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Anemia, Iron Deficiency, Meat Consumption, and Hookworm Infection in Women of Reproductive Age in Rural area in Andhra Pradesh

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ABSTRACT

Iron deficiency anemia poses an important public health problem for women of reproductive age living in developing countries. A prospective study, comprised of written questionnaires and laboratory analysis of hemoglobin (Hb), ferritin, transferrin receptor, and stool hookworm egg count, was undertaken in 99 non-pregnant women. 37.53% were anemic (Hb < 12 g/dL), and 23.10% were iron deficient (ferritin < 15 ng/L). Hookworm infection was present in 51.3% of women, although heavy infection was uncommon (6.29%). Iron deficiency was more prevalent in anemic than non-anemic women (38.21% versus 14.08%, P < 0.001). Consumption of meat at least three times a week was more common in non-anemic women (51.15% versus 66.67%, P = 0.042). Mean ferritin was lower in anemic women (18.99 versus 35.66 ng/mL, P < 0.001). There was no evidence of a difference in prevalence (15.20% versus 17.23%, P = 0.629) or intensity (171.07 versus 129.93 eggs/g, P = 0.412) of hookworm infection between anemic and non-anemic women. Anemia, iron deficiency, and hookworm infection were prevalent in this population. Intake of meat was more clearly associated with hemoglobin and iron indices than hookworm. An approach to addressing iron deficiency in this population should emphasize on iron supplementation, deworming and better public heigine.

Keywords: Hookworm, anemia, meat consumption, iron deficiency.

INTRODUCTION

It has been estimated that more than one third of the world's women are anemic; the vast majority of this burden occurs in developing countries [1]. This produces an enormous public health impact, because anemia poses a significant mortality and morbidity risk, particularly to women of reproductive age and their children [2]. Iron deficiency is recognized as perhaps the most common cause of anemia worldwide. Even in the absence of anemia, mild to moderate iron deficiency has been associated with impaired physical and cognitive growth of children, [3] reduced work capacity of adolescents and adults, [4] and increased morbidity and mortality in pregnant women and their infants [5]. Iron deficiency exists in a continuum, from a reduction in body storage iron, to iron deficient erythropoiesis, and ultimately to iron-deficient anemia [6]. Women of reproductive age who are iron deficient but not anemic may become anemic during pregnancy as a consequence of increased iron requirements and expanded plasma volume. Hookworm (*Ancylostoma duodenale* and *Necator americanus*) infection causes chronic gastrointestinal blood loss and is the most common worldwide cause of iron deficiency [7]. The impact of hookworm infection on iron stores depends on the intensity of infection and the amount of iron consumed in the diet.

The World Health Organization has suggested that anemia is of "moderate" public health importance where its prevalence is between 20% and 39.9% and "severe" if it occurs in 40% or more of the population. It is recommended that an approach to iron deficiency anemia be especially targeted toward women of reproductive age who risk the attendant consequences of anemia during pregnancy [8]. Integrating this strategy into a community-based program has been found to be effective [9]. A recent meta-analysis has confirmed that antenatal iron supplementation improves both antenatal and post-natal hemoglobin concentration and that side effects of iron supplementation appear more common in women who receive daily iron [10]. For countries in the Western Pacific Region, where anemia prevalence constitutes a public health problem, a strategy of weekly iron supplementation has been recommended, with hookworm control where prevalent [11].

Hookworm infestation is more common in coastal area people in the region of West Bengal. It is commonly seen in Darjeeling, Jalpaigun, Hooghly and Calcutta.The prevalence of hookworm infestation in India is 51.3%.

Thus a prospective, observational study was conducted to assess the prevalence of anemia and factors associated with iron deficiency and anemia in non-pregnant women of reproductive age. This study represents the assessment of hookworm infestation in india and to address the relation between anemia and hookworm infection in these women of reproductive age [12].

MATERIALS AND METHODS

The study was conducted among 99 patients with hookworm infestation in women of reproductive age in rural setup of RR district near Hyderabad, in department of Microbiology in Bhaskar Medical College, Andhra Pradesh.

The institutional Ethics Committee approved the study protocol, informed consent form and the case report form. The study was a prospective, observational study. Duration of the study period is 2 days with Albendazole 400mg, single dose. Inclusion criteria included non-pregnant women from 18-60 years, who had bloody stools with or without anaemia, and all were in good general health, as established by physical, clinical examination and laboratory investigations. Subjects with hypersensitivity to Albendazole were excluded from the study. These women belonged to weak economic streta.

Laboratory methods. Blood and stool samples were collected from each participant. The intensity of hookworm infection was evaluated from the fecal specimen using standard Kato Katz methodology [13] and expressed as eggs per gram of feces (epg). Intensity of hookworm infection was classified according to WHO guidelines: 0 epg classified as nil, up to 2,000 epg classified as light, 2,000–4,000 epg as moderate, and > 4,000 epg classified as severe [14]. The count of eggs per gram of stool has been previously shown to rise with overall gastrointestinal hookworm burden [15]. Hemoglobin was evaluated in the field from finger prick blood, using a hemoglobinometer. A 3-mL sample of venous blood was collected using a closed collection system into tubes containing fast clotting agent. The tubes were spun at 4,000 rpm for 20 minutes at room temperature, after which serum was collected and stored at –4°C. The samples were transported on dry ice to Hyderabad University. Serum ferritin was measured using a sandwich immunoenzymatic assay. Soluble transferrin receptor was evaluated using enzyme-linked immunoassay (ELISA). The ratio of transferrin receptor to log (to base 10) serum ferritin (TfR-F index) was calculated from these results [16]. Serum C-reactive protein (CRP) levels were assessed by an ELISA method. We chose a level of > 10 mg/L to represent a state of inflammation, because this has been previously reported as an appropriate clinical cut-off [17].
Anemia was defined as a hemoglobin concentration of < 12 g/dL and iron deficiency as a serum ferritin of < 15 ng/mL, except where otherwise stated, in concordance with WHO recommendations for women of reproductive age [8]. Transferrin receptor levels of 2.3 µg/mL or above were considered abnormal, based on the manufacturer's reference interval (0.8–2.3 mg/L). The TfR-F index has been previously shown to provide an excellent indicator of iron reserves, with depletion of iron stores implied once the ratio exceeds 1.8 [18]. We adopted this cut-off as an alternative indicator of iron deficiency.

Survey. Participating women were asked to complete a questionnaire covering potential demographic risk factors for iron deficiency and hookworm infection, including dietary meat consumption, ethnic group, education, number of children, domestic sanitary facilities, and frequency of wearing shoes. Current pregnancy status was also assessed. Local village health workers assisted the survey team in administering the survey.

Statistical analysis. The data were entered in the field into spreadsheets using Microsoft Excel (Microsoft Corp., Redmond, WA). The spreadsheets were imported into Stata (Intercooled Stata 9.2 for Windows; StataCorp, College Station, TX) for further analysis. The distribution of laboratory variables was assessed.

Hemoglobin values were approximately normally distributed, enabling use of the arithmetic mean. Ferritin, transferrin receptor, and the TfR-F index were right-skewed; thus, these were log-transformed for subsequent analysis. Hookworm eggs per gram data were right skewed, with a considerable number of zero values. Thus, egg count data were analyzed using the Poisson distribution, incorporating the clustered nature of the survey (by village), with comparisons between groups made by calculating the incidence rate ratio [14] . Median meat consumption was determined.

The prevalence of anemia, iron deficiency, hookworm infection, and iron deficiency anemia were calculated using the cut-offs defined above. Average iron indices, hookworm burden, and meat consumption were calculated and compared between those women who were anemic and those who were non-anemic; the analysis was based on a linear regression model that incorporated the effect of clustering by village. Prevalences of iron deficiency, hookworm infestation, and meat consumption were also compared between anemic and non-anemic groups. Comparison of significance of differences between meat consumption in the anemic and non-anemic groups was performed using a two-sample Wilcoxon rank-sum (Mann-Whitney) test.

Multiple regression analysis incorporating the cluster design of the survey was performed to evaluate the effect of hookworm eggs per gram, meat consumption, and the demographic variables of age, number of children, and level of education on hemoglobin and log transformation of iron indices (ferritin, transferrin receptor, and TfR-F index). The regression equation used the log(eggs per gram) value as described above, and as such, the results for association with hookworm are reported after transformation by taking the inverse logarithm.

RESULTS

The demographic and socio-economic data for the 99 women of reproductive age who participated in the study are presented in Table 1 Two women had CRP levels > 10 mg/L, but because neither had hemoglobin < 12 g/dL and thus did not have evidence of anemia of chronic disease, they were included in the analysis.

TABLE -1 Demographic and socioeconomic data of patients

Parameter	Number (%)
N	99
Marital status	80 (79.20)
Number of children	2(0-5)
Education	
Illiterate	6(5.94)
To grade 5	20(19.80)
Grade 6-9	50(49.50)
Grade 10-12	18(17.82)
Higher	6(5.94)
Frequency of wearing shoes	
Never	10(9.9)
Occasionally	50(49.50)
Always	40(39.60)

The distinct and total means for hemoglobin, iron studies, meat consumption, and stool parasite counts, together with prevalences of anemia, iron deficiency, and hookworm infection, are presented in Table 2. The geometric mean eggs per gram was 144.05 (95% CI 105.53–190.01).

The prevalence of anemia, defined as a hemoglobin < 12 g/dL, was 37.40, and the prevalence of iron deficiency (defined as a serum ferritin concentration < 15 ng/mL) was 23.08. Borderline iron depletion (serum ferritin between 15 and 30 ng/mL) was observed in 22.18 of participants. The prevalence of soluble transferrin receptor concentration above the cut-off of 2.3 mg/mL was 22.05%, similar to iron deficiency. The TfR-F index was < 1.8 for 19.90 of the participants.

The overall prevalence of hookworm infection was 51.3%; however, most participants 60%, had a light worm burden.

Table- 2 Hematological parameters, meat intake and hook worm infestation in patients

Parameters	**Patients**
Hemoglobinn Mean (95% CI) Proportion anemic(%) <12g/dL <10g/dL <7g/dL	99 12.20 (12.04, 12.37) 37.40 (32.30,42.89) 8.27 (5.38, 11.20) 1.12 (0.01, 2.25)
Ferritin (ng/mL) Mean (95% CI) Proportion Ferritin < 15ng/mL Ferritin <30ng/mL Ferritin 15 to 30 ng/mL	 28.15 (25.20, 31.40) 23.08 (18.50, 27.60) 45.30 (39.89, 50.72) 22.18 (17.67, 26.69)
Transferrin receptor (mg/mL) Mean (95% CI) Proportion Transferrin receptor > 2.3	 1.68 (1.60, 1.76) 22.05 (17.58, 26.49)
TfR-F index Mean (95% CI) TfR index > 1.8	 1.20 (1.15, 1.30) 19.90 (15.54, 24.25)
Meat intake Median (range)	 3(0,4)
Hook worm (epg) Mean (95% CI)	 144.05 (105.53, 190.01)

A comparison of indices of iron stores, hookworm infection, and meat consumption between anemic and non-anemic women is shown in Table 3. The mean indices of iron stores among the anemic group were significantly different to those of the non-anemic group. Median meat consumption was also lower among the anemic population (P = 0.001). However, there was no evidence of significant difference in hookworm eggs per gram [incidence rate ratio, 1.20; 95% confidence interval (CI), 0.91–2.21; P = 0.25].

TABLE—3 Comparison between the prevalence of iron deficiency, meat consumption, and hookworm infection in anemic and non-anemic women

Variable	Anemic (Hg<12g/dL) Mean (95% CI)	Non-anemic (Hg>12g/dL) Mean (95% CI)	p-value
Ferritin Percent ferritin < 15ng/mL Percent ferritin < 30ng/mL Ferritin 15 to < 30ng/mL	 35.28 (28.09, 40.60) 60.76 (53.10, 70.58) 24.08 (17.08,30.16)	 13.16 (9.28, 17.84) 36.45 (27.56, 42.18) 21.54 (14.98, 26.98)	 <0.001 <0.001 0.56
Transferrin receptor Percent transferring receptor >2.3	 37.96 (28.78, 45.67)	 12.46 (8.02,16.85)	 <0.001
TfR-F index Percent TfR-F index > 1.8	 36.50 (27.89, 44.56)	 17.08 (11.43, 20.97)	 <0.001
Meat consumption Pecent meat consumption > 3 servings/wk	 50.96 (41.31, 58.68)	 68.90 (60.45, 74.30)	 0.05
Hookworm eggs per gram	168.08 (102.89, 289.60)	128.78 (89.90, 180.67)	0.32

A comparison between the prevalence of iron deficiency, meat consumption, and hookworm infection in anemic and non-anemic women is shown in Table 3. Of 38 anemic participants in this analysis, 35.28 had a ferritin level < 15 ng/mL, compared with 13.16 who were not anemic ($P < 0.001$). There was no evidence of difference in prevalence of borderline iron deficiency (serum ferritin, 15–30 ng/ mL) between anemic and non-anemic women. Similar differences between the two groups were observed for the other markers of iron deficiency. The proportion of participants consuming meat more than three times per week was greater among the non-anemic population ($P = 0.05$). Prevalence of moderate or severe hookworm infection was not significantly different between the anemic and non-anemic groups. In particular, severe hookworm infection (> 4,000 epg) was not significantly more common among anemic women (6.85%) than non-anemic women (5.64%; $P = 0.32$).

There was weak evidence of inverse association with meat consumption (coefficient, –0.15; 95% CI, –0.36, 0.00; $P = 0.05$). Hookworm, hemoglobin, and iron indices were not associated with frequency wearing of shoes or type of latrine used

DISCUSSION

We found that the prevalence of anemia and iron deficiency was 31% in reproductive age group women. Iron deficiency was more common among anemic women, although less than one half of the cases of anemia could be attributed to iron deficiency. Both iron deficiency and anemia were associated with a lower weekly consumption of meat. Hookworm infection was common and, although not associated with anemia in this population, was correlated with iron deficiency. Demographic indicators such as age, number of children, and education were not associated with iron deficiency or anemia.

Associations between hemoglobin and iron with meat consumption were not part of the *a priori* hypothesis. However, the association is robust ($P < 0.05$) and physiologically feasible. In this study, iron deficiency was more prevalent among the anemic population, but only approximately one third of women with anemia had a low serum ferritin. The true prevalence of iron deficiency anemia may be higher than this because ferritin is an acute phase protein that may be elevated in inflammation. To address this, we used alternative indices of iron deficiency—soluble transferrin receptor and the TfR-F index [16] and confirmed that the prevalence of iron deficiency was similar using the TfR (> 2.3 µg/mL), the TfR-F index (> 1.8), and serum ferritin (< 15 ng/mL), both in the overall and in the anemic groups. This suggests that the newer indices are comparable to ferritin for detecting iron deficiency in this population. Combining the results of both serum ferritin and the TfR-F index provides a higher prevalence of iron deficiency, both overall and among the anemic group, implying that the cases detected by each method do not completely overlap. Iron depletion (ferritin = 15–30 ng/mL) was present in 23.58% of anemic women (and a similar proportion of the non-anemic sample), raising the possibility that iron supplementation may have a greater than expected impact in the long term because both anemic and non-anemic women would be at risk of developing or exacerbating iron deficiency anemia should their iron requirements increase, for example, during pregnancy.

Inadequate iron intake, particularly caused by reduced access to heme iron, which is chiefly found in meat and is highly bioavailable, can contribute to iron deficiency [19].Our analysis showed that meat intake was associated with iron status, and there was a strong trend toward a positive correlation with hemoglobin. This suggests that failure of adequate iron intake is an important contributor to iron deficiency in this region. Although demographic factors were not associated with outcomes of iron deficiency or anemia, there was an association between education and increasing meat intake. This suggests better educated women can afford, or make it a priority to include, meat in their diet. These findings provide a rationale to include regular iron fortification or supplementation in any strategy aimed at alleviating iron deficiency

This study showed that hookworm infestation is prevalent in rural setup around Hyderabad with 78.14% of women in the study are infected. However, heavy infection (> 4,000 epg) was relatively uncommon. The mean hookworm burden remained "light," even if the uninfected population was removed from the analysis, suggesting that hookworm burden was low among those who were infected. This may be explained by recent school-based deworming activities conducted in the area. Increasing intensity of hookworm infestation was related to iron deficiency, suggesting that hookworm is a contributing factor to gastrointestinal blood loss in women.

The lack of an association with anemia may be caused by the low prevalence of moderate and heavy hookworm infection and the large number of anemic women who were not iron deficient, suggesting other causes of anemia may be prevalent in this population. Other nutritional deficiencies [20], and vitamin A [21] have been such as of folate, vitamin B_{12}, shown to contribute to anemia. Inflammation can produce an anemia of chronic disease, characteristically associated with an elevated CRP and ferritin [17]. However, the finding of a normal CRP in > 99% of the study group makes it unlikely that this had an impact on the results. Genetic hemoglobinopathies, such as α-thalassemia and hemoglobin E may also cause anemia. These have been found to be common among certain populations in Southeast Asia [22] and may have contributed to anemia in some of the study participants.

Our study confirmed that iron deficiency anemia is a major public health problem in women of reproductive age in rural setup around Hyderabad. Lack of dietary iron and hookworm infection both contribute to iron deficiency, and therefore, a program of iron supplementation and deworming would be beneficial in this area. Further study to define the other causes of anemia will be important for planning national anemia prevention strategies.

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