RISK FACTORS ASSOCIATED WITH ANEMIA, IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA IN RURAL NEPALI PREGNANT WOMEN

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Abstract. We conducted a cross sectional study to investigate risk factors associated with severe anemia [hemoglobin (Hb) < 8.0 g dl^{-1}] and poor iron status among Nepali pregnant women. Socio-demographic, anthropometric, health and dietary data were collected from 3,531 women living in the southeastern plains of Nepal. Stool samples were analyzed for intestinal helminthes. Dark adaptation was assessed using the Night Vision Threshold Test (NVTT). Hb levels were measured in all subjects to detect anemia and the soluble transferrin receptor (sTfR) was measured among a subsample of 479 women. The iron status categories were: 1) normal (Hb≥11.0 g/dl and sTfR≤8.5 mg/l); 2) anemia without iron deficiency (Hb<11.0 g/dl and sTfR≤8.5 mg/l); 3) iron deficiency without anemia (Hb≥11.0 g/dl and sTfR>8.5 mg/l); and 4) iron deficiency anemia (IDA): (Hb<11.0 g/dl and sTfR>8.5 mg/l). Factors associated with severe anemia and poor iron status were determined using logistic regression. Hookworm infection increased the risk for developing severe anemia [adjusted odds ratio (AOR): 4.26; 95% CI 1.67-10.89; $p<0.01$] and IDA [relative risk ratio (RRR): 2.18; 95% CI 1.14-4.16; $p<0.05$]. Impaired dark adaptation was a common risk factor for iron deficiency with and without anemia. Intake of iron supplements as tablets and/or tonic was protective against severe anemia, anemia without iron deficiency and IDA. Dietary heme iron was significantly associated with iron deficiency without anemia (RRR: 0.1; 95% CI 0.02-0.47; $p<0.01$). These results indicate the risk factors varied by classification and multiple approaches are needed to reduce anemia and associated nutrient deficiencies.

Keywords: anemia, iron deficiency, pregnant women, Nepal

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INTRODUCTION

Iron deficiency is the single largest cause of anemia in developed countries; however, it is not always the only or primary cause of anemia in developing

countries (WHO, 2002). Anemia in these countries can result from other nutritional deficiencies, such as folate and vitamin B_{12}, malaria, other infectious and parasitic diseases (Verhoeff *et al*, 1999; WHO, 2002; Ayoya *et al*, 2006). Additionally, several studies have found an association between vitamin A deficiency (VAD) and anemia (Christian *et al*, 1998; Bondevik *et al*, 2000; Dreyfuss *et al*, 2000; van den Broek *et al*, 2000). Severe anemia during pregnancy reduces the woman's ability to survive obstetric complications during and after childbirth and may result in premature and/or low birth weight babies (Allen, 1993, 2000; Scholl and Rielly, 2000; Steer, 2000).

In the present study, we identified risk factors associated with severe anemia among a large sample of pregnant women living in the rural plains of Nepal. We also determined whether risk factors for anemia without iron deficiency, iron deficiency without anemia and iron deficiency anemia (IDA) differed across this population. Identifying differences between IDA and anemia due to other factors would enable program managers to design effective intervention strategies that target the underlying causes of anemia. It could also allow the distribution of necessary treatments, commonly in short supply in developing countries, to the most vulnerable groups of pregnant women.

MATERIALS AND METHODS

Pregnant women were recruited during their regular antenatal visits from six health facilities located in two districts in the southeastern plains *(terai)* of Nepal: Parsa and Sunsari. Eligible women were in their second or third trimester of pregnancy, between 18 and 45 years old. Exclusion criteria included women who were blind, pregnant <13 weeks gestation, unable to understand the instructions or were not willing to participate. A total of 3,531 pregnant women were enrolled in the study over a 12 month period.

The study protocol was approved by the institutional review boards of the University of Arizona and the Nepal National Research Council.

Socio-demographics and health-related data

On enrollment, demographic information was obtained: date of birth, marital status, education level and employment status. A history of recent illness was obtained: respiratory illnesses, diarrhea and fever. Women were asked whether they had received treatment for intestinal helminths or malaria during the previous six months prior to enrollment. A current helminth infection was confirmed with a stool test using the Kato-Katz method (WHO, 1994). Infected participants were treated with a single dose of Albendazole 400 mg per Nepali Ministry of Health (MOH) regulations. Parity and gestational age were obtained from each participant. The gestational age was estimated from the last menstrual period. Weight, height and mid-upper arm circumference (MUAC) were measured using standardized methods (CDC, 2004).

Dietary intake assessment

Information was obtained about intake of iron tablets and tonic (micronutrient-containing energy drink). Interviewers administered a semi-quantitative food frequency questionnaire (FFQ) that emphasized dietary sources of iron and consisted of 33 food items commonly consumed by the study population. The serving units were based on locally used serving utensils. Food items were placed in these utensils and weighed in order to

determine the gram equivalent of each food item. We measured intake of heme iron in animal foods and non-heme iron in animal and plant foods. Enhancers of iron absorption included foods rich in vitamin C and inhibitors included dietary phytates and tea (rich in tannins). Iron, vitamin C and phytate content per 1 g of food were determined using the USDA National Nutrient Database (USDA Food Search, version1.0, SR18). Then, for each participant, daily dietary heme, non-heme and total iron, vitamin C and phytate intake in grams were calculated. Tea consumption was reported as yes or no.

Assessment of iron and vitamin A status

The participant's dark adaptation was assessed by questionnaire and the Night Vision Threshold Test (NVTT) (Duncan *et al*, 2000; Taren *et al*, 2004). NVTT scores ranged from 0 to 7. A woman with a score ≤ 6 was considered to have night blindness; the lower the score the more severe the night blindness. Women identified with night blindness received 4 weekly doses of vitamin A 7,500 µg (25,000 International Units) following Nepal's MOH standard protocol. Hemoglobin (Hb) was measured (n = 3,509) using a HemoCue photometer (HemoCue; Leo Diagnostics, Helsingborg, Sweden). Anemic participants (Hb<11.0 g/dl) were given 30 tablets of iron supplement and instructed to take one tablet (60 mg) per day.

The first 479 consecutive women enrolled in the study (June - July 2005) provided capillary blood samples by finger prick. The samples were placed on filter paper, dried at room temperature away from UV light, placed in air tight zip-lock plastic bags with desiccants and then stored in a refrigerator (2-8°C) until shipped for analysis. Retinol was measured in the dried blood spots (DBS) using high performance liquid chromatography (Craft *et al*, 2000) with a between-assay coefficient of variation (CV) of 8%. Soluble transferrin receptor (sTfR), retinol binding protein (RBP), C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) were measured using enzyme-linked immunosorbent assays (Erhardt *et al*, 2004) with CVs of 7.5, 5.7, 8.2 and 5.7%, respectively. We defined severe anemia as Hb<8.0 g/dl and iron deficiency as sTfR>8.5 mg/l and vitamin A deficiency (VAD) as a retinol<1.05 µmol/l. A CRP>5 mg/l and AGP>1 mg/l indicated acute and chronic infection, respectively.

Statistical analysis

The first analysis was completed using the whole samples from 3,509 women to determine factors associated with severe anemia. The second analysis was conducted with a subsample of 479 consecutive women who provided complete biochemical measurements to determine factors associated with iron deficiency and/or anemia. The characteristics of participants, including age, gestational age, anthropometric measurements, Hb and NVTT score, did not differ significantly between the subsample and the whole sample groups.

Descriptive statistics included frequencies and/or means (with standard deviations) for maternal socio-demographics, anthropometric, health and biochemical variables. Hookworm egg count data were grouped into the World Health Organization (WHO) standard categories (WHO, 1994) for the descriptive part of the analysis and then were analyzed by groups increasing by 1,000 eggs per gram (epg) of feces for bivariate and multivariate analyses of severe anemia. Quantiles of distribution were used to categorize anthropometric and dietary variables.

Associations between variables and severe anemia as a binary outcome were tested using the chi-square (χ^2) test. Predictors of severe anemia were determined using a multivariate logistic regression model with the Allen-Cady modified backward selection method (Anderson *et al*, 1972). In light of findings from previous studies (Verhoeff *et al*, 1999; Bondevik *et al*, 2000; Dreyfuss *et al*, 2000; Suega *et al*, 2002; Aikawa *et al*, 2006; Ayoya *et al*, 2006), age, literacy, parity, gestational age, hookworm infection intensity and *Trichuris trichiura* (*T. trichiura)* infection were accounted for in the model regardless of statistical significance for face validity and to rule out confounding. Next, all variables that had a significance of $p<0.2$ on bivariate analysis were ranked in order of importance and entered into the model. They were then subjected to removal until the first variable meeting criterion for retention ($p<0.05$) was encountered. Based on several diagnostic tests, the model lack of fit and inadequacy were ruled out.

To determine whether risk factors of anemia with and without iron deficiency and iron deficiency without anemia differed, we created an outcome variable (iron status) with four mutually exclusive categories using the subsamples of 479 women: 1) normal (Hb≥11.0 g/dl and sTfR≤8.5 mg/l); 2) anemia without iron deficiency (Hb<11.0 g/dl and sTfR≤8.5 mg/l); 3) iron deficiency without anemia (Hb≥11.0 g/dl and sTfR>8.5 mg/l); and 4) iron deficiency anemia (IDA) (Hb<11.0 g/dl and sTfR>8.5 mg/l). The sets of variables significantly ($p<0.2$) associated with at least one iron status outcome category were entered into the multinomial logistic regression model. Age and gestational age were kept in the model for face validity regardless of statistical significance. We selected the one model that most uniformly predicted iron status outcomes. The normal category was selected as the comparison group and relative risk ratios (RRR) with 95% confidence intervals (CIs) were calculated for each outcome category. Diagnostic tests were performed separately on individual models for iron status outcomes; they showed good overall fit and adequacy of the models.

All data were analyzed using STATA (version 8.0; StataCorp LP, College Station, TX).

RESULTS

The characteristics of study participants are presented in Table 1. The participants were on average young, thin and stunted. The majority were illiterate and unemployed. Hookworm infection was the most prevalent followed by *Ascaris lumbricoides (A. lumbricoides)* and *T. trichiura.*

About 9% of the participants had impaired dark adaptation based on the NVTT. More than 50% of participants reported the intake of supplements in the form of tonic, iron tablets or both. The average Hb concentration was 10.9±1.6 g/dl, just below the WHO cutoff level for anemia (WHO, 1998). The prevalence of severe anemia (Hb<8.0 g/dl) was 4.2% (Table 1).

One third of the women in the subsample (n=479) were considered iron deficient (sTfR>8.5 mg/l) and half had a poor vitamin A status (retinol<1.05 µmol/l). Acute (CRP>5 mg/l) and chronic (AGP>1 mg/l) infections were present in 17% and 1.3% of the participants, respectively (Table 1).

Risk factors for severe anemia (n = 3,509)

With unadjusted analysis (Table 2), severe anemia was significantly more

Table 1
Socio-demographic, anthropometric and health characteristics of study participants.

Characteristic	% [a]
Age (yrs) (*n* = 3,531)	
< 20	14.2
20-30	81.3
> 30	4.5
Literate (*n* = 3,531)	
No	79.0
Yes	21.0
Household size (*n* = 3,531)	
1-4	25.9
5-10	58.6
> 10	15.5
Work for wages (*n* = 3,531)	
No	87.4
Yes	12.6
Parity (*n* = 3,531)	
0	34.9
1-4	61.5
≥ 5	3.6
Gestational age (wks) (*n* = 3,531)	
< 26	56.0
≥ 26	44.0
Visits to antenatal clinic (*n* = 3,531)	
0	38.9
1-2	51.5
≥ 3	9.5
Weight (kg) (*n* = 3,530)	
Mean (SD)	46.0 (5.8)
Height (cm) (*n* = 3,530)	
Mean (SD)	150.9 (5.6)
≤ 147.3	25.0
147.4-150.9	25.0
151.0-154.5	25.2
> 154.5	24.8
MUAC (cm) (*n* = 3,530)	
Mean (SD)	22.3 (2.0)
≤ 21.0	26.2
21.1-22.2	23.6
22.3-23.6	25.2
> 23.6	25.0
Hookworm infection (*n* = 3,003)	
Negative (0 egp)	70.9
Light (1-1,999 epg)	27.5
Moderate (2,000-3,999 epg)	1.1
Heavy (≥ 4,000 epg)	0.5

Characteristic	% [a]
Ascaris lumbricoides infection (*n* = 3,003)	5.5
Trichuris trichiura infection (*n* = 3,003)	1.3
Illness past 4 wks (*n* = 3,531)	
Respiratory infection	16.2
Diarrhea	8.4
Fever	14.5
NVTT score (*n* = 3,529)	
7 (passed NVTT)	90.7
5-6	5.8
≤ 4	3.5
Iron supplements (*n* = 3,531)	
No	48.5
Tonic	10.9
Iron tablets	30.9
Iron tablets + tonic	9.7
Hb (g/dl) (*n* = 3,509)	
Mean (SD)	10.87 (1.57)
≥ 11.0	51.0
10.0-10.9	23.6
8.0-9.9	21.2
< 8.0	4.2
sTfR (mg/l) (*n* = 461)	
Mean (SD)	7.93 (5.53)
≤ 8.5	69.4
> 8.5	30.6
Retinol (µmol/l) (*n* = 466)	
Mean (SD)	1.12 (0.42)
< 0.7	13.5
0.7-1.05	31.6
> 1.05	54.9
RBP (µmol/l) (*n* = 461)	
Mean (SD)	0.92 (0.26)
< 0.7	20.4
0.7-1.05	50.3
> 1.05	29.3
CRP (mg/l) (*n* = 461)	
Mean (SD)	3.19 (4.97)
≤ 5.0	83.1
> 5.0	16.9
AGP (mg/l) (*n* = 461)	
Mean (SD)	0.44 (0.18)
≤ 1.0	98.7
> 1.0	1.3

SD, standard deviation; MUAC, mid-upper arm circumference; epg, eggs per gram (of feces); NVTT, night vision threshold test; Hb, hemoglobin; sTfR, soluble transferrin receptor; RBP, retinol binding protein; CRP, C reactive protein; AGP, α_1-acid glycoprotein.

[a] Values are given as percentages except where indicated mean (SD) is reported.

[b] Biochemical measurements were performed on a subset of the study population. The subset consisted of the first 479 consecutive women enrolled in the study.

prevalent among women 20 to 30 years old who were illiterate, worked for wages, had a small MUAC, had hookworm infection, failed the NVTT and did not take any iron supplements. After adjusting for variables in the multivariate model (Table 2), associations with severe anemia remained statistically significant for age [adjusted odds ratio (AOR): 2.05; 95% CI 1.00-4.21; $p<0.05$], working for wages (AOR: 1.94; 95% CI 1.23-3.09; $p<0.01$), gestational age ≥26 weeks (AOR: 1.50; 95% CI 1.03-2.20; $p<0.05$) and MUAC>23.6 cm (AOR:0.47; 95% CI 0.26-0.84; $p<0.05$). The risk for severe anemia increased with increasing intensity of hookworm infection; pregnant women with a moderate to heavy hookworm load (≥2,000 epg) were 4.26 (95% CI 1.67-10.89; $p<0.01$) times more likely to develop severe anemia than uninfected women. *T. trichiura* showed a trend for increased prevalence of severe anemia ($p=0.06$) among infected participants. Women who failed the NVTT with a score ≤4 increased the odds for severe anemia by 2.28 (95% CI 1.14-4.59; $p<0.05$) (Table 2).

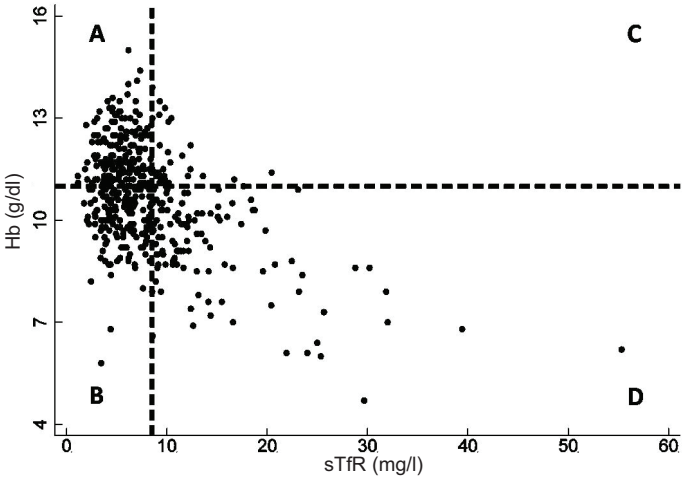

Fig 1–Association between serum soluble transferrin receptor (sTfR) and hemoglobin (Hb). Pearson $r = -0.49$ ($p < 0.001$). The interrupted lines inside the graph represent cutoff concentrations for sTfR (8.5 mg/l) and Hb (11.0 g/dl). Quadrants include the following observations: A) normal (38.7%); B) anemia without iron deficiency (30.6%); C) iron deficiency without anemia (7.0%); D) iron deficiency anemia (23.7%).

The average daily total dietary iron intake was 13.2±9.7 mg. Participants had low heme iron (0.3±0.7 mg/day) and high phytate intake (3.9±2.2 g/day). The prevalence of severe anemia did not differ significantly by intake of heme, non-heme and total iron, phytates and tea. Intake of iron supplements was protective against developing severe anemia (AOR: 0.54; 95% CI 0.34-0.86; $p<0.01$). Tonic, when taken in addition to iron tablets, appeared to have an added protective effect (AOR: 0.31; 95% CI 0.12-0.80; $p<0.05$) (Table 2).

Risk factors for anemia without iron deficiency, iron deficiency without anemia and IDA (n = 479)

The iron status outcome categories (prevalence rates) were: normal (38.7%), anemia without iron deficiency (30.6%), iron deficiency without anemia (7.0%) and IDA (23.7%) (Fig 1). The results of multinomial logistic regression analysis indicate NVTT score, height and iron supplement intake were common risk factors for any two of the three abnormal iron status categories (Table 3). The NVTT score was a strong risk factor for iron deficiency regardless of the presence of anemia. Relative to the normal iron status group, women with a NVTT score of 5

Table 2
Risk factors for severe anemia (Hb<8 g/dl) among a sample of rural Nepali pregnant women.

	Unadjusted[a]		Adjusted[b]		
	n	% Hb<8 g/dl	*n*	AOR	95% CI
Age (yrs)					
< 20	499	2.2	433	1.0	
20-30	2,854	4.6	2,382	2.05[d]	1.0-4.21
> 30	156	3.8[d]	87	2.17	0.67-7.03
Literate					
No	2,773	4.7	2,273	1.0	
Yes	736	2.3[e]	629	0.6	0.34-1.07
Work for wages					
No	3,069	3.9	2,540	1.0	
Yes	440	6.5[d]	362	1.94[e]	1.23-3.09
Parity					
0	1,219	3.4	1,060	1.0	
1-4	2,162	4.9	1,842	1.08	0.69-1.69
≥ 5	128	0.8[d]	98	-[c]	
Gestational age (wks)					
< 26	1,970	3.7	1,663	1.0	
≥ 26	1,539	4.9	1,239	1.50[d]	1.03-2.20
MUAC (cm)					
≤ 21.0	921	5.9	742	1.0	
21.1-22.2	833	4.2	692	0.69	0.42-1.15
22.3-23.6	884	4.5	737	0.95	0.59-1.52
> 23.6	871	2.2[e]	731	0.47[d]	0.26-0.84
Hookworm infection					
0 epg (negative)	2,127	3.4	2,053	1.0	
1-999 epg	744	5.1	720	1.40	0.93-2.11
1,000-1,999 epg	81	11.1	81	3.02[e]	1.39-6.59
≥ 2,000 epg	48	12.5[f]	48	4.26[e]	1.67-10.89
Trichuris trichiura infection					
No	2,960	4.1	2,863	1.0	
Yes	40	10.0	39	2.30	0.75-7.07
NVTT score					
7 (passed NVTT)	3,184	3.8	2,619	1.0	
5-6	202	7.9	176	1.73	0.93-3.21
≤ 4	123	8.9[f]	107	2.28[d]	1.14-4.59
Iron supplements					
No	1,707	5.4	1,411	1.0	
Tonic	378	4.5	312	1.13	0.65-1.96
Iron tablets	1,087	2.8	899	0.54[e]	0.34-0.86
Iron tablets + tonic	337	2.1[e]	280	0.31[d]	0.12-0.80

Hb-hemoglobin; AOR-adjusted odds ratios; CI, confidence interval; MUAC, mid-upper arm circumference; epg, eggs per gram (of feces); NVTT, night vision threshold test.
[a] χ^2 test for differences in proportions of women with Hb<8 g/dl between categories.
[b] AORs were obtained from a multivariate logistic regression model. 95% CIs that do not include 1 are considered statistically significant according to the χ^2 test.
[c] No subject with parity ≥ 5 was severely anemic; therefore, 98 observations were not used in the model because parity ≥ 5 predicted failure perfectly.
Significant at [d]$p<0.05$; [e]$p<0.01$; [f]$p<0.001$.

Table 3
Risk factors for iron deficiency, anemia, and iron deficiency anemia compared to subjects with normal iron status among rural Nepali pregnant women: a multinomial logistic regression model.

	Anemia without iron deficiency Hb<11 g/dl sTfR≤8.5 mg/l (*n*=141)		Iron deficiency without anemia Hb≥11 g/dl sTfR>8.5 mg/l (*n*=32)		Iron deficiency anemia Hb<11 g/dl sTfR>8.5 mg/l (*n*=109)	
	RRR[a]	95% CI	RRR[a]	95% CI	RRR[a]	95% CI
Age (yrs)						
< 20	1.0		1.0		1.0	
20-30	1.02	0.50-2.06	0.47	0.17-1.32	1.54	0.64-3.70
> 30	1.74	0.29-10.38	-[b]		6.38	0.87-46.51
Gestational age (wks)						
< 26	1.0		1.0		1.0	
≥ 26	0.89	0.52-1.52	1.08	0.46-2.55	1.63	0.87-3.04
Height (cm)						
≤ 147.3	1.0		1.0		1.0	
147.4-150.9	0.45[c]	0.21-0.93	0.71	0.20-2.58	0.40[c]	0.18-0.90
151.0-154.5	0.70	0.35-1.41	1.53	0.47-4.98	0.34[c]	0.14-0.78
> 154.5	0.82	0.39-1.73	1.55	0.43-5.54	0.76	0.33-1.76
Hookworm infection						
No	1.0		1.0		1.0	
Yes	1.38	0.76-2.49	1.37	0.53-3.54	2.18[c]	1.14-4.16
NVTT score						
7 (passed NVTT)	1.0		1.0		1.0	
5-6	0.79	0.25-2.52	4.73[c]	1.30-17.15	1.42	0.46-4.41
≤ 4	4.79	0.87-26.16	4.64	0.36-60.47	5.88[c]	1.03-33.57
Heme iron (mg/d)						
0.0	1.0		1.0		1.0	
≤ 0.5	1.16	0.62-2.15	0.34[c]	0.12-0.99	0.78	0.38-1.59
> 0.5	0.64	0.33-1.22	0.10[d]	0.02-0.47	0.48	0.23-1.03
Iron supplements						
No	1.0		1.0		1.0	
Tonic	0.26[d]	0.10-0.71	0.29	0.05-1.57	0.45	0.18-1.10
Iron tablets	0.52[c]	0.29-0.94	0.69	0.27-1.79	0.14[e]	0.07-0.30
Iron tablets + tonic	0.37[c]	0.15-0.90	0.37	0.08-1.61	0.11[e]	0.03-0.37

Hb, hemoglobin; sTfR, soluble transferrin receptor; RRR, relative risk ratio; CI, confidence interval; NVTT, night vision threshold test.
[a]RRR estimated from the multinomial logistic regression model (base category: subjects with normal iron status; Hb≥11g/dl and sTfR≤8.5 mg/l; *n*=178; 95% CIs that did not include 1 were considered statistically significant according to the χ^2test.
[b]No subject > 30 years was iron deficient without anemia.
Significant at [c]$p<0.05$; [d]$p<0.01$; [e]$p<0.001$.

or 6 had 4.73 (95% CI 1.30-17.15; $p<0.05$) times the risk for iron deficiency without anemia compared to those who had a score of 7. Failing the NVTT with a score ≤4 increased the risk for developing IDA (RRR: 5.88, 95% CI 1.03-33.57; $p<0.05$). Taller women had a lower risk for developing non-iron-deficient anemia and IDA than shorter ones ($p<0.05$). Tonic and iron tablets were protective against anemia and IDA and had a stronger protective effect when taken together. Hookworm infection doubled the risk (RRR: 2.18, 95% CI 1.14-4.16; $p<0.05$) for IDA. RRR for iron deficiency significantly decreased with increasing daily intake of heme iron (RRR: 0.10, 95% CI 0.02-0.47; $p<0.01$). The same trend existed for IDA but was not significant (RRR: 0.48, 95% CI 0.23-1.03) (Table 3).

DISCUSSION

In this study of rural Nepali pregnant women, hookworm infection, lack of iron supplement intake, impaired dark adaptation and a diet deficient in heme iron were associated with increased risk of severe anemia and iron deficiency. These risk factors differed among non-iron-deficient anemic, iron-deficient anemic and iron-deficient non-anemic women.

The prevalence rates of severe anemia and iron deficiency in our population were much lower than those reported in a previous study (4.2% *versus* 7% and 31% *versus* 81%, respectively) conducted in the *terai* of Nepal from 1994 through 1997 (Dreyfuss *et al*, 2000). This could be due to two reasons. First, iron supplementation coverage in Nepal increased during the years preceding our study (UNICEF, 2006), which could have resulted in a decrease in iron deficiency prevalence. Second, the iron deficiency prevalence reported in a previous study (Dreyfuss *et al*, 2000) could have been overestimated because it was based on serum ferritin levels, which decrease after the first trimester (Baynes, 1996). Two recent studies using different cutoffs for ferritin reported estimates of iron deficiency among Nepali pregnant women that are more consistent with our findings (Bondevik *et al*, 2000; Jiang *et al*, 2005). The sTfR, measured in this study, may be a more sensitive and specific marker of iron deficiency during pregnancy; sTfR levels increase with tissue iron deficiency and with increased erythropoiesis (Ahluwalia, 1998; Beguin, 2003) and unlike conventional laboratory tests, are less likely to be confounded by gestation and acute inflammatory states (Carriaga *et al*, 1991; Ferguson *et al*, 1992).

Globally, 50% of anemia cases are attributed to iron deficiency (WHO, 2002). However, in our study, iron deficiency accounted only for less than half of anemia observed. Our data show borderline significant positive associations with elevated CRP and AGP levels and non-iron-deficient anemia, suggesting the anemia in these women could have been associated with malaria or other chronic infections. Since both heme iron and vitamin B_{12} are present exclusively in animal foods, the low heme iron intake in this study may also indicate low vitamin B_{12} intake.

The prevalence of hookworm infection was lower than that reported in a previous study in Nepal (Dreyfuss *et al*, 2000). Improved distribution of deworming drugs to pregnant women (UNICEF, 2006) could have accounted for this decrease. Interestingly, 75% of hookworm infections occurred in women who worked in the field. Therefore, in addition to deworming, sanitation control programs and the use of footwear should be emphasized.

Heme iron intake, even if minimal, was significantly protective against iron deficiency, but not IDA, the more severe form of iron deficiency. It is possible the iron needs during pregnancy cannot be met by dietary iron alone (Fernandez-Ballart, 2000). Iron supplementation was protective against severe anemia and IDA. Only half of our participants used iron supplements, possibly because of: 1) limited access to iron tablets; 2) decreased awareness of anemia; 3) side effects of iron supplements; and 4) fears of adverse effects on the baby. Thus, having access to ante-natal care (ANC) and education are critical to initiating and continuing iron supplementation. Consumption of tonic should be considered, particularly in areas with a limited supply of iron tablets.

NVTT, a measure of dark adaptation, is a reliable and valid indicator of vitamin A status (Taren *et al*, 2004). In our study women with impaired dark adaptation were more likely to be iron deficient, similar to other studies (Christian *et al*, 1998; Verhoeff *et al*, 1999; Bondevik *et al*, 2000; Dreyfuss *et al*, 2000; van den Broek *et al*, 2000). Supplementation studies have found vitamin A intake increases Hb and reduces the risk of anemia (Suharno *et al*, 1993; Chawla and Puri, 1995; Muslimatun *et al*, 2001; Tanumihardjo, 2002). Vitamin A deficiency is involved in the pathogenesis of anemia through several mechanisms including : 1) decreased erythropoiesis (Jelkmann *et al*, 1997); 2) impaired mobilization of iron stores (Jang *et al*, 2000; Strube *et al*, 2002); and 3) depressed immunity to infection and a consequent increase in the anemia of infection (Thurnham, 1993).

This study had several strengths, including the use of sTfR as a measure of iron deficiency and a large sample size, which makes generalization to similar populations possible. This study also had several limitations. First, we did not directly assess other possible underlying causes of anemia, such as malaria, chronic inflammation and megaloblastic and dilutional anemia. Second, misclassification of participants as iron-deficient or non-iron-deficient could have resulted because sTfR is affected by altered erythropoiesis (Beguin, 2003) and can be confounded by active malaria infection (Ahluwalia, 1998). Finally, the subsample of 479 participants enrolled consecutively over two successive months might not be representative of the whole study population because of seasonal variation in the risk of anemia.

We demonstrated that among rural Nepali pregnant women there are several shared risk factors for iron deficiency, with and without anemia. Major risk factors included hookworm infestations, impaired dark adaptation, lack of iron supplement intake and a diet low in heme iron. The multifactorial profile of anemia suggests iron supplementation alone is not sufficient to correct this health problem in this population and additional approaches are required, including: identification and treatment of night blindness, a regular monitoring system for iron and vitamin A supplementation, nutrition education and control of intestinal helminthes infections.

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