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ORIGINAL COMMUNICATION

Anemia in pregnancy in rural Tanzania: associations with micronutrients status and infections

SG Hinderaker[1,2]*, BE Olsen[1,2], RT Lie[4], PB Bergsjø[3], P Gasheka[2], GT Bondevik[1], R Ulvik[5] and G Kvåle[1]

[1]*Centre for International Health, University of Bergen, Norway;* [2]*Haydom Lutheran Hospital, Tanzania;* [3]*Department of Obstetrics and Gynecology, Haukeland Hospital, University of Bergen, Norway;* [4]*Section for Medical Statistics, University of Bergen, Norway; and* [5]*Laboratory of Clinical Biochemistry, Haukeland Hospital, University of Bergen, Norway*

Objective: We studied the association between anemia in pregnancy and characteristics related to nutrition and infections.
Design: Cross-sectional study.
Setting: Four antenatal clinics in rural northern Tanzania.
Subjects/methods: A total of 2547 women were screened for hemoglobin (Hb) and malaria plasmodia in capillary blood and for infections in urine. According to their Hb, they were assigned to one of five groups and selected accordingly, Hb < 70 g/l (n = 10), Hb = 70 – 89 g/l (n = 61), Hb = 90 – 109 g/l (n = 86), Hb = 110 – 149 g/l (n = 105) and Hb ≥ 150 g/l (n = 50). The 312 selected subjects had venous blood drawn, were interviewed, and their arm circumference was measured. The sera were analyzed for ferritin, iron, total iron binding capacity (TIBC), cobalamin, folate, vitamin A, C-reactive protein (CRP), and lactate dehydrogenase (LD). Transferrin saturation (TFsat) was calculated. Urine was examined by dipsticks for nitrite.
Main outcome measures: Unadjusted and adjusted odds ratio (OR and AOR) of anemia with Hb < 90 g/l.
Results: Anemia (Hb < 90 g/l) was associated with iron deficiency (low s-ferritin; AOR 3.4). The association with vitamin deficiencies were significant in unadjusted analysis (low s-folate; OR 3.1, low s-vitamin A; OR 2.6). Anemia was also associated with markers of infections (elevated s-CRP; AOR 3.5, urine nitrite positive; AOR 2.4) and hemolysis (elevated s-LD; AOR 10.1). A malaria positive blood slide was associated with anemia in unadjusted analysis (OR 2.7). An arm circumference less than 25 cm was associated with anemia (AOR 4.0). The associations with less severe anemia (Hb 90 – 109 g/l) were similar, but weaker.
Conclusions: Anemia in pregnancy was associated with markers of infections and nutritional deficiencies. This should be taken into account in the management of anemia at antenatal clinics.
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Introduction

Anemia is regarded as a major risk factor for an unfavorable outcome of pregnancy. Anemia has been associated with premature labor and low birth weight (Brabin *et al*, 1990; Scholl *et al*, 1992), and maternal and perinatal mortality (AbouZahr & Royston, 1991; Murphy *et al*, 1986), but some of these associations are not firmly established (Rasmussen & Øian, 1993). Severe anemia (Hb below 70 g/l) has consistently been associated with maternal mortality (Rush, 2000).

Based on these associations and the high prevalence of iron deficiency anemia, supplementation programs were expected to reduce poor outcome of pregnancy, but the results were often disappointing (Rush, 2000). This was often attributed to program implementation weaknesses,

*Correspondence: SG Hinderaker, Centre for International Health, University of Bergen, N-5021 Bergen, Norway.
E-mail: sven.hinderaker@cih.uib.no
Guarantors: SG Hinderaker and G Kvåle.
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but more profound anemia, accounting for most of the anemia-related increased risk of maternal death, is likely to have complex, multiple causes (Beaton, 2000). A study from Malawi showed that 60% of iron-deficient women had other deficiencies as well, and many had signs of inflammation (van den Broek & Letsky, 2000). Similarly, a study from Tanzania identified iron deficiency, malaria, hookworms, and other infections as major causes of anemia (Massawe *et al*, 1999). Both of these studies used bone marrow aspiration as a gold standard for defining empty iron stores, and consequently using a control group of non-anemic women was not acceptable.

The objective of this article was to study anemia in pregnancy and its relationship with micronutrient status and infections, by comparing anemic with non-anemic women.

Methods

Data collection

Data collection was done at four antenatal clinics run by the Haydom Lutheran Hospital. Between February 1995 and March 1996 we examined Hb, thick blood slide, and urine of 2547 women during their first antenatal attendance, independent of their stage of pregnancy. The study area and the prevalence of anemia was described in a previous paper (Hinderaker *et al*, 2001). In order to study possible causes of anemia, we selected sub-samples from various Hb strata. The study subjects were sampled from the total series as shown in Table 1 in order to include all with Hb < 70 g/l, a balanced number of subjects from the strata Hb 70–89 g/l, Hb 90–109 g/l, Hb 110–149 g/l, and all with Hb ≥ 150 g/l. For the three middle strata, we selected the first woman to present with the appropriate Hb for a stratum, and a second woman in a stratum was not selected until a woman was registered in both of the other two strata. Of the 414 women we selected, 92 women did not consent to further investigations, and 10 were later proven not to be pregnant, leaving 312 subjects for further analysis.

At their first antenatal attendance, the selected subjects had venous blood drawn and gave specimens for urine and stool examinations. They went through an interview, focusing on domestic and socioeconomic factors. We used arm circumference as a proxy for body weight, since it is largely independent of gestational age (Krasovec & Anderson, 1991; Liljestrand & Bergström, 1991). Mid-upper arm circumference was measured to the nearest 0.5 cm by standardized methods, and values below 25.0 cm were used in the analyses as an indicator of low body weight.

Table 1 Outline of selection procedure

Hb stratum (g/l)	*Total*	*< 70*	*70–89*	*90–109*	*110–149*	*150+*
Eligible	2547	17	114	428	1932	95
Selected	414	11	74	120	142	67
Blood taken	322	11	62	91	105	54
Analyzed	312	11	59	89	103	50

Tanzanian antenatal clinic policy included free provision of daily iron and folic acid tablets, and weekly prophylactic chloroquine tablets, but the supply was insufficient. Instead, free tablets of iron (ferrous sulfate 200 mg daily) and folic acid (0.1 mg daily) for a month were provided by the project for subjects with Hb below 100 g/l. Severely anemic women were referred to the hospital. If a blood slide showed malaria parasitemia, she was given a standard therapeutic course of chloroquine tablets free of charge (600 mg base first and second days, 300 mg third day). Free tablets of nitrofurantoin (100 mg twice daily for a week) were given to women with urinary tract infections (judged by the urine test results and symptoms). The midwives at the antenatal clinics took care of the further management, and sick women were referred to the hospital. No remuneration was given to participants, other than free treatment corresponding to the test results.

Laboratory methods

Hb was measured with a HemoCue photometer (HemoCue® AB, Ängelholm, Sweden). The venous blood was left to coagulate for at least 30 min, then separated, and the serum was frozen and brought to the Laboratory of Clinical Biochemistry, Haukeland Hospital, in Bergen, Norway, and analyzed for vitamin A, cobalamin, folate, iron (Fe), ferritin, total iron binding capacity (TIBC), C-reactive protein (CRP), lactate dehydrogenase (LD), and haptoglobin. s-Vitamin A was measured by high performance liquid chromatography (Aksnes, 1994). s-Cobalamin, s-folate and s-ferritin were measured by magnetic separation assays (MSA) using a Technicon Immuno 1 System (Technicon Instruments, Bayer Corp., New York, USA). s-Iron, TIBC and s-LD were measured spectrophotometrically using a Technicon Chem-1 System (Technicon Instruments, Bayer Corp., New York, USA). Transferrin saturation (TFsat) was calculated by the formula: TFsat (s-iron×100/TIBC)%. s-CRP was measured by an immunoturbidometric assay (Orion Diagnostica, Espoo, Finland). s-Haptoglobin was measured with a Behring Nephelometer Analyser II (Dade Behring, Deerfield, Illinois, USA).

Anemic women with Hb < 90 g/l and women with Hb 90–109 g/l were compared to women with Hb ≥ 110 g/l. Hb < 70 g/l were too few to justify separate analysis. In addition we used the following definitions for abnormal values: s-vitamin A below 0.70 μmol/l, s-cobalamin below 150 pmol/l, s-folate below 4.5 nmol/l, s-ferritin below 15 μg/l and above 75 μg/l, s-Fe below 11 μmol/l, s-TIBC above 72 μmol/l, s-TFsat below 16%, s-CRP 10 mg/l and above, s-LD above 450 U/l, s-haptoglobin below 0.4 g/l (Brekke, 1997; Nielsen *et al*, 1990). There were many missing values for s-haptoglobin, because serum quality was insufficient for this analysis in some of the samples. For ferritin, we did not use the upper cut-off value indicated by the laboratory, because very few subjects had s-ferritin above 160 μg/l. Instead, we used the 97.5 percentile of non-anemic participants as upper cut-off value (75 μg/l). As an indicator of empty iron stores we used s-ferritin <15 μg/l among women with s-CRP <10 mg/l, and s-ferritin <50 μg/l in women with s-CRP ≥ 10 mg/l (Cook & Skikne, 1989).

During storage in Tanzania the sera melted once (duration 1 day at temperature around 0–4 C°) because of electricity failure. During transport some of the sera melted.

Examinations for the detection of malaria parasitemia were done at the laboratory at Haydom Lutheran Hospital. Thick blood slides were stained with Field stain and read at high power magnification at the laboratory. Parasites and leukocytes were counted, and the number of high power fields examined corresponded to 100 leukocytes. In a negative blood slide, no parasites were observed. The thick blood slides did not allow species identification of plasmodia, but the dominating species in the area was *P. falciparum*.

Urine was examined at the antenatal clinics using a reagent strip for nitrite (Nephur-Test®, Boehringer-Mannheim GmbH, Mannheim, Germany), and the definitions of positive specimens followed the instructions of the manufacturer.

Computing and statistics

For data entry we used EpiInfo 5.0 and 6.04b (Dean *et al.*; 1994). For analysis of the data we converted all files from EpiInfo 6.04b to SPSS version 9.0 (SPSS, 1999).

Descriptive analysis included means, and standard deviations of the laboratory variables in subgroups defined by Hb (Hb ≥ 110 g/l, Hb 90–109 g/l, Hb < 90 g/l). The mean values of biochemical variables by the degree of anemia were explored by analysis of variance. The associations between anemia and its determinants were examined by multiple logistic regression analysis and expressed as odds ratios (OR) and adjusted odds ratios (AOR), with Hb < 90 g/l and Hb 90–109 g/l as dependent variables and Hb ≥ 110 g/l as reference category. The OR was regarded as an approximation for relative risk. The OR for each variable in Table 3 were adjusted for the other variables in the table. However, we excluded arm circumference and s-vitamin A from the final regression model because of many missing values and little effect on the estimates. TFsat, calculated from s-Fe and s-TIBC, was also excluded. Age, tribe, place of residence, altitude, literacy, acreage, parity and gestational age had little effect on the estimates in the preliminary analyses, and therefore they were not included in the final regression model. Pearson's correlation coefficients (*r*) were used as measurement of correlation. All significance levels were 0.05 and two-tailed.

Ethical considerations

The research protocol was approved by The National Committee for Research Ethics in Medicine in Norway and by The Commission for Science and Technology in Tanzania. Prior to the field study, the Regional Development Officer, the District Commissioners, and the leaders of wards and villages had given consent to the study. The local people had been informed about the study through gatherings in the villages, and the participants also consented individually.

Results

The median age of the participants was 25 y and median gestational age was 5 months. Most of the background characteristics (maternal age, altitude of residence, acreage, illiteracy, parity and gestational age) were similar among anemic and non-anemic women. There were more women of datoga tribe among the anemic than among non-anemic women, but this had little influence on the analyses.

Mean values of the biochemical characteristics are shown in Table 2, and Table 3 shows the ORs of anemia for each determinant.

Nutritional factors

Anthropometric measurements. An arm circumference less than 25.0 cm was found in 25, 36 and 55% of women with

Table 2 Mean values of biochemical indexes and arm circumference, by level of Hb, among pregnant women in Tanzania

	Hb ≥ 110 g/l		*Hb 90–109 g/l*		*Hb < 90 g/l*		
	n[a]	*Mean (s.d.)*	n[a]	*Mean (s.d.)*	n[a]	*Mean (s.d.)*	P-*value*[b]
arm circumference (cm)	115	26.0 (1.9)	54	25.4 (2.0)	53	24.8 (2.5)	0.003
s-Ferritin (μg/l)	153	22 (21)	89	35 (63)	70	90 (164)	<0.001
s-Fe	153	14.9 (6.0)	89	12.1 (7.9)	69	14.9 (12.0)	0.03
s-TIBC	153	80.5 (16.8)	89	84.3 (22.4)	69	85.8 (22.8)	0.14
TFsat (%)	153	19.9 (10.1)	89	16.5 (12.2)	69	21.1 (21.5)	0.08
s-Folate (nmol/l)	153	8.9 (4.8)	89	8.2 (6.7)	70	7.9 (9.1)	0.50
s-Cobalamin (pmol/l)	153	243 (94)	89	231 (94)	70	230 (92)	0.53
s-Vitamin A (μmol/l)	137	0.93 (0.33)	73	0.84 (0.29)	51	0.75 (0.29)	0.004
s-CRP (mg/l)	153	8.2 (11.0)	89	12.9 (20.2)	70	22.6 (24.2)	<0.001
s-LD (U/l)	153	294 (94)	89	334 (122)	69	484 (300)	<0.001
s-Haptoglobin (g/l)	44	0.62 (0.32)	31	0.54 (0.43)	36	0.33 (0.39)	0.003

[a]There were different numbers of missing values.
[b]*P*-values for difference between Hb strata, obtained in one-way analysis of variance.

Table 3 Associations between Hb level and indicators of nutritional deficiencies and infection among pregnant women in Tanzania

	Hb ≥ 110		Hb 90–109		Hb < 90	
	n	(%)	OR[a] (95% CI)	AOR[b] (95% CI)	OR[a] (95% CI)	AOR[b] (95% CI)
Markers of nutritional status						
Arm circumference < 25 cm[c]	28	(25.0)	1.7 (0.9, 3.4)	1.2 (0.5, 2.9)	3.6 (1.8, 7.2)	4.0 (1.3, 12.6)
s-Ferritin < 15 μg/l[d]	79	(51.6)	1.4 (0.8, 2.5)	1.0 (0.4, 2.1)	2.4 (1.2, 4.9)	3.4 (1.0, 11.3)
s-Fe < 11 μmol/l	38	(24.8)	3.0 (1.7, 5.2)	3.6 (1.8, 7.3)	2.6 (1.4, 4.8)	5.7 (2.1, 15.4)
s-TIBC > 72 μmol/l	98	(64.1)	0.9 (0.5, 1.5)	0.8 (0.4, 1.7)	0.9 (0.5, 1.7)	0.8 (0.3, 2.2)
TFsat < 16%	63	(41.2)	1.8 (1.0, 3.0)	1.9 (0.9, 3.8)	1.5 (0.8, 2.6)	2.3 (0.9, 6.3)
s-Folate < 4.5 nmol/l	21	(13.7)	1.4 (0.7, 2.8)	1.0 (0.5, 2.2)	3.1 (1.6, 6.0)	1.8 (0.7, 4.6)
s-Cobalamin < 150 pmol/l	22	(14.4)	1.3 (0.6, 2.6)	1.3 (0.6, 2.8)	1.6 (0.8, 3.4)	1.1 (0.4, 3.2)
s-Vitamin A < 0.7 μmol/l[c]	31	(22.6)	1.5 (0.8, 2.8)	1.6 (0.8, 3.5)	2.6 (1.3, 5.1)	2.2 (0.8, 6.0)
Markers of infection						
s-CRP > 10 mg/l	35	(22.9)	1.7 (1.0, 3.1)	1.5 (0.8, 2.8)	4.5 (2.5, 8.2)	3.5 (1.5, 8.2)
s-Ferritin > 75 μg/l[d]	4	(2.6)	6.4 (1.9, 21.8)	4.2 (1.1, 15.8)	29.6 (8.8, 100)	19.8 (4.2, 92.4)
Malaria in blood slide	25	(17.2)	1.0 (0.5, 2.1)	0.8 (0.4, 1.8)	2.7 (1.4, 5.3)	1.7 (0.7, 4.2)
s-LD > 450 U/l	7	(4.6)	2.6 (1.0, 7.2)	2.6 (0.8, 8.4)	11.1 (4.5, 27.5)	10.0 (2.8, 35.9)
U-Nitrite positive	55	(36.7)	0.9 (0.5, 1.6)	1.0 (0.5, 1.8)	2.5 (1.4, 4.4)	2.4 (1.0, 5.5)

[a]OR = unadjusted odds ratio. The reference group is women with normal values of the variable studied.
[b]AOR = adjusted odds ratio, adjusted for all the variables in the table except Tfsat, arm circumference, and s-vitamin A.
[c]There were 92 missing values for arm circumference, and 51 missing values for s-vitamin A.
[d]Ferritin 15–75 μg/l was reference category.

Hb ≥ 110 g/l, Hb 90–109 g/l and Hb < 90 g/l, respectively. Mean arm circumference decreased with increasing degree of anemia (Table 2). The AOR of anemia (Hb < 90 g/l) was increased among women with arm circumference below 25 (Table 3). Arm circumference was not significantly correlated to s-Fe, s-TIBC, s-folate, s-B 12, s-vitamin A, a positive malaria blood slide and a positive u-nitrite test. However, we found a significant, negative correlation with s-CRP ($r = -0.18$, $P = 0.0l$), s-ferritin ($r = -0.15$, $P = 0.03$), and s-LD (-0.18, $P = 0.01$). The association between anemia and arm circumference was strongest among the women with an elevated s-CRP.

Iron status. Empty iron stores were found in 59, 65 and 62% of women with Hb < 90, Hb 90–109, and Hb ≥ 110 g/l, respectively. The AOR of anemia with Hb < 90 g/l was increased among women with s-ferritin below 15 μg/l compared to those with s-ferritin between 15 and 75 μg/l (Table 3). There was a significant correlation between iron deficiency and a low s-vitamin A among the women with a s-CRP below 10 mg/l ($r = 0.25$, $P = 0.001$).

Vitamins. Low s-folate (below 4.5 nmol/l) was observed in 14, 18 and 33% of women with Hb ≥ 110 g/l, Hb 90–109 g/l, and Hb < 90 g/l, respectively. We observed a significantly increased OR for anemia (Hb < 90 g/l) among women with a s-folate below 4.5 nmol/l. The association was weaker and not significant in multivariate analysis (Table 3).

Low s-cobalamin (below 150 pmol/l) was found in 14, 18, and 21% of women with Hb ≥ 110 g/l, Hb 90–109 g/l and Hb < 90 g/l, respectively. None of the women had a s-cobalamin below 52 pmol/l. S-cobalamin was not significantly associated with anemia.

Low s-vitamin A (below 0.70 μmol/l) affected 23, 30 and 43% of women with Hb ≥ 110 g/l, Hb 90–109 g/l and Hb < 90 g/l, respectively. Almost 20% of non-anemic women with a normal s-CRP (below 10 mg/l) had low s-vitamin A. We observed a significant decrease in mean s-vitamin A with increasing degree of anemia (Table 2). A s-vitamin A below 0.7 μmol/l was significantly associated with anemia (Hb < 90 g/l), but the association was weaker after adjustment (Table 3). s-Vitamin A was correlated to s-CRP ($r = -0.14$, $P = 0.02$). Among women with s-CRP

< 10 mg/l, s-vitamin A increased with increasing s-ferritin ($r = 0.26$, $P = 0.001$).

Most of the women had at least one micronutrient deficiency, and many women had combined deficiencies, the commonest combination being iron and vitamin A deficiencies. The proportion of women without deficiency was 38, 26 and 20% of women with Hb ≥ 110 g/l, Hb 90–109 g/l, and Hb < 90 g/l, respectively.

We found a statistically significant interaction between s-CRP and iron status ($P = 0.03$). The associations between anemia and micronutrients deficiencies were stronger among the women with s-CRP < 10 mg/l, whereas among the women with elevated s-CRP the associations were not significant.

Markers of infections and related factors

An elevated s-CRP and an elevated s-ferritin were used as indicators of infection (Scholl, 1998). There was a strong correlation between s-CRP ≥ 10 mg/l and s-ferritin > 75 μg/l ($r = 0.4$, $P < 0.0001$).

We found a s-CRP ≥ 10 mg/l in 23, 34 and 57% of the women with Hb ≥ 110 g/l, Hb 90–109 g/l, and Hb < 90 g/l, respectively. The mean s-CRP increased with increasing degree of anemia (Table 2). The AOR of anemia (Hb < 90 g/l) was significantly increased among women with elevated s-CRP (Table 3). Similarly, the AOR of anemia was significantly increased among women with s-ferritin above 75 μg/l (Table 3).

Elevated s-LD above 450 U/l was found in 5, 11 and 35% of women with Hb ≥ 110, Hb 90–109, and Hb < 90 g/l, respectively. Mean s-LD increased with increasing degree of anemia (Table 2). The AOR of anemia (Hb ≥ 110 g/l) was highly increased among women with s-LD above 450 U/l (Table 3), the association was weaker for anemia with Hb 90–109 g/l. s-LD displayed significant correlation with s-CRP ($r = 0.35$, $P < 0.001$), s-ferritin ($r = 0.51$, $P < 0.001$), s-haptoglobin ($r = -0.37$, $P < 0.001$), and malaria parasitemia ($r = 0.20$, $P < 0.001$). Haptoglobin (not shown in Table 3) was measured in the sera of only 111 women, and low s-haptoglobin (< 0.4 g/l) was found in 21, 48 and 75% of women with Hb ≥ 110, Hb 90–109 and Hb < 90 g/l, respectively.

Malaria parasitemia was detected in 17, 17 and 36% of women with Hb ≥ 110, Hb 90–109 and Hb < 90 g/l, respectively. A positive malaria slide was significantly associated with anemia in univariate analysis (Table 3), but not significantly in multivariate analysis. A positive blood slide was correlated with an elevated s-LD ($r = 0.21$, $P = 0.0004$), but not with an elevated s-CRP nor a low s-ferritin. The mean parasite density among the 64 positive slides was 2.9 (range 2–10) plasmodia per 100 white blood cells,

A positive u-nitrite was found in 37, 35 and 59% of women with Hb ≥ 110, Hb 90–109, and Hb < 90 g/l, respectively. The AOR for anemia was increased in women with a positive u-nitrite (Table 3). A positive u-nitrite was significantly associated with an elevated s-CRP ($r = 0.16$, $P < 0.004$).

Among the 162 stool specimens, we found one with hookworm ova, 7 with *Giardia Lamblia* trophozoites, one with tapeworm ova, one with *Entamoeba histolytica* cysts and one with *Entamoeba histolytica* trophozoites. No specimens showed *Ascaris Lumbricoides* ova.

Discussion

In this study among pregnant women in rural Tanzania, anemia was associated with various signs of infections and to some micronutrients deficiencies.

Nutrition

Anthropometric measurements. Women with a small arm circumference had a higher risk of anemia with Hb < 90 g/l. Restricting analysis to women without signs of infections, the arm circumference was not associated with anemia, whereas the association was stronger among the women with signs of infection. Arm circumference was negatively correlated to the biochemical indicators of infection and acute phase response, but not with the presence of malaria parasites or urinary tract infections. Prolonged infections may have led to both anemia and wasting and therefore an apparent association between the arm circumference and anemia. Arm circumference was not associated with micronutrients deficiencies.

Iron status. Depleted iron stores were common among non-anemic as well as anemic women. This supports findings from studies worldwide showing that the iron stores of most women are exhausted during pregnancy unless supplemented (Kiwanuka *et al*, 1999; Milman *et al*, 1991). In our study, the iron-depleted women had a higher risk of anemia (Hb < 90 g/l) than iron-replete women. Iron supplementation in pregnancy has been shown to have a beneficial effect on anemia (Milman *et al*, 1999; Panth *et al*, 1990; Suharno *et al*, 1993), which justifies antenatal routine iron supplementation in areas with high prevalence of iron deficiency (Carroli *et al*, 2001).

Folate. A s-folate below 4.5 nmol/l was more common in this area than in a similar study in Nepal (Bondevik *et al*, 2000). A s-folate below 4.5 nmol/l was associated with increased risk of anemia, but the association was weaker and not significant in multivariate analysis. The association between anemia and low s-folate was probably underestimated in our study. Firstly, folate concentration is much higher inside the erythrocytes than in plasma. The women with the lowest Hb had the highest proportion of sera with signs of hemolysis, as indicated by an elevated s-LD. During hemolysis, intra-erythrocytic folate leaks into the plasma, and s-folate may rise temporarily and mask a folate deficiency preferentially among the women with the lowest Hb.

Secondly, food intake may substantially influence the s-folate so that it varies from day to day. A random variation of s-folate would lead to weaker and less significant associations. Thus, taken together, the increased risk of anemia among the folate deficient women was probably real, and supports the current Tanzanian recommendations of giving folic acid supplementation to pregnant women.

Cobalamin. The prevalence of cobalamin deficiency (s-cobalamin below 150 pmol/l) was much lower than in a similar study among Nepali women (Bondevik *et al*, 2000). Studies in Africa have shown a relatively low prevalence of cobalamin deficiency (Brabin *et al*, 1986; Coetzee *et al*, 1994; Liljestrand *et al*, 1986). In our study, the OR for anemia (Hb < 90 g/l) was not significantly increased among cobalamin deficient women, and the importance of cobalamin deficiency for causing anemia in our study population was probably minor.

Vitamin A. A high proportion of women had low s-vitamin A and this may reflect a real vitamin A deficiency, since the prevalence was high (20%) even among non-anemic women without signs of infection. In populations where the prevalence of low vitamin A (less than 0.70 μmol/l) is higher than 15%, vitamin A deficiency should be regarded as a public health problem (Arroyave *et al*, 1989). The prevalence was higher than among non-anemic pregnant women in Nepal (4.8%; Bondevik *et al*, 2000), but lower than the overall prevalence in Indonesia (33%; Suharno *et al*, 1992).

The increased risk of anemia (Hb < 90 g/l) for women with low s-vitamin A was not statistically significant, but does not contradict the findings of the study in Nepal (Bondevik *et al*, 2000), where the risk estimate was higher. Also, a randomized controlled trial among anemic pregnant women in Indonesia showed that vitamin A supplementation increased the Hb, and 35% became non-anemic without any iron supplementation (Suharno *et al*, 1993).

A low s-vitamin A was associated with signs of infections (s-CRP < 10 g/l), also shown in other studies (Friis *et al*, 1997). An acute phase response can depress s-vitamin A (Hautvast *et al*, 1998), and malaria has been associated with low s-vitamin A, even when it is subclinical (Das *et al*, 1996). Das *et al*, explained the association by a decrease in the carrier molecule retinol binding protein, which can extravasate during inflammation, and may also leak into the urine. Vitamin A deficient rats had decreased resistance to plasmodia (Krishnan *et al*, 1976; Stoltzfus *et al*, 1989), but an intervention study in Ghana showed no effect of vitamin A supplementation on malaria morbidity.

We found an association between low vitamin A and low iron stores, which has been shown in other studies (Suharno *et al*, 1992). It could be due to impaired mobilization of iron from stores (Roodenburg *et al*, 1994). Animal studies have shown that vitamin A deficiency does not decrease iron absorption (Sijtsma *et al*, 1993), and that iron deficiency decreases vitamin A mobilization. Intervention studies have shown that vitamin A and iron is better than iron alone in alleviating anemia (Kolsteren *et al*, 1999; Panth *et al*, 1990; Suharno *et al*, 1993). Routine supplementation of vitamin A for pregnant women should be considered, particularly targeting women with low Hb. Vitamin A supplementation for fertile women in susceptible areas is currently under debate, and care must be taken in order not to cause teratogenic effects (Azais Braesco & Pascal, 2000; Rothman *et al*, 1995). The International vitamin A consultative group (IVACG) recommended daily 10 000 IU or weekly 25 000 IU to pregnant women in areas with high prevalence of vitamin A deficiency (International Vitamin A Consultative Group, 1998).

Infections and related factors

Many women, particularly the women with the lowest Hb, had signs of infections. We observed an association between an elevated s-CRP and anemia, as well as between an elevated s-ferritin and anemia, which is observed in other studies as well (Massawe *et al*, 1999). Treatment of the infection would be the rational approach in managing anemia in these women. Intestinal parasites potentially causing malnutrition were rare in this area.

Microscopically detected malaria parasitemia was associated with higher risk of anemia (Hb < 90 g/l). The association was not statistically significant in multivariate analysis. This could be expected since variables strongly related to malaria infection were included in the model. Malaria is frequently reported as a major cause of anemia in studies from Africa (Garner & Gulmezoglu, 2000), and is related to acute phase markers (Hurt *et al*, 1994). Blood slide microscopy may, however, not be a useful marker of malaria disease in persons with acquired immunity (Smith *et al*, 1993), and this would reduce the association with anemia. The predictive values of blood slide results may be particularly low under strained operational conditions. s-LD, a marker of hemolysis and associated with malaria parasitemia, may be a more sensitive marker of active malaria disease in endemic areas (Jakobsen *et al*, 1997). We found a significant 10-fold increased risk of anemia (Hb < 90 g/l) among the women with signs of hemolysis. Taken together, our results suggest malaria as a major contributor to anemia with Hb below 90 g/l.

Urinary tract infections were associated with anemia (Hb < 90 g/l) and with an elevated s-CRP. In a study from Nepal using urine microscopy to detect infections, their point estimate for risk of anemia (Hb < 90 g/l) was similar to ours (doubling of risk), but they had wide confidence intervals (0.45 – 8.49). In a previous study, we showed that urinary tract infections were very common in this setting (Olsen *et al*, 2000), and should be given more attention at antenatal visits. Apart from the association with anemia, urinary tract infections in pregnancy increase the risk of pyelonephritis, amnionitis, premature labor, low birth weight and perinatal death (Schieve *et al*, 1994), and deserves more attention at antenatal clinics (Carroli *et al*, 2001).

Other infectious diseases commonly associated with anemia, like leishmaniasis and schistosomiasis, were non-existent in the area. Only 12 patients with sickle cell disease were identified at Haydom Lutheran Hospital during 1995–1996 (inpatients and outpatients), and only 38 cases of hookworm were recorded (Haydom Lutheran Hospital (HLH), 1998). There were two HIV positive sera among 733 women examined at antenatal clinics at HLH in 1995–1996 (unpublished data from HLH, 2000).

In this study, the selection of the women was non-random, and some of the selected women refused to participate and were not included in the final analysis. This may influence the representativity of prevalence figures, but the associations between biochemical factors and anemia will likely not be affected. The varying stages of pregnancy at the antenatal visits complicated the assessment of cut-off points for abnormal values of several variables by physiological hemodilution, but the gestational age was similar at the three levels of Hb, and preliminary analysis with adjustment for gestational age did not change the estimates.

Conclusion

Our results show that anemia with Hb below 90 g/l was associated with nutritional factors such as iron deficiency, folate deficiency and vitamin A deficiency. Anemia with Hb below 90 g/l was also associated with signs of infections, including malaria and urinary tract infections. Women with Hb below 90 g/l should be investigated for other causes of the anemia aside from iron deficiency. Proven measures to reduce malaria in pregnancy, like impregnated bed-nets and antimalaria prophylaxis, should be promoted at antenatal clinics, and screening for urinary tract infections and subsequent treatment should be an integral part of the service given (Carroli *et al*, 2001). Simple iron supplementation programs may have failed to focus on the women with more profound anemia who have increased risk of poor outcome, and a diet based approach to meet the requirements of non-anemic and mildly anemia may have been underestimated (Beaton, 2000). However, promoting a good and varied diet and bed-nets is futile if the women do not have the sufficient means to practice accordingly. Improving socioeconomic conditions remain the permanent solution of poverty related conditions.

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