microorganisms including *Gardnerella vaginalis*, *Mobiluncus spp.*, *Peptostreptococcus spp.*, *Mycoplasma hominis*, *Ureaplasma urealyticum* which are common cause of BV (1, 4, 8–11).

The vaginal pH is also important factor determining the presence of TV and anaerobic microorganisms (1, 6–8). The protozoan changes the local pH via phagocytosis of lactobacilli (1, 2, 4). This reduction of hydrogen peroxide producing lactobacilli in normal flora makes more suitable milieu for BV-associated microorganisms (1, 2, 4, 12, 13). The vaginal pH for TV and anaerobic agents is greater than 4.5, often pH is 6–6.5 (1, 4, 7, 12). BV has also been associated with numerous adverse pregnancy outcomes such as preterm delivery, preterm rupture of membranes, post partum endometritis, late miscarriage, and pelvic inflammatory diseases (8–11, 14–16).

Although there are some reports indicating the presence of TV and anaerobic microorganisms together, there are very few studies pointing out this relation by using cervicovaginal smears (1, 11, 17). The aim of the study is to detect whether there is an association between trichomoniasis and BV.

MATERIAL AND METHODS

Six hundred patients were seen at the Gynecology and Obstetrics Clinics of Hacettepe University for routine gynecologic exami-

nation. Cervicovaginal smears were taken from each patient with a wooden spatula, and fixed with a hair spray without drying in the air. They were stained by the Papanicolaou technique after fixation. All the patients included in this study were not pregnant. The statistical analysis was performed with χ^2 test.

To detect BV, cytologic criteria as follows were used: (1) the presence of clue cells, (2) an absence of vaginal lactobacilli, (3) the absence of polymorphonuclear neutrophilic leukocytes (PMNLs), and (4) profuse free cocci scattered among cornified type epithelial cells giving a nebulous appearance (8–11). TV was also diagnosed as oval and spreaded in shaped and unflagellated due to the fixation. The most important criterion was the observation of the nucleus of TV stained darker than cytoplasm and of elliptical shape (6).

RESULTS

Cervicovaginal smears obtained from 600 women were examined cytologically. Patients were separated into four groups according to the presence and absence of TV and BV, but only two groups (Group 1 and Group 2) were compared in this study. The number and proportional distribution of these groups are shown in Table 1.

Thirty-six (6%) of 600 smears were detected as having TV [TV(+)]. The 36 smears were examined again with a great care for detecting BV(+) cases. BV(+) smears were diagnosed according to four cytologic criteria mentioned in Material and Methods. Sixteen (44%) of 36 TV(+) smears [Group 1 (2.7%)] and 31 of 564 TV (–) smears [Group 2 (5.2%)] were detected as having BV.

Table 1. Number and percentages of women according to the presence of TV and BV

Number of group	Diagnosis	Number of women
Group 1	TV (+) BV (+)	16 (2.7%)
Group 2	TV (-) BV (+)	31 (5.2%)
Group 3	TV (+) BV (-)	20 (3.3%)
Group 4	TV (-) BV (-)	533 (88.8%)
Total		600 (100%)

Table 2. Cytologic findings detected in Group 1 and Group 2

Cytologic Diagnosis	No. of cases (n=16) TV(+)BV(+) (Group 1)	No. of cases (n=31) TV(-)BV(+) (Group 2)
PMNLs	10 (62.5%)	-
Erythrocytes	7 (43.75%)	7 (22.6%)
Döderlein bacilli	2 (12.5%)	-
Metaplasia	3 (18.75%)	1 (3.23%)

Table 3. Detail data associated with genital complaints of Group 1 and Group 2

Genital complaints	No. of cases (n=16) TV(+)BV(+) (Group 1)	No. of cases (n=31) TV(-)BV(+) (Group 2)
Homogenous VD	5 (31.25%)	6 (19.35%)
White-cheesy VD	1 (6.25%)	4 (12.9%)
Yellow-foamy VD	1 (6.25%)	-
Brown VD	-	2 (6.45%)
Itching+VD	1 (6.25%)	2 (6.45%)

The other cytologic findings such as the presence of erythrocytes, PMNLs, Döderlein bacilli and metaplasia observed in group 1 and 2 are presented in Table 2.

The clinical complaints of the patients including group 1 and 2 were also evaluated in this study. The detailed data concerning genital complaints are presented in Table 3.

DISCUSSION

TV is an anaerobic protozoan lacking of mitochondria which obtains its energy from the metabolism of polyamines, especially putrescine production from arginine (1, 6). Some microorganisms can also grow in anaerobic milieu including *Gardnerella vaginalis*, *Mobilincus* spp., *Peptostreptococcus* spp., *Mycoplasma hominis* as causative agents of BV (8–11). In our study, we would like to discuss whether there is a relationship between TV and BV.

According to some authors, the absence of PMNLs is one of the most important criterion to diagnose BV (9, 10, 18), but we found PMNLs in 10 (62.5%) of 16 BV(+) cases. It can be therefore suggested that the presence of PMNLs might be associated with TV. Although trichomonads secrete chemotactic protein for

phagocytosis of PMNLs, it is seen that this parasite could not be able to ingest all PMNLs in the environment. This finding is consistent with some of those published (1, 2, 19).

In normal vaginal flora, *Lactobacillus* species can account for 95% of the total number of organisms and they are responsible for preventing the growth of other pathogen vaginal organisms via production of lactic acid and hydrogen peroxide (2, 8, 10, 11, 13). It is believed that the equilibrium and the composition of the vaginal ecosystem changes following the depopulation of lactobacilli (2, 8–13, 20). The reason of our detecting lactobacilli in only 2 of 16 BV(+) cases with trichomoniasis might be the increase of vaginal pH. There are numerous reports indicating trichomonads have the ability to phagocytize lactobacilli (2). It may be postulated that the proportion of lactobacilli may be decreased because of the overgrowth of TV.

It is well known that metaplasia occurs due to injury or chronic irritation caused by an inflammatory process or a mechanical trauma (21). In our study, metaplasia was observed in 3 (18.75%) of 16 TV(+)BV(+) cases. Due to the presence of trichomonads with metaplastic cells in 3 women, metaplasia might occur as a result of the TV related inflammatory process. However we also detected metaplasia in one woman with bacterial vaginosis using IUCD and we therefore assumed that metaplasia might have occurred because of the presence of the mechanical trauma as a result of IUCD in BV(+) patients.

According to Table 1, erythrocytes were present in 7 (43.75%) of 16 cervicovaginal smears. There are numerous reports showing the erythrophagocytic activity of the parasite and suggesting that TV may use hemoglobin as an iron source (1, 2, 4, 19, 22). It has been reported that *Gardnerella vaginalis* can also utilize hemoglobin (9). In our study, we also observed erythrocytes in 7 (22.6%) of 31 BV(+) smears. The observation of profuse cocci as phagocytosed in the cytoplasm of TV has also been indicated by Demirezen (23). Consequently it can be suggested that iron in erythrocytes might be engulfed by both free cocci and trichomonads for growth and multiplication.

Vaginal discharge is a common clinical symptom accompanying disorders caused by some infectious agents (5, 9, 24, 25). As is seen in Table 3, 7 (43.75%) of 16 patients with both TV and BV(+) cases had vaginal discharge. While 5 (71.43%) of 16 had homogenous vaginal discharge, 1 (14.28%) of 16 had white-cheesy, 1 (14.28%) of 16 had yellow foamy vaginal discharge. Moreover, 1 patient (6.25%) had itching including vaginal discharge. It is known that anaerobic bacteria associated with BV also cause an abnormal vaginal discharge via production of glycosidases and proteases to degrade the protective mucus gel and thereby change the vaginal fluid viscosity (24, 25). In our study, 12 (38.7%) of 31 patients with BV had vaginal discharge. Among them, 6 (50%) of 12 women had homogenous vaginal discharge. According to these results, it can be postulated that the presence of vaginal discharge, particularly homogenous discharge, is a significant clinical criterion supporting the diagnoses of both BV(+) and TV(+) cases.

In conclusion, trichomoniasis is a common infection that involves an alteration in the genital microflora in which lactobacilli are replaced by some microorganisms commonly found in BV. So cervicovaginal smears of patients with *Trichomonas vaginalis* should be examined carefully for the presence of other infectious agents associated with BV.

REFERENCES

1. Wang J: Trichomoniasis. Prim Care Update Ob/Gyns. 2000;7: 148–153.
2. Rendon-Maldonado JG, Espinosa-Cantellano M, Gonzales-Robles A, Martinez-Palomo A: Trichomonas vaginalis: In vitro phagocytosis of lactobacilli, vaginal epithelial cells, leukocytes and erythrocytes. Exp Parasitol. 1998;89: 241–250.
3. Safi Z, Demirezen S, Beksaç MS: The relationship between *Trichomonas vaginalis* and some clinical findings. Gynecol Obstet Reprod Med. 2000;6: 185–187.
4. Fiori PL, Rappelli P, Addis MF: The flagellated parasite *Trichomonas vaginalis*: new insights into cytopathogenicity mechanisms. Microbes Infect. 1999;2: 149–156.
5. Bates S: Vaginal discharge. Curr Obstet Gynaecol. 2003;13: 218–223.
6. Demirezen S: Trichomonas vaginalis in vaginal smears of women using intrauterine contraceptive device. Cent Eur J Publ Health. 2001;4: 176–178.
7. Rashad AL, Toffler WL, Wolf N, Thornburg K, Kirk EP, Ellis G, Whitehead WE: Vaginal Po_2 in healthy women and in women infected with *Trichomonas vaginalis*: Potential implications for metronidazole therapy. Am J Obstet Gynecol. 1992;166: 620–624.
8. Wang J: Bacterial vaginosis. Prim Care Update Ob/Gyns. 2000;7: 181–185.
9. Demirezen S: Review of cytologic criteria of bacterial vaginosis: Examination of 2,841 Papanicolaou-stained vaginal smears. Diagn Cytopathol. 2003;29: 156–159.
10. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M: Bacterial vaginosis: a public health review. Br J Obstet Gynaecol. 2001;108: 439–450.
11. Nieves B: Bacterial vaginosis. Anaerobe. 1999;5: 343–345.
12. Milani M, Molteni B and Silvani I: Effect on vaginal pH of a polycarbophil vaginal gel compared with an acidic douche in women with suspected bacterial vaginosis: a randomized, controlled study. Curr Ther Res Clin Exp. 2000;61: 781–788.
13. Boris S, Barbes C: Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect. 2000;2: 543–546.
14. Nelson DB, Bellamy S, Gray TS, Nachamkin I: Self-collected versus provider-collected swabs for the diagnoses of bacterial vaginosis: An assessment of validity and reliability. J Clin Epidemiol 2003;56: 862–826.
15. Ugwumadu A, Manyonda I, Reid F, Hay P: Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: randomised controlled trial. Lancet. 2003;361: 983–988.
16. Yudin M, Landers DV, Meyn L, Hillier SL: Clinical and cervical cytokine response to treatment with oral or vaginal metronidazole for bacterial vaginosis during pregnancy: a randomized trial. Obstet Gynecol. 2003;102: 527–534.
17. Rappelli P, Carta F, Delogu G, Addis MF, Dessi D, Cappuccinelli P, Fiori PL: *Mycoplasma hominis* and *Trichomonas vaginalis* symbiosis: multiplicity of infection and transmissibility of *M. hominis* to human cells. Arch Microbiol. 2001;175: 70–74.
18. Donders GGG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B: Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG. 2002;109: 34–43.
19. Demirezen S, Safi Z, Beksaç S: The interaction of *Trichomonas vaginalis* with epithelial cells, polymorphonuclear leukocytes and erythrocytes on vaginal smears: light microscopic observation. Cytopathology. 2000;11: 326–332.
20. Demirezen S: The lactobacilli – Candida relationship in cervico-vaginal smears. Cent Eur J Publ Health. 2002;10: 97–99.
21. Koss GL: Morphologic response of cells to injury. In: diagnostic cytology and its histopathologic bases. Winters R, Orem E, Gibbons T. J.B. Lippincott company. 1992: 112–113.
22. Ryu JS, Choi HK, Min DY, Ha SE, Ahn MH: Effect of iron on the virulence of *Trichomonas vaginalis*. J Parasitol. 2001;87: 457–460.
23. Demirezen S: Phagocytosis of rod-shaped bacteria and cocci by *Trichomonas vaginalis*: light microscopic observations. Acta Cytol. 2001;45: 1088–1099.
24. Bro F, Anderson BS: Vaginal discharge – diagnosis and treatment in general practice. Ugeskrift for Laeger. 2002;164: 5245–5249.
25. Olmsted SS, Meyn LA, Rohan LC, Hillier SL: Glycosidase and proteinase activity of anaerobic gram-negative bacteria isolated from women with bacterial vaginosis. Sex Transm Dis. 2003;30: 257–261.

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