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Full Length Research Paper

Nutritional status, bacterial vaginosis and cervical colonization in women living in an urban slum in India

Rajkumar Hemalatha*, Baru Anantha Ramalaxmi, Gummuluri Krishna Swetha, Dasari Madusudhan Rao, Sesha Charyulu, and Dinesh Kumar

National Institute of Nutrition, Jamaio-Osmania PO, Hyderabad – 500007, Andhra Pradesh, India.

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The prevalence of bacterial vaginosis and cervical colonization, and association of bacterial vaginosis with serum nutrients were determined in women living in slum areas of Hyderabad, India. Bacterial vaginosis was diagnosed based on Nugents' score. Cervical infections with human papilloma virus, herpes simplex virus type 2*, Neisseria gonorrhea, Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum* were determined by PCR. Of the 260 women who participated in the study, 31% (81) had bacterial vaginosis and 48.8% (127) had intermediate flora based on Nugents' score. Only 184 vaginal samples were processed for candidiasis, of which 66 showed *Candida albicans*, accounting for a prevalence of 36.0%. PCR analysis of cervical swabs obtained from 50 women with acute cervicitis showed the following trend of prevalence of various organisms: 30% *U. urealyticum*, 10% *M. hominis, 2%* herpes simplex virus and Human papilloma virus, while *C. trachomatis* and *N. gonorrhea* were not detected in any. In the 50 women without cervicitis, 6 (12%) had human papilloma virus, while other organisms were not detected. All the women with cervical colonization (*U. urealyticum, M. hominis and* herpes simplex virus type 2) and 6 of 7 women with human papilloma virus had bacterial vaginosis or intermediate flora. Thirty percent of the women were undernourished (body mass index <18.5), while all the women in the study were anemic (hemoglobin <12 g/dl). More than 50% of the women in the study had low serum iron, while more than 90% had low serum zinc levels. But vitamin A deficiency was prevalent in 3% of the study subjects. Low concentrations of serum iron, zinc and vitamin A were significantly associated with bacterial vaginosis.

Key words: Bacterial vaginosis, cervicitis, *Ureaplasma urealyticum, Mycoplasma hominis*, vitamin A, iron, zinc.

INTRODUCTION

Bacterial vaginosis (BV) is the most common cause of lower genital tract infections in women of reproductive age group and is associated with increased susceptibility to sexually transmitted infections, preterm deliveries and HIV infection (Angela, 2008; Sabina et al., 2002; Allsworth and Peipert, 2007; Sewankambo et al., 1997; Hillier, 1998). It is characterized by a disturbance in normal vaginal flora with loss of lactobacilli and increasing numbers of anaerobes and gram negative rods. BV is often asymptomatic and relapses are frequent (Jyoti et al., 2010). Several factors such as race, smoking, chronic stress, vaginal douching and contraceptive use are associated with BV (Nansel et al., 2006), yet the etiology is not very well understood. In recent years, nutrition is also being hypothesized to be another putative risk for BV (Verstraelen et al., 2005; Beth et al., 2007). Subclinical iron deficiency and low levels of serum vitamin D have been shown to be independently associated with prevalence of BV (Verstraelen et al., 2005; Lisa et al., 2009). Similarly, low dietary intake of micronutrients and high intakes of fat have been associated with BV (Neggers et al., 2007).

*Corresponding author. E-mail: rhemalathanin@yahoo.com. Tel: 040-27191297. Fax: 040-27019074.

Abbreviations: BV, Bacterial vaginosis; **BMI,** body mass index; **HPV,** human papilloma virus; **HSV-2,** herpes simplex virus type 2.

BV is very common and ranges from 11 to 62% in different populations in India (Neeraja et al., 2009; Patel et al., 2006; Aggarwal et al., 1999; Bang et al., 1989). In a study conducted in Karnataka, India, prevalence of bacterial vaginosis was 20% and another study from Haryana showed 48% prevalence of BV (Rao et al., 2004; Bhalla et al., 2007). Though BV is widely reported and recurrences are common, no information is available on nutritional status of women with BV in India, despite the fact that a sizable proportion of women in India are undernourished (Fred et al., 2009). In the current study, we evaluated BV and cervical colonization and studied the association of BV with serum concentration of nutrients.

MATERIALS AND METHODS

A cross-sectional community based study was carried out among the population of slum areas of low socioeconomic status in Hyderabad city, India. The study was approved by Institutional Ethical Committee. Households were selected by systematic random sampling method. House-to-house survey was carried out to select women, who were asymptomatic, 20 to 40 years, HIV negative, non-pregnant and living with husband and who were between 8^{th} to 10^{th} day of menstrual cycle. 300 women fulfilled our eligibility criteria, of which 285 agreed to participate in the study. These 285 women were ferried to the health centers located in the area. In the health centers, demographic and clinical data were collected using a structured questionnaire and after excluding those using oral antibiotics/ contraceptives/ vaginal medication in the last 10 days and those who had sexual intercourse in the last 2 days, we had 262 women. Height and weight were measured to calculate body mass index (BMI) (weight in kg/ height in m^2). General and gynecological examinations were done to evaluate reproductive health. Vaginal specimens, and cervical swabs were obtained after taking a written consent, but 2 women had bleeding during examination and were excluded. Thus 260 specimens were processed for diagnosing BV based on Nugents' score. All enrolled women denied using douches or tampons and were nonsmokers.

Gram stain

After pelvic and speculum examination, vaginal smears were collected for wet mount and gram stain. Gram stained smears were scored for gram negative and gram positive bacteria, clue cells, yeast and pus cells (leukocyte counts) to diagnose bacterial vaginosis or vaginitis. Wet mount was prepared to screen for *Trichomonas vaginalis*. BV and intermediate flora were diagnosed based on vaginal evaluation by Nugents' score. Three different bacterial morphotypes - lactobacilli, *Gardnerella*-like species (including *Gardnerella vaginalis, Bacteroides* species, *Prevotella* species, and *Porphyromonaz* species), and *Mobilincus* species were quantitatively evaluated according to the Nugent's score method (Nugent et al., 1991). Women with Nugent scores of 0 to 3 were categorized as normal flora. Women with scores of 4 to 6 were classified as intermediate flora and women with Nugent score 7 or more were enrolled as BV.

Multiplex PCR for cervical pathogens

Cervical swabs from 50 women with cervicitis (>30 WBC/HPF) and 50 women without cervicitis were screened for cervical colonization. Cervical swabs were processed for human papilloma virus (HPV), herpes simplex virus type 2 (HSV-2), *Neisseria gonorrhea*, *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Genomic DNA was isolated from the cervical swab samples using Bioserve Genomic DNA isolation kit. The genomic DNA was subjected to multiplex PCR for the detection of HPV*, M. hominis, C. trachomatis,* HSV-2, *N. gonorrhoea and U. urealyticum*. The PCR products were resolved on a 2% Agarose gel.

Micronutrients in the serum

For comparing micronutrient status of women with and without BV, blood samples were obtained from 52 women with normal vaginal flora, 52 women with intermediate flora and 80 women with BV. BV cases were age and socioeconomic status matched with controls. A sample size of 50 was decided based on the results of a previous study (Verstraelen et al., 2005). Iron, zinc, calcium, magnesium, and copper were analyzed by atomic absorption spectroscopy (AAS) and vitamin A was determined by high-performance liquid chromatography (HPLC). For iron estimation, serum was treated with one volume of 20% (w/v) trichloro acetic acid (TCA) and heated to ensure release of transferrin bound iron and then centrifuged. The supernatant was diluted with 3 volumes of deionized water and then analyzed by AAS at 248.3 nm. For zinc, one part of the serum sample was diluted in 5 parts of deionized water and mixed well. The supernatant was read at 213.9 nm. Vitamin A was detected at 325 nm using a sensitive ultra violet (UV) detector. Retinyl acetate was used as internal standard to account for processing losses for vitamin A analysis. Serum lipid profile (high density lipoprotein cholesterol (HDL-C) and triglycerides (TG)) were analysed by kits obtained from Biosystems (Barcelona, Spain).

Statistical analysis

Log-transformed micronutrient mean values were compared between women with BV and without BV by using a Student's t test. Pearson chi-squared test was used to study the association of BV with micronutrient status. To assess the relationship between serum nutrients levels and vaginal flora, Pearson's correlation was done. Logistic regression was used to assess the relation of each nutrient variable to BV. P-values < 0.05 were considered as statistically significant. Statistical analysis was performed using SPSS statistical software (SPSS Inc, Chicago, IL, USA).

RESULTS

The mean age of the women was 27.9 years. All the women were of low socioeconomic group, semi-literate and apparently normal. Of the 260 women, 31% had BV and 48.8% had intermediate flora based on Nugents' score. Only 184 vaginal samples were processed for candidiasis, which was prevalent in 36.0%. Nearly 33% had acute cervical erosion and 47% had tender fornices on one/both sides (Table 1). On clinical examination, 23 (28%) women with BV and 36 (28%) women with intermediate flora had vaginal discharge, while 11 (21%) women with normal vaginal flora had vaginal discharge.

PCR analysis of cervical swabs obtained from 50 women with acute cervicitis showed 15 (30%) and 5 (10%) women positive for *U. urealyticum* and *M. hominis* respectively. HSV-2 and HPV were prevalent in 2% of the

Table 1. Reproductive tract infections (RTI) in women living in slum.

Laboratory-diagnosed RTI	Total Nos.	Number positive	Percentage positive
Bacterial vaginosis (BV)	260	81	31.1
Intermediate vaginal flora	260	127	48.8
Vaginal candidiasis	184	66	36.0
Human papilloma virus	100	7	7
Herpes simplex virus type 2	100	1	1
Mycoplasma hominis	100	5	5
Ureaplasma urealyticum	100	15	15
Chlamydia trachomatis	100	None	0
Neisseria gonorrhea	100	None	0
Clinically diagnosed RTI			
Vaginal discharge	260	67	26.0
Acute cervical erosion	260	87	33.4
Both fornices tender (PID)	260	58	22.3
Single fornix tender (PID)	260	65	25.0

Nugent's classification was followed for BV and intermediate vaginal flora. Nugent score: 0 to 3 is normal flora, 4 to 6 is intermediate flora and equal to or greater than 7 score is BV. Candida infection was based on gram stain. Human papilloma virus, herpes simplex virus type 2, *Neisseria gonorrhea*, *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* were detected by polymerase chain reaction (PCR) of samples obtained from cervix.

Table 2. Serum nutrients in undernourished (BMI <18.5) women.

Parameter	Less than 18.5 BMI (n=56)	More than or equal to 18.5 BMI (n=128)	Total mean of nutrients	Proportion with deficiency
Hemoglobin (g/dl) (n=175)	11.4 ±0.14	11.7 ±0.09	11.6 ±0.07	2.28% (<9 g/dl)
Calcium (mg/dl) (n=171)	8.74±0.09	9.05±0.11	8.9±0.08	54.5% (<9 mg/dl)
Vitamin A (µg/dl) (n=174)	48.22±3.21	53.80±2.56	52.3±1.66	2.9% (<20 µg/dl)
Zinc (µg/dl) (n=181)	47.25±2.85	48.03±1.74	47.7±1.27	90.6% (<70 µg/dl)
Copper (µg /dl) (n=176)	70.97±3.32	75.45±2.67	78.5±1.99	58.5% (<80 µg/dl)
Iron (µg/dl) (n=184)	68.24±6.78	70.38±4.72	72.6±3.95	49.5% (<60 µg/dl)

Values are mean ± standard error (SE). BMI = body mass index.

women with cervicitis, while *N. gonorrhoea* and *C. trachomatis* were not detected in any (Table 1). In 50 women without cervicitis, 6 (12%) had HPV, while other organisms were not detected. All women with cervical colonization (*U. urealyticum, M. hominis* and HSV-*2*) had BV or intermediate flora and 6 out of 7 HPV positives were associated with BV.

The mean (standard error; SE) BMI was 21.30 (0.387), and 30% of the women had BMI less than 18.5. All the women in the study had hemoglobin less than 12 g/dl. More than 50% of the women in the study had low serum iron, copper and calcium, while low serum zinc was prevalent in 90% of the study subjects (Table 2). However, serum vitamin A was within normal range in 97% of the subjects. Women with low BMI (<18.5) had lower concentrations of serum nutrients (Table 2).

For comparing micronutrient status of women with and without BV, 80 women with BV were taken as case, and 52 women with normal vaginal flora were taken as controls. BV cases were age and socioeconomic status matched with controls. As indicated in Table 3, the mean serum vitamin A, zinc and iron concentrations were significantly (P<0.005) lower in the BV compared to the normal vaginal flora and intermediate flora. Similarly, higher proportion of women with vitamin A, zinc and iron deficiencies had significantly (p<0.05) higher prevalence of BV (Figure 1). When correlation analysis was done taking Nugents' score as continuous variable, low levels of serum vitamin A and zinc were associated with higher Nugents' score, while serum iron deficiency showed a trend (Table 4). However, logistic regression analysis showed 2 fold higher risk (OR 1.95; 95% confidence

Table 3. Mean of serum nutrients in women with BV, intermediate flora and normal vaginal flora.

Parameter	Normal vaginal flora (n=52)	Intermediate flora (n=52)	BV posiitive (n=80)	P values
Heamoglobin (gm/dl)	11.7±0.14	11.7±0.14	11.5±0.12	>0.05
Calcium (mg/dl)	8.81±0.21	9.05±0.10	8.88±0.07	>0.05
Vitamin A (µg/dl)	57.99±3.42	53.2±2.44	47.95±2.55	0.007
Zinc (µg/dl)	50.61±2.22	52.45±2.6	42.58±1.75	0.021
Copper (µg/dl)	81.98 ± 3.58	77.59±4.20	75.33± 3.67	>0.05
Iron(µg/dl)	87.08±7.5	72.28±5.51	63.03±6.62	0.004
Total cholesterol (mg/dl)	138.95±4.98	124.84±4.51	138.55±5.61	>0.05
Triglyceride (mg/dl)	104.3±7.56	86.44±5.93	96.08±5.55	>0.05
High density lipoprotein (HDL) (mg/dl)	36.24±2.07	33.02±1.11	35.0±1.11	>0.05

Values are mean ± standard error (SE). Mean micronutrient concentrations were compared in women with normal vaginal flora, intermediate flora and BV using a Student's t test.

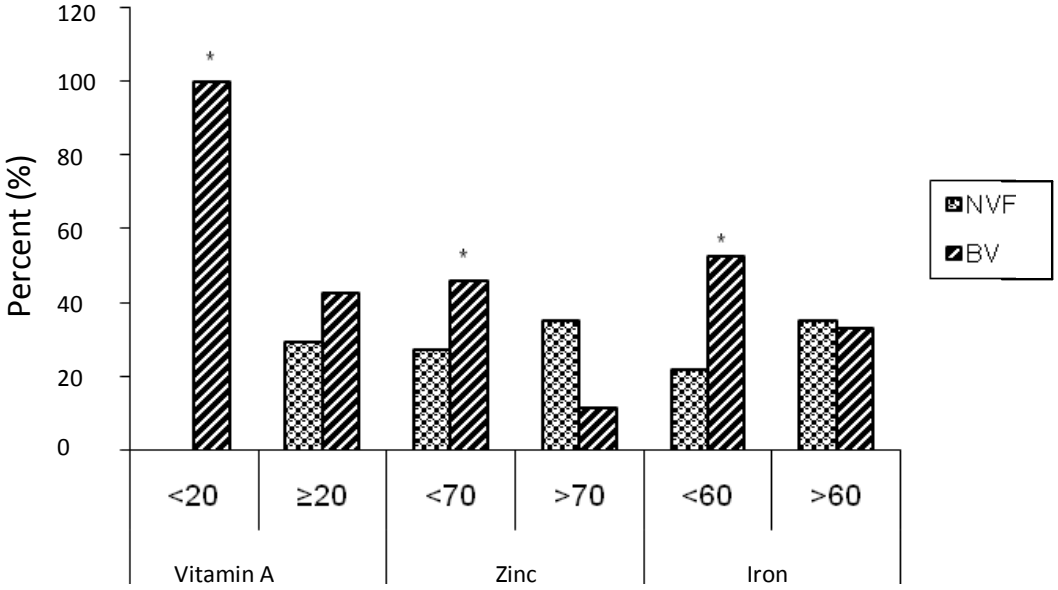

Figure 1. Prevalence (%) of BV in women with micronutrient deficiency. A Pearson chi-squared test was used to study the association of BV with micronutrient status. Y axis depicts percentage prevalence of BV and X axis depicts cutoff values for deficient and normal concentration of nutrients. BV: bacterial vaginosis, NVF: normal vaginal flora. *P<0.05 BV significantly higher in women with vitamin A, zinc and iron deficiency. Vitamin A, zinc and iron are expressed as µg/dl.

Table 4. Correlations of vitamin A, zinc and iron with Nugents score (BV).

Parameter	R values	P values
Vitamin A	-0.196**	0.009
Zinc	-0.207**	0.005
Iron	-0.138	0.062

** P<0.01. This table depicts correlation analysis with Nugent's score and nutrients as continuous variable. Low concentrations of serum vitamin A, zinc and iron are associated with higher Nugents' score.

interval, 1.01 to 3.75) of BV in women with iron deficiency. Serum copper and calcium concentrations were not associated with BV. Lipid profile such as total cholesterol, triglyceride (TG) and high density lipoprotein (HDL) were similar in women with or without BV (Table 3).

DISCUSSION

In the current study, on asymptomatic women, not complaining of vaginal discharge, BV was prevalent in

31% and there was modest association between altered vaginal flora and microbial colonization of the cervix. Low serum concentrations of iron, zinc and vitamin A were significantly associated with BV.

Women with cervicitis (>30 WBC/HPF) had *U. urealyticum, M. hominis* and HSV-2 colonization, but HPV colonization was not associated with cervicitis. Nevertheless, all cervical colonization (*U. urealyticum, M. hominis* and HSV-2*)* including HPV were associated with BV or intermediate flora. Cervicitis may be caused by infections with *U. urealyticum, M. hominis* and sexually transmitted infections, such as chlamydia, gonorrhea and trichomonias (Rodrigues et al., 2011). HPV also causes cervicitis, but not all strains of HPV cause cervicitis. Probably, HPV colonization in women without cervicitis in the current study was due to those strains that do not cause cervicitis.

Most women with BV suffer with multiple recurrences despite antibiotic therapy (Jyoti et al., 2010). Mycoplasma and ureaplasma colonisation have consistently been found in women with BV and have been linked with recurrent BV (Keane et al., 2000*;* Angela, 2008). Though the current study was not designed to address the issue of recurrent BV and *M. hominis* or U. urealyticum, all the women with cervical *M. hominis* and *U. urealyticum* colonization had BV. Few studies in India have reported prevalence of mycoplasma and ureaplasma infections. Our findings on mycoplasma and ureaplasma colonization are similar to Brabin et al. (1998), but lower than that reported in pregnant women (Choudhury et al., 1994); however, none of these studies attempted correlation of BV with these infections. In the subsample of 100 women that we tested, none were positive for *C. trachomatis* or *N. gonorrheae* infections. Similarly, a study from Mumbai (India) registered a very low prevalence of *C. trachomatis* (0.5%) and *N. gonorrheae* (none). In contrast, Singh et al. (2003) from Delhi (India) reported a very high prevalence of *C. trachomatis* (29%), but the study was on symptomatic patients. *C. trachomatis* or *N. gonorrheae* prevalence is low in India but this is not surprising, given the conservative attitudes about extramarital relationships in India.

About one third of the women in the present study had chronic energy deficiency (CED) (BMI< 18.5) and surprisingly, all the study subjects were anemic. Even in a small sample size as this, the study showed a clear correlation of BMI and serum nutrients with lower concentration of all nutrients studied in CED women. More than 60% prevalence of zinc deficiency had been reported by others from India (Kapil et al., 2003; Priyali et al., 2008); comparatively, a higher prevalence was registered in the present study. Similarly, copper, serum iron and calcium levels were low in majority of the study subjects; however, vitamin A was deficient only in 3% of the subjects.

In an earlier study, BV was similar in undernourished (BMI 16.5 to 18.5) and well-nourished women (BMI >18.5), but was significantly higher in women with severe under-nutrition (BMI <16.5) (Yashodhara et al., 2006). The findings of the current study are in agreement with the observations of the aforementioned study. Correlations of vitamins A and D with reproductive infections have been reported by others (Beth et al., 2007; Belec et al., 2002; Lisa et al., 2009). Association of BV with high fat and low micronutrients intake was reported by Neggers et al. (2007). Low levels of serum micronutrients such as zinc, iron and vitamin A were highly correlated with BV in the current study. As far as our knowledge goes, this is the first study demonstrating low serum zinc and vitamin A in women with BV. Beth et al. (2007) had reported increased risk of HPV infection with low serum zinc concentration, but there are no studies relating zinc with BV. Subclinical iron deficiency has been shown to be independently associated with BV during early pregnancy (Verstraelen et al., 2005). In the current study, logistic regression showed two fold higher risk of BV with iron deficiency in non-pregnant women.

Micronutrients such as vitamin A, zinc and iron play an integral part in both cell mediated and humoral immunity (Bhaskaram, 1997). Vitamin A plays a key role in maintaining the integrity of all the epithelial surfaces in the body, such as the skin, the lining of the respiratory tracts, digestive tracts and the vagina. In vitamin A deficiency, squamous and keratinized epithelial cells replace mucus-secreting cells, thus making these surfaces vulnerable to external environment and foreign invaders. Iron and zinc are important minerals for several enzymes and metabolic pathways. Iron and zinc deficiencies impair cell mediated immunity, delayed hypersensitivity and leukocyte functions (Bhaskaram, 1997). The ability of leukocytes to kill ingested bacteria is impaired. Not much is known about recurrences and relapses of BV, though local vaginal immunity is thought to play an important role in the development of BV (Yashodhara et al., 2006). Micronutrient deficiencies can affect vaginal immune function and may contribute to recurrences and relapses of BV, however, it needs to be explored in a prospective cohort study.

The major weakness of the study is the cross sectional nature, however, this study has been useful in identifying serum nutrients association with BV that can be more rigorously studied using a prospective cohort design. Iron deficiency indicators such as serum transferrin receptors and ferritin were not determined. Serum transferrin receptors and ferritin are important when serum iron levels are >60 µg/dl. However, when serum iron levels are <60 µg/dl, there is no need for additional indicators of iron deficiency. The time of sample collection was a definite strength, because all the women in the current study were recruited during estrogen phase; thus minimizing the hormonal influence on vaginal flora.

BV is the most common vaginal infection, and its impact on the health of women is substantial. Nevertheless, its treatment and prevention remain

difficult. Women with BV suffer with multiple recurrences despite antibiotic therapy. There is a great need for a prospective study that identifies the risk factors for BV and recurrence of BV in India. Research is also warranted in finding good preventive measures and dietary interventions if any for BV.

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