Treatment response to standard of care for severe anemia in pregnant women and effect of multivitamins and enhanced anthelminthics

Parul Christian, Farhana Shahid, Arjumand Rizvi, Rolf DW Klemm, and Zulfiqar A Bhutta

ABSTRACT

Background: Severe anemia (hemoglobin <70 g/L) in pregnancy may increase the risk of maternal and perinatal mortality.

Objectives: We assessed response to standard treatment with high-dose iron–folic acid for 90 d and single-dose (500 mg) mebendazole among severely anemic pregnant women in periurban Karachi, Pakistan. In addition, we evaluated the efficacy of 2 enhanced treatment regimens.

Design: We screened pregnant women (n = 6288) for severe anemia and provided them all with the standard treatment. To test the efficacy of 2 additional treatments, women were randomly assigned to standard treatment alone (control) or with 100 mg mebendazole twice daily for 3 d or 90 d of daily multivitamins or both using a 2 × 2 factorial design.

Results: Prevalence of severe anemia was high (10.5%) during pregnancy. Prevalence of geohelminths and malaria was low. Treatment response was defined as hemoglobin ≥100 g/L at the 90-d or ≥25 g/L at the 60-d follow-up visit. The standard-of-care treatment resulted in a response rate of 49% at follow-up, although an adherence of ≥85% elicited a higher response (67%). The effect of the additional treatments was weak. Although response was higher in the enhanced groups than for the standard treatment at the final assessment, the differences were not statistically significant. However, hemoglobin concentration increased significantly in all groups and was higher in the enhanced mebendazole group compared with the standard group (P < 0.05).

Conclusions: Iron deficiency was high in this population, and the standard-of-care treatment alone (control) or with 100 mg mebendazole twice daily for 3 d or 90 d of daily multivitamins or both using a 2 × 2 factorial design.

INTRODUCTION

Anemia continues to be a public health problem of global proportions. Severe anemia (hemoglobin <70 g/L) is of particular concern because it poses a significant health and mortality risk. Pregnant women and young children (6–24 mo of age) are the 2 groups at highest risk. Severe anemia in pregnant women is associated with an elevated risk of maternal and perinatal mortality (1, 2) and an increased risk of low birth weight and preterm birth by 3–5-fold (3). Furthermore, maternal iron status is associated with fetal and infant iron stores and incidence of anemia in infancy (4). Antenatal iron-folic acid supplementation reduces low birth weight and preterm in both developed and developing country settings (5–7).

Prevalence rates of anemia (hemoglobin <110 g/L) among pregnant women, currently ranging between 40% and 50% in South Asia, continue to be among the highest in the world (8). Prevalence rates of severe anemia are the highest in sub-Saharan Africa followed by South Asia, although in some populations in South Asia, such as Nepal, prevalence of severe anemia in the third trimester has been recorded at ~9% (9). An important cause of anemia is Plasmodium falciparum infection that contributes to the incidence and severity of anemia in regions of the world where it is endemic. Iron deficiency may largely contribute to anemia in South Asia where diets are predominantly cereal based, low in animal products, and high in foods that inhibit iron absorption such as phytate, fiber, and tannins (10). In addition to iron, deficiency of vitamins such as folic acid, vitamin A, vitamin C, riboflavin, and vitamin E can also inhibit erythropoiesis (11). Controlled trials of malaria chemoprophylaxis were shown to increase hemoglobin and reduce severe anemia in pregnant women (12, 13). Geohelminths, especially hookworm, are also an important contributor of iron deficiency anemia.

The current international recommendation for treatment of severe anemia in pregnant women is daily high-dose iron (120 mg) and folic acid (400 μg) for 3 mo (14). Where P. falciparum malaria is endemic, antimalarial treatment is recommended.

1 From the Center for Human Nutrition, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD (PC and RDWK), and the Department of Pediatrics, Aga Khan University, Karachi, Pakistan (FS, AR, and ZAB).

2 Supported by the Thrasher Research Fund, Salt Lake City, UT, the Bill and Melinda Gates Foundation, Seattle, WA, and the Sight and Life Research Institute at the Johns Hopkins School of Public Health. Multivitamin supplements for the women were generously supplied by Nutrilite, Access Business Group, Buena Park, CA. Iron-folic acid supplements were generously supplied by the Government of Pakistan’s National Programme for Family Planning and Primary Health Care. The mebendazole formulations were generously supplied in blister packages by GlaxoSmithKline, Karachi, Pakistan.

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Received August 12, 2008. Accepted for publication December 9, 2008. First published online January 28, 2009; doi: 10.3945/ajcn.2008.26826.
Where hookworm is prevalent (20–30%), anthelminthic treatment is recommended in the second and third trimesters of pregnancy. The recommended single-dose (500 mg) benzimidazole compound, however, was found to have a low efficacy for treating hookworm (15).

Few studies have examined the effectiveness of the current treatment recommendations for severe anemia, neither has the efficacy of a different regimen of anthelmintics nor the additional benefit of multivitamin supplementation been tested. Thus, the primary objective of the study was to evaluate the effectiveness of the current standard of care and test the efficacy of an alternative dose of mebendazole and daily multivitamins in the treatment of severe anemia in pregnant women in a periurban, poor population of Karachi, Pakistan.

SUBJECTS AND METHODS

Study design and population

To test the effectiveness of the standard of care (iron-folic acid and single-dose mebendazole) for treatment of severe anemia, we examined treatment response at the end of the treatment period. To test the enhanced regimens a 2 × 2 factorial, randomized, controlled study design was used. The 4 treatment groups were as follows: group A received iron-folic acid, mebendazole 500 mg (single dose), and placebo; group B received iron-folic acid, mebendazole 100 mg twice daily for 3 d, and placebo; group C received iron-folic acid, mebendazole 500 mg (single dose), and multivitamins; and group D received iron-folic acid, mebendazole 100 mg twice daily for 3 d, and multivitamins.

Group A represents the standard-of-care control. We hypothesized that compared with the standard of care, mebendazole at 100 mg twice daily for 3 d (improved anthelminthic treatment) or daily multivitamins for 3 mo would result in a response rate higher by ≥25%.

The study was conducted in 5 sites of periurban Karachi called Ibrahim Hyderi, Ali Akhbar Shah Goth, Rehri Goth, Bilal Colony, and Lalabad. Three of these sites were fishing populations and located on the seashore. The study was conducted between April 2004 and May 2007. Women and children residing in these periurban areas harbor high rates of undernutrition and infection and were previously included in studies conducted by researchers at the Aga Khan University. These populations undergo a regular census and household updates, including enumeration of migrations of families in and out of the settlements.

Study interventions

Iron and folic acid

The iron-folic acid supplements for pregnant women used in the trial were antenatal supplements containing 50 mg of elemental iron in the form of ferrous fumarate and 0.5 mg folic acid being used by the Pakistan’s National Program for Family Planning and Primary Health Care. To provide the treatment dosage for severe anemia of 120 mg iron, women were given and asked to take 2 tablets each day. The total daily dosage of iron (100 mg) that women received in the study was therefore slightly lower than the recommended 120 mg. Because each tablet also contained 0.5 mg of folic acid, women received twice that amount daily.

Anthelminthic treatment

Anthelminthic treatment consisted of 6 doses (to be taken twice a day for 3 d) of 100-mg mebendazole tablets packaged in a blister pack. The control standard-of-care blister pack consisted of one 500-mg mebendazole tablet and 5 tablets of the same shape, size, and color being inactive. This allowed for the investigators, field staff, and participants to remain masked to whether women received the single-dose regimen or the 3-d regimen. The manufacturers were instructed to number each dose from 1 through 6, and the tablet marked 1 was to be the active 500-mg tablet. The blister packs were labeled with unique treatment codes assigned by the manufacturer and kept in sealed envelopes in Baltimore and Karachi.

Multivitamin supplements

The multivitamin arm of the trial had a placebo control. Thus, multivitamin and placebo supplements for pregnant women were colored red and identical in shape, size, and color. Bottles coded 1 and 2 corresponding to one of the 2 supplement types were assigned by the manufacturer and kept in sealed envelopes in Baltimore and by the manufacturers. The vitamins included were vitamins A (770 μg retinol equivalents), E (15 mg tocopherol equivalents), B-12 (2.6 μg), C (85 mg), B-2 (1.4 mg), each at a US Institute of Medicine Recommended Daily Allowance for pregnancy. These amounts were used in the absence of local recommendations or evidence for alternative dosages that would be effective.

Screening and enrollment

All women of reproductive age were enumerated at the time of the census in each of the study sites and were visited every 3–4 mo to ascertain pregnancy and conduct anemia screening for identifying cases of severe anemia (hemoglobin < 70 g/L). The date of the last menstrual period among pregnant women was assessed with the use of recall. Pregnant women whose gestational age of pregnancy was ≥20 wk underwent hemoglobin testing on the spot to screen for severe anemia. Pregnant women who were <20 wk of gestation were scheduled for a hemoglobin test only after they had reached 20 wk of gestation. This was done for 2 reasons: 1) because gestational age is a strong determinant of hemoglobin concentration in pregnancy, uniformity of gestational age at enrollment would help reduce the variation in this measurement; and 2) severe anemia is more likely to occur in the latter part of pregnancy; thus, assessing hemoglobin too early in pregnancy was not likely to yield many cases of severe anemia. A finger stick blood draw was conducted to assess hemoglobin concentration with the use of a HemoCue machine (HemoCue Inc, Mission Viejo, CA). Enrollment of severely anemic pregnant women was accomplished over a period of 2.5 y (April 2004 to December 2006) during ten 3–4-mo cycles of screening for new pregnancies and anemia. After the initial screening, severely anemic women were invited to the study clinic (one located in each site) on the following day. Moderately and mildly anemic women identified during anemia screening received the government iron-folic acid tablets and were asked to take 1 tablet/d.
Enrollment clinic visit

At the clinic, severely anemic pregnant women underwent a clinical assessment by the study medical officer. No lower limit of hemoglobin was used for exclusions. However, subjects were excluded if they presented with one or more of the following conditions: labored breathing, edema, or a severe illness symptom according to the international guidelines (14). Those subjects were referred to and assisted to reach and receive treatment at a hospital. Severely anemic women with a gestational age of ≥36 wk were also not eligible for enrollment and were provided with iron-folic acid treatment if they did not have any clinical symptoms or a referral to a hospital if found to have one of the above-mentioned clinical symptoms. Eligible women were enrolled in the study after obtaining written consent. Consenting women were randomly assigned, in blocks of 4, to 1 of the 4 treatment arms of the study. A list containing 800 numbers, ranging from 1 to 4, and generated in randomly arranged blocks of 4, was created. Each of the numbers 1–4 was randomly associated with the mebendazole (codes M and U) and multivitamin (codes 1 and 2) treatment codes.

Another finger stick capillary blood collection was done for malaria testing, and dried blood spot (DBS) collection on filter paper was done for retinol and protein analysis. Millipore collection cards (no. 903; Schleicher and Schuell, Keene, NH) were spotted with capillary whole blood from the finger, dried overnight at ambient temperatures, placed in individual envelopes, packed in a zip-closure bag with loose silica desiccant packages, transported to the Aga Khan University laboratory on the day after the collection, and stored at −20°C freezers until they were analyzed later. Women were also asked to bring a sample of their morning stool specimen to test for helminths. For those women who were found to have malarial parasitemia, treatment was provided within a week of receiving the laboratory results. Women who were positive for *P. falciparum* were treated with sulfadoxine pyrimethamine, and women with *Plasmodium vivax* were given chloroquine according to the local guidelines.

A questionnaire was administered at the time of the clinic visit to assess household socioeconomic status; previous pregnancy history; self-reported history of morbidity symptoms in the past 30 d; a 7-d frequency of consumption of food, including those that were rich in iron or were known to inhibit or promote iron absorption; use of tobacco, alcohol, and other substances; a 7-d work activity history; and antenatal care received during pregnancy. Women’s weight and height were also measured at this time with the use of standard anthropometric methods. Weight was taken on an adult weighing scale to the nearest 0.1 kg, and height was measured using an adult height board.

Follow-up visits

According to the study protocol and international guidelines (14), hemoglobin among participants was again assessed at 7 and 28 d of treatment to identify and refer nonresponders, defined as subjects whose 7-d hemoglobin concentration was 3 g/L less than their baseline concentration or whose hemoglobin had not improved by ≥3 g/L by the 28-d assessment. After the 28-d visit women were tested for hemoglobin at ~60 and 90 d after enrollment and treatment. Although the 60-d visit was done in the home, the final visit was conducted at the clinic and also involved an endpoint interview, anthropometric measurements, and blood spot collection on filter paper. The home-based visits and tests were performed by a team of trained phlebotomists, whereas the research physicians conducted all the clinic-based assessments. Stool specimens were collected at 28 d and at the final clinic visit. Among women who delivered before the end of the 90-d treatment, hemoglobin measured at the visit before the delivery was used for evaluating treatment effects.

Monitoring treatment adherence

The first of the 6 doses of mebendazole was administered immediately after randomization and during the baseline clinic visit. Because the tablet marked “1” was always the active one in the standard mebendazole group, the single-dose regimen and the first of the 100-mg dose regimen was always observed being taken by clinic staff. Participants were instructed to take the other dose later that day and 2 tablets/d for the remaining 2 d. Study staff visited women on those 2 d to assess compliance. At enrollment, women received a bottle containing 15 (of the multivitamin or placebo) tablets and were asked to take one tablet every day for the next 90 d. Similarly, women were given 30 iron-folic tablets at the outset and asked to consume 2 tablets/d. Thereafter, weekly visits were conducted by local female workers to monitor treatment adherence, record the number of both types of supplements consumed, and replenish the consumed supplements.

Laboratory analyses

Malaria testing was done with routine microscopy with the use of thick-and-thin blood smears fixed with ethanol and stained with Giemsa and read by light microscope with ×100 oil immersion lens. The number of malaria parasites (parasite density) was counted against leukocytes with the use of the thick smear, and identification of species was done with the use of the thin smear. The method for examining stool samples was the Kato-Katz cellophane quantitative thick smear technique described by Ash et al (16). *Ascaris lumbricoides*, *Ancylostoma duodenale–Necator americanus* (hookworm), and *Trichuris trichiura* eggs were counted and expressed as eggs per gram of fecal sample. DBS retinol analysis was performed at the micronutrient laboratory at the Aga Khan University with the use of a modified reverse-phase HPLC (series 200; Perkin-Elmer, Waltham, MA) after a simple extraction method described by Erhardt et al (17). A limited number (because of cost and sample constraints) of DBS samples were sent to Germany where they were analyzed later that day and 2 tablets/d for the remaining 2 d. Study staff visited women on those 2 d to assess compliance. At enrollment, women received a bottle containing 15 (of the multivitamin or placebo) tablets and were asked to take one tablet every day for the next 90 d. Similarly, women were given 30 iron-folic tablets at the outset and asked to consume 2 tablets/d. Thereafter, weekly visits were conducted by local female workers to monitor treatment adherence, record the number of both types of supplements consumed, and replenish the consumed supplements.

Outcome

No appropriate definitions of treatment response for severe anemia are available in the literature. Our a priori definition of treatment response was an increase in hemoglobin concentration by 25 g/L or hemoglobin >100 g/L at follow-up. Treatment response was examined at both the 60- and 90-d follow-up visits. Previously, daily dosages of iron of ≥120 mg were shown to increase hemoglobin concentration by 17–18 g/L in pregnant women.
women (19). Thus, we assumed a change of 25 g hemoglobin/L would likely occur, given the much lower baseline hemoglobin concentrations of <70 g/L in the study women. Especially in cases in which women were more advanced in gestation at entry in the trial, we expected that they would not be followed through 90 d of treatment before birth outcome and would be unable to achieve hemoglobin ≥100 g/L because of the shorter treatment duration. Thus, for such cases we considered any increase in hemoglobin >25 g/L to be an adequate treatment response. When hemoglobin was available for the 90-d follow-up visit, treatment response was defined as hemoglobin concentration ≥100 g/L.

Sample size
The sample size calculations were done with the use of treatment response as the outcome and a 2-sided test with α = 0.05 and β = 0.20. The probability of treatment success with standard of care of iron-folic acid (and single-dose mebendazole) was estimated to be 50%, because half of all cases of anemia globally are attributable to iron deficiency (20). We considered a 25% higher response rate (absolute response rate of 62%) in the enhanced treatment group to be of public health importance and likely to influence policy decisions about treatment of severe anemia. With these assumptions, and a loss to follow-up of 20%, the sample size required was 300 per group or a total of 600. A total of ≈4000 pregnant women were required to achieve this sample size of severely anemic women, assuming severe anemia prevalence of ≈12% in the study area. The 20% loss to follow-up accounts for losses because of miscarriages, stillbirths, out-migrations, and refusals.

Statistical analysis
Characteristics of women at enrollment were compared across the treatment groups. Adherence to treatment was assessed by calculating the proportion of all doses taken for the iron-folic acid and multivitamin tablets as well as for mebendazole. Analysis was done by intention to treat. Effectiveness of the standard of care was assessed by examining the treatment response in the control group and all the groups combined. Because mean adherence to iron-folic acid was low, treatment effectiveness was examined stratified on level of adherence. Treatment failure or nonresponse was defined according to the international guidelines (14) and assessed for each of the treatment groups at 7 and 28 d after treatment initiation. Because no interaction was found between the 2 enhanced treatments, we analyzed them separately with the use of logistic regression analysis to test for main effects. Linear mixed effects regression analyses were done separately for each type of treatment to examine the change in hemoglobin concentration over the duration of treatment (21). This method allows accounting for the within-subject correlation of repeated measurements in estimating the regression variables and their SEs. All analyses were conducted with the use of SAS version 9.1 (SAS Institute, Cary, NC).

Ethical approval
The study was approved by the institutional review boards at Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, and the Aga Khan University, Karachi, Pakistan, and annually reapproved at both institutions thereafter. Informed consent was obtained by reading the consent statements to women, and their signatures or thumb prints were obtained as written consent. Women were not compensated for participating in the study. A Data Monitoring and Safety Board was formed and met before the beginning of the study and then once every year throughout the study duration.

RESULTS

Study participation and follow-up
Over the course of 2.5 y, a total of 18,762 women of reproductive age were visited for pregnancy surveillance followed by screening for severe anemia of 6288 consenting pregnant women (Figure 1). The prevalence of severe anemia among pregnant women was 10.5% (n = 663). Of 600 women eligible for enrollment, 547 (91.2%) were enrolled and completed an enrollment interview. Approximately 270 women were randomly assigned to each of the 2 enhanced treatment arms of the trial. Of these women, 512 (94%), 449 (82%), 374 (68%), and 295 (54%) were available for the 7-, 28-, 60-, and 90-d assessments (data not shown). Attrition over the course of the 90-d treatment was due to refusal for blood draw, ranging from 3% to 12% at various visits; birth outcome, including miscarriage, stillbirth, or live birth, ranging from 1% to 25% from the 7-d to the 90-d visit; not meeting the woman, ranging from 2% to 13%; and some permanent migrations (<1%). A total of 393 women (71.8%) of the total enrolled had at least one 60- or 90-d hemoglobin assessment after treatment.

FIGURE 1. Pregnancy and severe anemia surveillance and enrollment in the study. *Includes labored breathing, edema, or a severe illness symptom.
Baseline characteristics

At baseline, characteristics of the enrolled subjects did not differ by treatment group except parity (Table 1). Women were \( \approx 27 \) y of age and \( \geq 50\% \) had a parity of \( \geq 4 \). The mean gestational age at enrollment was 23 wk. Literacy was low, ranging between 12\% and 15\%, and >85\% of women had not received any schooling. A large majority had consumed fish and tea at least once in the past week, whereas consumption of citrus fruits and dark green leafy vegetables was less common. Meat and poultry were consumed at least once in the past week by 50–60\% of the women. The mean weight and body mass index (in kg/m\(^2\)) of the women was \( \approx 51 \) kg and 22, respectively.

Baseline prevalence of geohelminths, malaria, subclinical infection, and nutritional deficiency

At enrollment only a small proportion of women had any geohelminth infections as diagnosed by examination of eggs in the stool specimen (Table 2). A difference was observed in the rates of those women infected by treatment group (13.7\% in the standard group compared with the enhanced mebendazole group 3.9\%; \( P < 0.001 \)). The predominant helminth species was \( A. lumbricoides \) and was found in \( \approx 80\% \) of all the positive cases. Hookworm and \( T. trichiura \) were less common. Malarial parasitemia was low at 4–5\%, with the most resulting from \( P. falciparum \).

Baseline prevalence of elevated \( z_1 \)-acid glycoprotein (>1 g/L) was low across all treatment groups, although prevalence of elevated CRP (>5 mg/L) was high and significantly higher in the standard group than in the enhanced mebendazole group (32.6\% compared with 17.3\%; \( P < 0.05 \)). However, the prevalence did not differ between the no multivitamin group and the multivitamin group (29.6\% compared with 20.9\%; \( P = 0.20 \)). DBS analysis for retinol, retinol-binding protein, and TIR showed concentrations that did not differ by treatment group. A large proportion of women, ranging between 68\% and 75\%, had high DBS TIR (\( \geq 9 \) mg/L), indicating iron deficiency. The prevalence of low DBS retinol concentration (<0.7 \( \mu \)mol/L) was also high.

The median adherence to treatment of daily iron-folic acid and the multivitamin supplement was low across the treatment groups, ranging from 64\% to 72\% (data not shown). Median adherence to treatment of mebendazole was high at \( \geq 95\% \). Adherence to all 3 treatments did not differ by treatment group.

Effectiveness of standard of care

Treatment response in the control group receiving the standard of care was 49\% and varied by adherence to iron-folic acid treatment (\( P < 0.05 \)). The treatment response in all groups combined was 56\% and also varied by treatment adherence to iron-folic acid (\( P < 0.001 \); Table 3). Changing the definition with cutoffs of \( \geq 10 \), \( \geq 20 \), and \( \geq 30 \) g/L for treatment response at

### TABLE 1

Baseline characteristics of severely anemic pregnant women enrolled in the study

<table>
<thead>
<tr>
<th></th>
<th>Standard mebendazole ( (n = 275) )</th>
<th>Enhanced mebendazole ( (n = 272) )</th>
<th>No multivitamin ( (n = 273) )</th>
<th>Multivitamin ( (n = 274) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>( 27.2 \pm 6.2 )</td>
<td>( 26.9 \pm 5.8 )</td>
<td>( 27.2 \pm 5.9 )</td>
<td>( 26.9 \pm 6.1 )</td>
</tr>
<tr>
<td><strong>Gestational age (wk)</strong></td>
<td>( 23.2 \pm 4.9 )</td>
<td>( 23.9 \pm 4.8 )</td>
<td>( 23.5 \pm 5.1 )</td>
<td>( 23.5 \pm 4.7 )</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>( 51.5 \pm 9.6 )</td>
<td>( 51.0 \pm 8.3 )</td>
<td>( 51.1 \pm 9.3 )</td>
<td>( 51.1 \pm 8.8 )</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>( 153.3 \pm 5.9 )</td>
<td>( 153.2 \pm 6.3 )</td>
<td>( 153.2 \pm 6.5 )</td>
<td>( 153.3 \pm 5.6 )</td>
</tr>
<tr>
<td><strong>MUAC (cm)</strong></td>
<td>( 24.4 \pm 2.8 )</td>
<td>( 23.9 \pm 2.6 )</td>
<td>( 24.2 \pm 2.6 )</td>
<td>( 24.2 \pm 2.8 )</td>
</tr>
<tr>
<td><strong>BMI (kg/m(^2))</strong></td>
<td>( 21.9 \pm 3.6 )</td>
<td>( 21.7 \pm 3.2 )</td>
<td>( 21.7 \pm 3.4 )</td>
<td>( 21.8 \pm 3.4 )</td>
</tr>
<tr>
<td><strong>Parity [n (%)]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>( 26 (9.6) )</td>
<td>( 17 (6.5) )</td>
<td>( 19 (7.1) )</td>
<td>( 24 (9.0) )</td>
</tr>
<tr>
<td>1–3</td>
<td>( 104 (38.4) )</td>
<td>( 129 (49.2) )</td>
<td>( 126 (47.2) )</td>
<td>( 107 (40.2) )</td>
</tr>
<tr>
<td>( \geq 4 )</td>
<td>( 141 (52.0) )</td>
<td>( 116 (44.3) )</td>
<td>( 122 (45.7) )</td>
<td>( 135 (50.8) )</td>
</tr>
<tr>
<td><strong>Hemoglobin [n (%)]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 g/L</td>
<td>( 34 (12.4) )</td>
<td>( 29 (10.7) )</td>
<td>( 34 (12.4) )</td>
<td>( 29 (10.6) )</td>
</tr>
<tr>
<td>50–59 g/L</td>
<td>( 46 (16.7) )</td>
<td>( 52 (19.1) )</td>
<td>( 36 (13.2) )</td>
<td>( 62 (22.6) )</td>
</tr>
<tr>
<td>60–69 g/L</td>
<td>( 195 (70.9) )</td>
<td>( 191 (70.2) )</td>
<td>( 203 (74.4) )</td>
<td>( 183 (66.8) )</td>
</tr>
<tr>
<td><strong>Diet in past week [n (%)]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat or poultry</td>
<td>( 169 (61.5) )</td>
<td>( 153 (56.2) )</td>
<td>( 168 (61.5) )</td>
<td>( 154 (56.2) )</td>
</tr>
<tr>
<td>Fish</td>
<td>( 221 (79.0) )</td>
<td>( 215 (80.4) )</td>
<td>( 217 (79.5) )</td>
<td>( 219 (79.9) )</td>
</tr>
<tr>
<td>Milk or milk products</td>
<td>( 152 (55.9) )</td>
<td>( 156 (56.7) )</td>
<td>( 155 (56.8) )</td>
<td>( 153 (55.8) )</td>
</tr>
<tr>
<td>Dark green vegetables</td>
<td>( 129 (46.9) )</td>
<td>( 121 (44.5) )</td>
<td>( 119 (43.6) )</td>
<td>( 131 (47.8) )</td>
</tr>
<tr>
<td>Tea</td>
<td>( 248 (90.2) )</td>
<td>( 247 (90.8) )</td>
<td>( 249 (91.2) )</td>
<td>( 246 (89.8) )</td>
</tr>
<tr>
<td>Citrus fruits</td>
<td>( 71 (25.8) )</td>
<td>( 60 (22.1) )</td>
<td>( 68 (25.0) )</td>
<td>( 63 (23.0) )</td>
</tr>
<tr>
<td><strong>Schooling [n (%)]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never attended school</td>
<td>( 218 (87.6) )</td>
<td>( 208 (84.9) )</td>
<td>( 211 (85.1) )</td>
<td>( 215 (87.4) )</td>
</tr>
<tr>
<td>1–9 y</td>
<td>( 25 (10.0) )</td>
<td>( 32 (13.1) )</td>
<td>( 29 (11.7) )</td>
<td>( 28 (11.4) )</td>
</tr>
<tr>
<td>( \geq 10 )</td>
<td>( 6 (2.4) )</td>
<td>( 5 (2.0) )</td>
<td>( 8 (3.2) )</td>
<td>( 3 (1.2) )</td>
</tr>
</tbody>
</table>

1. MUAC, midupper arm circumference.
2. Mean ± SD (all such values).
3. \( P < 0.05 \) (chi-square test).
the 60-d assessment yielded response rates of 68.1%, 54.3%, and 47.9%, respectively, in the control group (data not shown). Nonresponse at 7 and 28 d after treatment was 11.5% and 23.6%, and 47.9%, respectively, in the control group (data not shown).

**Treatment response to enhanced regimens**

Treatments with multivitamins but not enhanced mebendazole significantly increased treatment response at the first visit after treatment (relative risk: 1.64; 95% CI: 1.07, 2.51) which occurred at a median time of 57 d (interquartile range: 7 d) (Table 5). Neither enhanced treatment had a significant effect on treatment response over and above the standard of care at the second visit that occurred at a median time of 85 d (interquartile range: 8 d). Response rate at either visit as a result of each of the 2 enhanced treatments was 25–35% higher but not statistically significant.

**Change in hemoglobin concentration with the use of linear mixed effects regression model** showed a significant increase over the course of treatment in all groups; the increase was significantly higher ($P < 0.05$) in the enhanced mebendazole group than in the standard mebendazole group but did not differ by multivitamin supplementation group (Table 6).

**TABLE 4**

Nonresponse to treatment of severe anemia among pregnant women by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Standard mebendazole</th>
<th>Enhanced mebendazole</th>
<th>No multivitamin</th>
<th>Multivitamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 7 d after treatment</td>
<td>254 (9.4)</td>
<td>258 (5.4)</td>
<td>248 (8.9)</td>
<td>132 (6.1)</td>
</tr>
<tr>
<td>At 28 d after treatment</td>
<td>225 (18.2)</td>
<td>224 (14.7)</td>
<td>222 (18.0)</td>
<td>227 (15.0)</td>
</tr>
</tbody>
</table>

$1$ Nonresponse defined as a hemoglobin decrease of 3 g/L from baseline.

$2$ All values are $n$; percentages are in parentheses. Chi-square test was NS between each treatment group.

**TABLE 3**

Effectiveness of iron–folic acid as standard of care in the treatment of severe anemia in pregnant women

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 94)</th>
<th>All groups combined (n = 393)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Treatment response$^1$</td>
<td>48.9</td>
<td>56.0</td>
</tr>
<tr>
<td>Compliance to iron-folic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;$65%</td>
<td>26.3$^2$</td>
<td>40.1$^2$</td>
</tr>
<tr>
<td>65–85%</td>
<td>63.6</td>
<td>65.0</td>
</tr>
<tr>
<td>$&gt;$85%</td>
<td>65.2</td>
<td>67.3</td>
</tr>
</tbody>
</table>

$1$ Defined as an increase of $\geq 25$ g hemoglobin/L from baseline at visit 1 or hemoglobin $\geq 100$ g/L at visit 2.

$2$ $P < 0.005$ (chi-square test).
TREATMENT OF SEVERE ANEMIA AMONG PREGNANT WOMEN

DISCUSSION

In this setting in periurban Karachi, the prevalence of severe anemia in pregnancy was high at 10% and comparable to prevalence rates found in sub-Saharan Africa where malaria is endemic. It is commonly recognized that the cause of severe anemia is multifactorial, involving causes such as malaria; helminthic infections, including hookworm; chronic infection; and other vitamin deficiencies. However, we found a low burden of both malaria and hookworm in this population of pregnant women although vitamin A deficiency and other infections were high. This is one of the first studies to evaluate the effectiveness of the current recommendation of treatment of severe anemia among pregnant women (14). The effectiveness of a daily large dose of iron-folic acid was >50% and was strongly associated with adherence to the iron-folic acid regimen, which was low on average despite the controlled research study environment. High rates of iron deficiency at baseline and significant and large increases in the concentration of TTR as a result of the iron-folic acid treatment suggest iron deficiency to be the predominant cause of anemia in this setting. Also note the high consumption of tea, a known inhibitor of nonheme iron absorption, in this population.

Compliance to iron-folic acid supplements during pregnancy has been a subject of much study and has frequently been at-

TABLE 5

<table>
<thead>
<tr>
<th>Treatment response with mebendazole and multivitamins compared with standard-of-care control among pregnant women</th>
<th>Standard mebendazole</th>
<th>Enhanced mebendazole</th>
<th>No multivitamin</th>
<th>Multivitamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>At visit 1</td>
<td>175</td>
<td>169</td>
<td>170</td>
<td>174</td>
</tr>
<tr>
<td>Total n</td>
<td>Responders to treatment [n (%)]</td>
<td>76 (43.3)</td>
<td>87 (51.5)</td>
<td>70 (41.2)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>1.38 (0.90, 2.11)</td>
<td>1.00</td>
<td>1.64 (1.07, 2.51)</td>
</tr>
<tr>
<td>At visit 2</td>
<td>141</td>
<td>125</td>
<td>122</td>
<td>144</td>
</tr>
<tr>
<td>Total n</td>
<td>Responders to treatment [n (%)]</td>
<td>66 (46.8)</td>
<td>65 (52.0)</td>
<td>56 (45.9)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>1.23 (0.76,1.99)</td>
<td>1.00</td>
<td>1.28 (0.79, 2.08)</td>
</tr>
<tr>
<td>Either visit</td>
<td>184</td>
<td>180</td>
<td>178</td>
<td>186</td>
</tr>
<tr>
<td>Total n</td>
<td>Responders to treatment [n (%)]</td>
<td>95 (51.6)</td>
<td>103 (57.2)</td>
<td>90 (50.6)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>1.25 (0.83,1.89)</td>
<td>1.00</td>
<td>1.35 (0.90, 2.05)</td>
</tr>
</tbody>
</table>

1 Treatment response defined as an increase of 25 g hemoglobin/L from baseline at visit 1 or hemoglobin ≥100 g/L at visit 2. OR, odds ratio.
2 Median follow-up: 57 d (interquartile range: 7 d).
3 Median follow-up: 85 d (interquartile range: 8 d).
4 Excluding women who were followed >92 d (n = 30).

supplementation programs in the developing world (22–27), although inadequate supply, poor quality of tablets, and other logistic issues may be the main cause of low coverage for supplementation under programmatic conditions (22). We used a high dosage of iron (100 mg) in this study, which may have resulted in more gastrointestinal distress than usual; side effects are known to increase with increasing dosage of iron (27). Supply of tablets was not an issue in the present study, and weekly home visits and supplement replenishment by study staff should have ensured higher adherence. The low adherence to treatment found in the study despite this indicates the need for other approaches to boost adherence. The slightly lower than the current recommended dosage of 120 mg of iron, chosen because of its easy availability may in part explain the lower than expected effectiveness of the standard-of-care treatment.

Vitamins, such as vitamin A, riboflavin, vitamin E, vitamin B-12, and vitamin C, may play a role in the occurrence of anemia and iron deficiency (12), but few studies have tested their efficacy in reducing the risk of anemia or enhancing treatment. The few studies that were done examined the effect of multiple micro-nutrient supplements also containing minerals such as zinc, copper, iodine, and magnesium and failed to show an improvement in hematologic or iron status over iron-folic acid alone (28, 29). We found vitamin A deficiency to be highly prevalent in this

TABLE 6

<table>
<thead>
<tr>
<th>Hemoglobin concentrations at baseline and at the last follow-up visit and change in hemoglobin in pregnant women by treatment group</th>
<th>Standard mebendazole</th>
<th>Enhanced mebendazole</th>
<th>No multivitamin</th>
<th>Multivitamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (g/L)</td>
<td>61 ± 9 (275)</td>
<td>62 ± 8 (272)</td>
<td>62 ± 8 (273)</td>
<td>61 ± 9 (274)</td>
</tr>
<tr>
<td>Last follow-up (g/L)</td>
<td>86 ± 24 (258)</td>
<td>90 ± 22 (261)</td>
<td>88 ± 22 (254)</td>
<td>88 ± 24 (265)</td>
</tr>
<tr>
<td>Change</td>
<td>0.40 (0.03)</td>
<td>0.48 (0.03)</td>
<td>0.43 (0.03)</td>
<td>0.45 (0.03)</td>
</tr>
</tbody>
</table>

1 Mean ± SD; n in parentheses (all such values).
2 Last hemoglobin measurement available for a woman at either 60- or 90-d follow-up visit.
3 Change in hemoglobin per day from baseline to follow-up with linear mixed-effects regression model and adjusted for gestational age was significant (P < 0.001) in all groups. The difference in change in hemoglobin was significant (P < 0.05) between standard and enhanced mebendazole; the difference in change in hemoglobin between no multivitamins and multivitamins was not significant.
4 Adjusted β coefficient; SE in parentheses (all such values).
group of severely anemic women. Approximately 75% across the treatment groups were vitamin A deficient (DBS retinol < 0.7 μmol/L). However, multivitamins containing vitamin A did not have an effect on vitamin A status. Perhaps a single Recommended Daily Allowance of vitamin A was insufficient to decrease vitamin A deficiency, although these results should be viewed with caution given the attrition rate. We were unable to assess the effect of other vitamins in the supplement because blood collection was limited to DBSs.

The effect of the enhanced mebendazole treatment regimen was modest, as expected, considering that the comparison group was women who received the recommended 500-mg single dose of mebendazole. Yet the alternate dosage of mebendazole that we tested may have acted in enhancing the increase in hemoglobin. We found a high prevalence of elevated CRP in this population (15–30%), suggestive of high rates of subclinical infection, another cause of anemia. Although mebendazole treatment did not lower the risk of elevated CRP, our data at follow-up were sparse and baseline imbalances in elevated CRP prevalence further precluded our ability to assess any mechanism operating through lowered inflammation due to the enhanced regimen.

The effect of mebendazole on severe anemia in the absence of hookworm infection has been previously recorded in a study among Zanzibari children < 2 y of age (30). The investigators hypothesized that incident infections causing inflammatory immune responses may result in decreased erythropoiesis. Mebendazole given at 100 mg twice daily over 3 d in the present study may have resulted in reduced inflammation that improved the trajectory of change in hemoglobin over the course of 3 mo as a result of treatment with iron-folic acid. The modest effect seen with supplementation with multivitamins containing vitamin A suggests that low concentrations of vitamin A may perhaps be due to the high burden of infection in this population. Subclinical infection is known to reduce the circulating concentrations of vitamin A that may not be responsive to vitamin A supplementation (31).

We were limited by women being lost to follow-up for the last posttreatment assessment because of having given birth. Although birth outcomes and rates did not differ by treatment group, with ~30% having a live birth and 2–3% a fetal loss in each group, this did result in attenuating the sample size and reducing our ability to find an effect of the enhanced regimens. Women lost to follow-up could have been more responsive to treatment, but this is hard to know because mean hemoglobin concentrations were comparable in women who had a birth outcome or not. The study protocol did not allow follow-up of women for hemoglobin assessment beyond birth outcome.

In this poor periurban setting in Karachi, Pakistan, the prevalence of severe anemia during pregnancy was high. The anemia appeared to be predominantly due to iron deficiency resulting from a diet comprising high-phytate wheat, low heme iron, and high levels of tea, thereby providing extremely low amounts of bioavailable iron. Neither malaria nor hookworm appeared to be an important cause of severe anemia, although chronic and subclinical infections may have been important contributors. We found that treatment response to the standard of care was 65% among women who took ≥85% of the iron-folic acid tablets. High adherence to treatment is likely to be highly predictive of treatment response, and programs may need innovative strategies to enhance compliance to supplement use. The additional enhanced treatments had modest benefit over and above the standard of care, and deworming as a strategy would probably not be considered in this setting of low geohelminth endemicity. Cost may be an important factor in adding other vitamins to the treatment, although this issue was not directly addressed in our study. Finally, anemia screening during pregnancy may also be important to target this high-risk population of pregnant women, who need special antenatal care and treatment. Promotion of dietary intake of heme iron and less consumption of tea or at least only between meals may also be warranted.

Pakistan Severe Anemia Treatment Trials was a joint undertaking of the Johns Hopkins University Bloomberg School of Public Health, Department of International Health and the Aga Khan University, Department of Pediatrics and Child Health, Karachi, Pakistan. In addition to the authors, the following persons helped with different aspects of the study: Ididdi Simba Khamis from the Public Health Laboratory and the Ivo de Carneri Foundation in Zanzibar provided training to conduct Kato Katz and malariometry, Asim Baig from the Department of Medicine and Microbiology at the Aga Khan University conducted quality control of the Kato Katz slides, Juergen Erhardt provided training to technicians at the Aga Khan to conduct the dried blood spot retinol analysis and also analyzed samples; Naveed Bhutto provided help with supervising the study, Chaman Lal and Taj Mohammad conducted the Kato Katz for detecting geohelminths and malariometry, the medical officers and community health volunteers conducted data collection and other clinic and field-based tests.

The authors’ responsibilities were as follows—PC: conceived the idea and research questions and is the guarantor for the study; ZAB and RDWK: helped in the design and implementation of the study and its conduct and provided help with data interpretation and editing the manuscript; FS: made primary contributions to the implementation plan, conduct, field supervision, and quality control of the trial; and AR: helped with data management, supervised data cleaning, and editing and preparation of the data for analysis. None of the authors declared a conflict of interest.

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