Maternal vitamin A and β-carotene supplementation and risk of bacterial vaginosis: a randomized controlled trial in rural Bangladesh

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ABSTRACT

Background: Bacterial vaginosis (BV) in pregnancy is linked to preterm birth, but its risk factors are not well understood. Micronutrient deficiencies may be associated with an increased risk of this condition.

Objective: We assessed the effect of weekly vitamin A or β-carotene supplementation during pregnancy until 3 mo postpartum on BV risk in rural northeastern Bangladesh.

Design: In this cluster-randomized, placebo-controlled trial, 33 clusters (n = 33) were randomly assigned to 3 groups. Women (n = 1812) were examined for BV by using self-administered swabs and the Nugent scoring method in early pregnancy, at 3 wk of gestation, and at 3 mo postpartum.

Results: The prevalence of BV in early pregnancy, before supplementation, was 7.6% (95% CI: 6.3%, 9.1%) overall. Neither the prevalence nor the incidence of BV in the third trimester differed by supplement group. However, the prevalence (OR: 0.71; 95% CI: 0.52, 0.98) and incidence (RR: 0.58; 95% CI: 0.41, 0.81) of BV at 3 mo postpartum was lower among women in the vitamin A group (9.1% and 6.7%, respectively) than in the placebo group (12.4% and 11.8%, respectively), but not in the β-carotene group. Both vitamin A and β-carotene reduced the prevalence and incidence of BV at both time points (ie, third trimester and 3 mo postpartum) by 30–40% compared with placebo (all P < 0.05).

Conclusions: Weekly vitamin A supplementation reduced the risk of maternal BV in this rural Bangladeshi population. Enhancement of vitamin A status before and during pregnancy may reduce the risk of BV in areas with vitamin A deficiency. This trial is registered at clinicaltrials.gov as NCT00198822. Am J Clin Nutr 2011; 94:1643–9.

INTRODUCTION

Bacterial vaginosis (BV), defined as a disruption in the vaginal microflora, is a condition that affects 6–41% of women of childbearing age in diverse settings (1–3). Among women with laboratory-diagnosed BV, 50% (4) to 84% (5) of cases are asymptomatic. Current guidelines call for the treatment of symptomatic BV with a 7-d regimen of 250 mg metronidazole, 3 times a day (6). Asymptomatic BV is associated with a 2-fold increased risk of preterm birth, although the risk has been observed to increase to nearly 3-fold when BV is detected before 16 wk of gestation (7). In a meta-analysis of 15 trials (n = 3888 women), BV treatment during pregnancy showed no overall effect on risk of preterm birth, except in women treated before 20 wk of gestation for whom there appeared to be significant risk reduction (OR: 0.63; 95% CI: 0.48, 0.84) (8).

The etiology of BV is largely unknown and its risk factors are varied. Alteration in vaginal pH and hormone concentrations during puberty, menstruation, and pregnancy and the practice of vaginal douching, sexual practices, and hygiene-related factors have been implicated. Recently, nutritional factors, including those related to micronutrients, have been linked to the risk of BV. Among US women, the severity of BV was observed to increase among those with lower intakes of folate, vitamin E, and calcium (9). Among women with HIV or at high risk of HIV, the risk of BV was inversely associated with lower concentrations of vitamins A, C, and E and β-carotene (10). Among low-income African American (but not white) adolescent pregnant women, the prevalence of BV was higher (57%) in those whose serum 25-hydroxyvitamin D concentrations were <20 nmol/L than in those with concentrations >80 nmol/L (23%) after adjustment for the presence of other sexually transmitted infections (11).

Among African American adolescents, the risk of BV was significantly associated with serum 25-hydroxyvitamin D concentrations <37.5 nmol/L (OR: 4.4; P = 0.02) (12). In a study in Central Africa, vitamin A deficiency based on low serum retinol concentrations (<0.70 μmol/L) was associated with an in-
Micronutrients that have been linked with BV are generally involved in regulating immune function and maintenance of epithelial integrity and may even influence hormonal factors known to affect the vaginal microbiome, which makes their contribution to promoting healthy vaginal flora an important yet less explored area of public health in undernourished populations. In rural northwestern Bangladesh, we conducted a study of BV in the context of a large maternal vitamin A and β-carotene supplementation trial that examined the effect on maternal and infant mortality (14–16). The current analysis examines the effect of maternal supplementation on the prevalence and incidence of BV during pregnancy and the postpartum period.

SUBJECTS AND METHODS

Study population and design

From 2001 to 2007, a double-blind, cluster-randomized, placebo-controlled trial of maternal vitamin A and β-carotene supplementation was conducted in the rural northwestern districts of Gaibandha and Rangpur in Bangladesh. Vitamin A in the form of a gelatin supplement was provided weekly at 7000 μg retinol equivalents (REs) in the form of retinyl palmitate and β-carotene at 42 mg—an amount equivalent to 7000 μg REs. All 3 supplements contained 5 IU vitamin E in oil, including the placebo. This amount of vitamin A, approximately the Recommended Dietary Allowance for women during pregnancy and lactation, was provided as a weekly dose. The study area, spread out over ~450 km², was divided into 596 communities called sectors, which were used as the unit of randomization for supplement allocation. The primary outcome of this trial in ~60,000 pregnant women was pregnancy-related mortality, which was not reduced with supplementation (14, 15). Sector randomization was done in blocks of 9 with identical coins marked 1, 2, and 3 being pulled from a container by senior study investigators. The sectors were assigned 3-digit numbers from 001 to 596, and the 9 marked coins were mixed and removed randomly, without replacement; each number pulled (1, 2, or 3) was assigned to the subsequent sector, in numeric sequence, 001–596. Study supplements were identical in appearance and were in 100-count dispensing bottles that were used by 596 local female staff for observed, direct dosing of pregnant women once a week. The bottles labeled with codes 1, 2, or 3 were relabeled by a senior accounts administrator not involved in the scientific investigation before being issued to fieldworkers. Permanent opaque stickers preprinted with the appropriate sector number, according to the allocation code, were used. Investigators, study participants, and data collection staff were all blinded to the allocation codes until the end of the parent trial.

Pregnancy surveillance, involving visits every 5 wk to all women of reproductive age living in the study area, was conducted to elicit menstrual history. In amenstrual women, a human chorionic gonadotropin–based urine test was used for pregnancy detection and enrollment in the study, after consent was obtained. Pregnancy surveillance was conducted by 596 trained local female staff also responsible for dosing pregnant women weekly with a sector-specific supplement from the time of pregnancy enrollment until 3 mo postpartum. Supplement receipt, compliance, pregnancy outcomes (including fetal losses and live births), and vital status of the women and their infants were tracked and recorded weekly in the study by these local female workers. Enrolled pregnant women were visited for an interview by trained female interviewers at enrollment, in the third trimester, and 3 and 6 mo postpartum. At baseline, data were collected on socioeconomic status, pregnancy history, 7-d dietary intake, 30-d and 7-d self-reported morbidity histories, substance use, antenatal care utilization, and work history. Histories of diet, morbidity, substance, and work were repeated at subsequent visits.

In a substudy area comprising 33 sectors (~6% of the study area, internally balanced by supplement allocation), selected for its relative access by navigable roads and proximity to the field headquarters, we conducted a more intensive nutritional and health assessment of women enrolled into the trial. This assessment included venous blood collection, comprehensive anthropometric measurements (including weight, height, midupper arm circumference, and triceps and subscapular skinfold thickness), and bioelectrical impedance testing twice during pregnancy (at enrollment in early pregnancy and at 32 wk of gestation) and 3 mo postpartum. In this substudy area, we introduced in August 2003 an assessment of BV using self-administered vaginal swabs at enrollment (presupplementation), in the third trimester of pregnancy, and 3 mo postpartum (home assessment visits). We collected vaginal swabs at each time point irrespective of whether women had contributed a swab in the previous visits, allowing us to maximize our total sample size for each cross-sectional assessment, especially at the third-trimester and 3-mo postpartum visits. The main objective of the BV assessment was to ascertain the burden of this condition and its responsiveness to routine (weekly) vitamin A or β-carotene supplementation in this malnourished, rural South Asian population.

The parent trial and its substudies were approved by institutional review boards at the Johns Hopkins Bloomberg School of Public Health (Baltimore, MD) and the Bangladesh Medical Research Council, Bangladesh. Oral informed consent from women was obtained for participation in the study.

BV diagnosis

Two self-administered swabs (polyester-tipped sterile applicator swabs; Puritan Medical Products) were collected at each visit: one for smearing onto a glass microscope slide for later Gram staining and reading and one to be stored frozen for later molecular analysis of vaginal microflora. Vaginal pH was assessed by vaginal insertion of a locally procured and sterilized plastic “pop-sickle” stick onto which a strip of semiquantitative colorimetric pH paper (Baker-PHIX pH papers with color scale) had been attached.

After the vaginal swab was collected and rolled out onto a glass slide, the slide was stored in a slide-box to dry while being transported to the study laboratory within a few hours of collection. At the laboratory, the slides were Gram-stained by a laboratory technician and read under an electric binocular microscope. BV was diagnosed by using the Nugent scoring criteria, which assigns a numerical score from 0 to 10 based on morphology and Gram stain of vaginal flora including Lactobacillus (decrease scored from 0 to 4), Gardnerella
vaginalis (increase scored from 0 to 4), and Mobiluncus (increase scored from 0 to 2) (17). Scores between 7 and 10 were classified as BV, and scores between 4 and 6 were classified as intermediate flora (17). A 7.5% sample of slides was read by ABL, who was also responsible for training the laboratory technicians. The proportion of slides subjected to random quality control decreased over time as technician capability improved. Discordant readings were reassessed, and a consensus score was reached by discussion. The Nugent score was recorded on a data sheet recording the various gram-specific morphotypes of organisms associated with BV, including Lactobacillus, Gardnerella/Bacteroides, and Mobiluncus. The presence of clue cells (vaginal epithelial cells covered with bacteria), yeast, white blood cells, and spermatozoa was also recorded. A slide quality score was also assigned, primarily for feedback to field teams in the event of poorly smeared slides being received in the laboratory.

The polymerase chain reaction swab was stored in M4RT media provided with the Amplicor 5mL Swab Specimen Kit (Roche Molecular Systems Inc). In the laboratory, the swab was gently vortex mixed in the conical tube provided with the kit and then removed from the liquid. The conical tube was centrifuged at 3200 rpm (1315 × g) for 1 min, after which 4 mL supernatant fluid M4RT was discarded. The remaining pellet was resuspended by gentle vortex mixing, and the suspension was transferred to a 1.5-mL cryovial for storage in liquid nitrogen (−196°C).

**Treatment of BV**

Once the laboratory-based diagnoses of BV was available, usually within 2–3 d of swab collection, this information was provided to a project research physician, who checked the identifiers of each woman against a list of identifiers sent from the field of women who had reported abnormal vaginal discharge in the past week. Women found to have symptomatic BV (defined by a Nugent score of 7 to 10 and complaints of abnormal vaginal discharge) were provided 250 mg oral metronidazole 3 times/d for 7 d as per the CDC recommendation (6). The treatment protocol entailed a senior female staff visiting the subject’s home with the treatment and explaining to her the treatment regimen. In addition, women were given a card-based reminder tool consisting of 21 perforated line drawings of tablets on a 7-d grid, distributed into morning, noon, and night quadrants that they pushed out every time they took a dose. At the end of 7 d, project staff visited the women to inquire about adherence to treatment and to do a pill count of remaining tablets, if any, or to collect the
empty blister pack. If there were any remaining tablets, the woman was encouraged to complete her treatment regimen. This protocol was followed for each of the 3 time-point assessments.

Statistics

The prevalence of BV at each of the 3 time periods and incidence rate between the baseline enrollment and third trimester and between the third trimester and 3 mo postpartum were calculated by supplementation group. Incidence proportion was calculated with the numerator defined as new BV cases among women without BV at the previous assessment and the denominator as all women with BV assessment. Differences in baseline characteristics of pregnant women and their households were examined by treatment group by using a chi-square test for categorical variables and a t test for continuous variables to examine baseline comparability. Adherence to the supplementation and BV treatment regimens was also examined by treatment group. Socioeconomic status was compared between supplement groups by using a household living standards index created by a principal components analysis of the entire study data set (18). ORs, RRs, and corresponding 95% CIs were calculated for prevalence rates and incidence proportions of BV, respectively, by using generalized estimating equation logistic regression analysis with a logit and log link, respectively, and assuming an exchangeable correlation structure to adjust for the cluster randomized study design. With the use of the same modeling approach, treatment effects were further adjusted for variables that were significantly different by treatment group as potential confounders. Because the living standards index was a more comprehensive measure of household wealth and was significantly associated with BV status, we adjusted for the living standards index in the multiple regression analysis rather than for individual variables such as television ownership or household electricity that were different by treatment group but were not associated with BV. All analyses were done as intent to treat. The analyses were done by using SAS version 9.1 (SAS Institute Inc).

RESULTS

Since the BV study start-up in August 2003, a total of 1485 consenting pregnant women were approached to contribute a vaginal swab, 7 of whom did not contribute a swab and 16 for.
TABLE 2
Adherence to treatment with metronidazole in women with symptomatic BV, by supplementation group

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin A</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>Accepted treatment (%)</td>
<td>100</td>
<td>90.9</td>
<td>90.6</td>
</tr>
<tr>
<td>Refused treatment (%)</td>
<td>0</td>
<td>0</td>
<td>3.1</td>
</tr>
<tr>
<td>Not met (%)</td>
<td>0</td>
<td>9.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Pills taken (n)</td>
<td>13 ± 8</td>
<td>15 ± 8</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>Third trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Accepted treatment (%)</td>
<td>81.8</td>
<td>100</td>
<td>81.8</td>
</tr>
<tr>
<td>Refused treatment (%)</td>
<td>0</td>
<td>0</td>
<td>9.1</td>
</tr>
<tr>
<td>Not met (%)</td>
<td>18.2</td>
<td>0</td>
<td>9.1</td>
</tr>
<tr>
<td>Pills taken (n)</td>
<td>15.1 ± 8.2</td>
<td>15.8 ± 6.7</td>
<td>13.1 ± 9.4</td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>23</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Accepted treatment (%)</td>
<td>95.6</td>
<td>88.2</td>
<td>95.8</td>
</tr>
<tr>
<td>Refused treatment (%)</td>
<td>0</td>
<td>0</td>
<td>4.2</td>
</tr>
<tr>
<td>Not met (%)</td>
<td>4.4</td>
<td>11.8</td>
<td>0</td>
</tr>
<tr>
<td>Pills taken (n)</td>
<td>17.8 ± 7.1</td>
<td>18.1 ± 6.3</td>
<td>15.4 ± 7.5</td>
</tr>
</tbody>
</table>

1 BV, bacterial vaginosis.
2 Calculated by ANOVA for continuous variables and by chi-square test for categorical variables.
3 Mean ± SD (all such values).

Overall, 453, 461, and 531 Nugent scores were available in the placebo, vitamin A, and β-carotene groups, respectively. No effect of supplementation was found with either intervention on symptomatic BV or mild BV (intermediate score: 4-6) (data not shown). Recurrence of BV (presence of BV at any of the 2 of the 3 times it was assessed) was not affected by vitamin A or β-carotene supplementation, with ~3.5% of prevalent cases

TABLE 3
Prevalence and incidence of BV during pregnancy and 3 mo postpartum, by supplementation group

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin A</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Prevalence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>462</td>
<td>25 (5.6)</td>
<td>—</td>
</tr>
<tr>
<td>Third trimester</td>
<td>336</td>
<td>25 (7.4)</td>
<td>0.84 (0.43, 1.66)</td>
</tr>
<tr>
<td>Postpartum</td>
<td>453</td>
<td>56 (12.4)</td>
<td>0.71 (0.52, 0.98)</td>
</tr>
<tr>
<td>Either</td>
<td>517</td>
<td>77 (14.9)</td>
<td>0.73 (0.53, 1.00)</td>
</tr>
<tr>
<td>Incidence ⁴</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third trimester</td>
<td>262</td>
<td>13 (5.0)</td>
<td>0.63 (0.28, 1.40)</td>
</tr>
<tr>
<td>Postpartum</td>
<td>255</td>
<td>30 (11.8)</td>
<td>0.58 (0.41, 0.81)</td>
</tr>
<tr>
<td>Either</td>
<td>317</td>
<td>43 (13.6)</td>
<td>0.60 (0.42, 0.86)</td>
</tr>
</tbody>
</table>

1 BV, bacterial vaginosis.
2 Generalized estimating equation logistic regression analysis was used to adjust for the design effect.
3 Numerator defined as new cases in those assessed to have a negative result for BV at the previous visit.
4 Denominator defined as new cases in those assessed to have a negative result for BV at the previous visit.

Baseline characteristics of women involved in the study showed few differences by treatment group (Table 1). Maternal age was about 22 y, and gestational age at enrollment was about 11 wk. Significant differences in the proportion of Muslims, membership in a local social welfare/microcredit nongovernmental organization, television ownership, electricity in the home, and percentage adherence to nutrient supplementation were observed between treatment groups. The prevalences of BV at enrollment were 5.6%, 7.9%, and 9.0% in the placebo, vitamin A, and β-carotene groups, respectively (P = 0.12; Table 1). Although most of the symptomatic patients with BV who were offered metronidazole treatment accepted it, the mean number of tablets was lower than the prescribed count of 21 tablets at each time point, but this difference was not significant between treatment groups (Table 2). Vitamin A deficiency assessed on the basis of a plasma retinol concentration <0.70 μmol/L was 14.2% in the third trimester in the placebo group; vitamin A supplementation significantly reduced this prevalence to 1.6% (15).

In the third trimester, the prevalence and incidence rates of BV in the vitamin A and β-carotene supplementation groups were not significantly different from those of the placebo group (Table 3). By 3 mo postpartum, prevalence and incidence were significantly lower among women in the vitamin A group than in those in the placebo group. The OR (95% CI) for the reduction in prevalence was 0.71 (0.52, 0.98) and the RR (95% CI) for the reduction in the incidence proportion was 0.58 (0.41, 0.81). Although lower, neither prevalence nor incidence at 3 mo postpartum was statistically significantly different between the β-carotene and placebo groups. Both vitamin A and β-carotene reduced the prevalence and incidence of BV at either time point by 30–40% when compared with the experience of the placebo group. The effect remained significant after adjustment for potential confounders found to be different at baseline, more clearly for vitamin A than for β-carotene (Table 4). No effect of supplementation was found with either intervention on symptomatic BV or mild BV (intermediate score: 4-6) (data not shown). Recurrence of BV (presence of BV at any of the 2 of the 3 times it was assessed) was not affected by vitamin A or β-carotene supplementation, with ~3.5% of prevalent cases...
DISCUSSION

In this rural setting in Bangladesh, we found that routine weekly supplementation of women with preformed vitamin A—or, to some extent, provitamin A β-carotene—reduced the prevalence and incidence of BV during pregnancy and the postpartum period. The prevalence rate of BV differed by stage of pregnancy and lactation. At baseline, before supplementation, the prevalence of BV in the first trimester was 7.6% (95% CI: 6.3, 9.1) overall. The prevalence of BV in the placebo group increased to 7.4% in the third trimester and to 12.4% at 3 mo postpartum. β-Carotene was less efficacious in reducing the risk of BV than was vitamin A, perhaps because of its lower bioefficacy related to inadequate conversion of β-carotene to retinol.

Our study used a double-masked randomized, controlled design to demonstrate that a weekly supplement of vitamin A, for which adherence was high, was efficacious in lowering the prevalence and incidence of BV during pregnancy through 3 mo postpartum. We know of no other study that has shown this previously, although several studies have observed an association between higher dietary intake or circulating concentrations of specific nutrients and decreased the risk of BV largely among US women and adolescents.

One study of African American women showed no difference between women with and without genital tract infections (including *G. vaginalis*) with regard to daily consumption of vitamin A, vitamin C, iron, and protein (19). However, mean intake of vitamin A, vitamin C, and protein was ≥60% above the recommended daily allowance, and *G. vaginalis* was detected through culture, which has been shown to be positive in ≤55% of colonized, noninfected women and is therefore an unreliable marker for infection. Another study of women recruited in a sexually transmitted disease clinic in the Central African Republic found no difference in vitamin A concentrations between women with and without BV; however, the women’s mean vitamin A concentration was within the normal range, which indicated that vitamin A deficiency was not a significant problem (13). A biologically plausible relation exists between nutrition and vitamin A deficiency and an increased risk of developing BV. BV is characterized by increased bacterial adherence to vaginal epithelial cells. Adequate vitamin A is necessary for genital tract mucosal integrity, because it affects the differentiation of the ectocervical epithelium (20, 21). Additionally, vitamin A deficiency is known to reduce epithelial barriers and host immune mechanisms, which leads to an increase in infections along epithelial surfaces such as the conjunctiva and respiratory, gastrointestinal, and genitourinary tracts (22). Vitamin A deficiency might increase the susceptibility to BV by reducing either epithelial integrity or host immune response to infection, or both, particularly during a nutritionally sensitive period such as pregnancy. It is also unclear whether vitamin A deficiency may disrupt the stability of the vaginal microbiome or whether supplementation contributes to the stabilization of healthy flora, dominated by *Lactobacillus* species. Further research is needed to determine the mechanism by which vitamin A deficiency can increase the risk of BV among pregnant and postpartum women.

Our study design, in which supplementation started in the first trimester and BV was assessed at baseline and at ~32 wk of gestation, cannot answer the question of whether an enhancement of vitamin A status can improve birth outcomes, such as preterm birth attributable to a reduction in the risk of BV. Studies have found that asymptomatic BV is associated with an increased risk of preterm birth when BV is detected before 16 wk of gestation (7), and BV treatment during pregnancy reduces the risk of preterm birth only when treatment is provided before 20 wk of gestation (8). Our study showed a reduction in the prevalence and incidence of BV in the late trimester and after delivery. To affect birth outcomes via reductions in the risk of BV, any nutritional intervention strategy would need to target an improvement in maternal status before and in the first half of pregnancy. Thus, in many low-resource settings, pregnancy detection or reporting does not occur until well into the second trimester, which leaves a narrow window of opportunity for any nutritional intervention to have an effect.

Our study had several strengths and few limitations. The randomized, controlled design of the study allowed us to examine
the causal relation between vitamin A (and β-carotene) supplementation and risk of prevalent or incident BV in rural Bangladesh. High rates of compliance with swab collection, high adherence with supplementation, and low loss to follow-up make the findings generalizable to similar South Asian contexts. However, the sample size of the study was driven by the number of individuals required to see differences in serum retinol concentrations of ≥0.25 SD between treatment groups and to account for a higher attrition among women in the third trimester; this resulted in low power (<30%) to observe reductions of ~20–30% in BV in the third trimester. Additionally, we did not find vitamin A or β-carotene to be effective at reducing the recurrence of BV, which was shown to be low (3.5%) in those treated with metronidazole, again perhaps because the study lacked sufficient power.

In conclusion, in a vitamin A–deficient setting, our study showed a beneficial effect of weekly antenatal supplementation with vitamin A in reducing the risk of BV during pregnancy and the postpartum period among rural Bangladeshi women. To affect birth outcomes, future studies of nutritional interventions may need to consider strategies for enhancing vitamin A status among women in first 16–20 wk of pregnancy.

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The authors’ responsibilities were as follows—PC: conceived the study and the study design, developed the protocol and procedures, provided oversight for study implementation, analyzed the data, interpreted the results, wrote the manuscript, and had primary responsibility for the final content; ABL (lead co-investigator): helped develop the study design and protocol, provided implementation oversight and staff training, provided quality control of the slides and comments, and edited the manuscript; HA (substudy coordinator and senior research physician): oversaw all aspects of data collection and quality control of the study and edited the manuscript; MJR: participated in forms development, study design, staff training, literature review, and development of procedures and treatment protocol and edited the manuscript; LW: helped with data management and statistical analysis and provided comments on the manuscript; MR (senior in-country investigator): provided management and scientific oversight for the larger trial and its substudies, provided clinical guidance during study implementation, and edited the manuscript; and KPW (principal investigator of the parent trial): provided comments and edited the manuscript. The authors had no conflict of interest to declare. Funding agencies had no role in the design or implementation of the study or in the analysis and interpretation of the data or in the preparation of the manuscript.

REFERENCES