

Iron, folate, and vitamin B₁₂ stores among pregnant women in a rural area of Haryana State, India

Priyali Pathak, Umesh Kapil, C.S. Yajnik, S.K. Kapoor, S.N. Dwivedi, and Rajvir Singh

Abstract

Background. Iron, folate, and vitamin B₁₂ deficiencies have adverse effects on pregnancy outcome. In India, data on the concomitant prevalence of these deficiencies among pregnant women are meager.

Objective. We conducted a community-based study to assess the prevalence of deficiencies of iron, folate, and vitamin B₁₂ among pregnant women in a rural block of Haryana State.

Methods. The study was approved by the ethics committee of the All India Institute of Medical Sciences, New Delhi. A total of 283 pregnant women were enrolled in the study. After oral informed consent had been obtained from the women, blood was drawn from the antecubital vein for estimation of the levels of serum ferritin by enzyme-linked immunosorbent assay (levels < 12 ng/mL were considered as indicative of poor iron stores); serum folate was determined by radioimmunoassay (levels < 3 ng/mL were considered as indicative of poor folate stores); and serum vitamin B₁₂ was estimated by the microbiologic method (levels < 200 pg/mL were considered as indicative of poor vitamin B₁₂ stores).

Results. The results indicated that 67.7%, 26.3%, and 74.1% of the women had poor iron, folate, and vitamin B₁₂ stores, respectively. Concomitant deficiencies of iron, folate, and vitamin B₁₂ occurred in 16.2% of the women. We found that 59.9% of the women were consuming less than 75% of the recommended daily caloric allowance

(2,175 kcal), indicating an overall poor food intake. This could be one of the predominant reasons for poor iron, folate, and vitamin B₁₂ stores among the women.

Conclusions. Our findings suggest that apart from iron and folate, vitamin B₁₂ deficiencies may play an important role in causing anemia.

Key words: Folate, India, iron, pregnant women, vitamin B12

Introduction

Iron, folate, and vitamin B₁₂ deficiencies have adverse effects on pregnancy outcome [1]. Iron deficiency during pregnancy results in poor fetal growth, prematurity, and even intrauterine death due to severe anemia. It is also associated with increased maternal morbidity and mortality [2]. Folate deficiency during pregnancy is associated with low birthweight and neural tube defects [2–4]. Strong associations of vitamin B₁₂ status with intrauterine growth retardation have been documented [5, 6].

In India, data on status of iron, folate, and vitamin B₁₂ deficiency are limited. Similarly, data on the concomitant prevalences of these deficiencies among pregnant women are meager. Limited data are available from hospital-based studies, and there are no data from community-based studies; hence, we conducted a community-based study to assess the prevalence of deficiencies of iron, folate, and vitamin B₁₂ among pregnant women in a rural block of Haryana State, India.

Methods

The study was conducted in a rural block of Faridabad District, Haryana State, India. Six villages were randomly selected from the block by a multistage sampling procedure. All pregnant women with 28 weeks or more

Priyali Pathak and Umesh Kapil are affiliated with the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi, India; C.S. Yajnik is affiliated with the Diabetes Unit, KEM Hospital Research Centre, Pune, India; S.K. Kapoor is affiliated with the Centre for Community Medicine, All India Institute of Medical Sciences, New Delhi; S.N. Dwivedi and Rajvir Singh are affiliated with the Department of Bio-Statistics, All India Institute of Medical Sciences, New Delhi, India.

Please direct queries to the corresponding author: Umesh Kapil, Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi 110 029, India; e-mail: umeshkapil@yahoo.com.

of gestation were enrolled. The study was approved by the ethics committee of the All India Institute of Medical Sciences, New Delhi. The objectives of the study were explained to the women, and oral informed consent was obtained. The following inclusion criteria were utilized: the pregnant women were at least 18 years or more of age, were not suffering from any chronic diseases, and were not suffering from any acute morbid condition on the day of the survey. Identification data (name, age, education, socioeconomic status, and duration of pregnancy) were also collected by a pretested, semistructured questionnaire. The socioeconomic status of the women was assessed by the Udai Parikh classification [7].

Blood was drawn from the antecubital vein and collected in amber-colored polypropylene tubes. The samples were transported in ice packs to the central laboratory at the All India Institute of Medical Sciences, New Delhi, for separation of serum by centrifuging within 2 to 3 hours of blood collection. The serum was subsequently collected in Eppendorf vials, labeled, and stored at -80°C until analysis. Estimates of serum ferritin, folate, and vitamin B_{12} were performed within 6 months of blood collection.

To assess the status of iron, serum ferritin levels were assessed by the standard enzyme-linked immunosorbent assay (ELISA) method [8] with the use of a Micro-well Enzyme Immunoassay Human Ferritin Quantitative EIA Kit (Bioplus). Serum ferritin levels less than 12 ng/mL were indicative of iron deficiency and hence poor iron stores [9]. The status of serum folate was assessed by estimating the serum folate levels by the standard radioimmunoassay method with the use of the Simul TRAC-SNB Radioassay Kit. Levels less than 3 ng/mL were considered as indicative of folate deficiency [10]. Serum vitamin B_{12} was estimated by the microbiologic method [11]; levels less than 200 pg/mL were considered as indicative of vitamin B_{12} deficiency [12]. Serum samples with known concentrations of ferritin, folate, and vitamin B_{12} were assayed with each batch as an internal quality control. The estimation was repeated for any batch in which the control sample values were over- or underestimated.

Caloric intake was assessed by the 24-hour dietary recall method [13]. Recommended dietary allowances of the Indian Council of Medical Research [14] were utilized to assess the adequacy of nutrient intake by the women.

Limitations of the study

Data on dietary intake of nutrients (iron, folic acid, and vitamin B_{12}) could not be assessed. However, the research team was able to calculate the caloric intake of the pregnant women as a measure of their overall food intake. Serum C-reactive protein levels could not be assessed because of limited resources. However, preg-

nant women with current morbidity were excluded in the present study. The research team could not follow up the women to determine the outcomes of their pregnancies because of limited resources.

Results

A total of 283 pregnant women (mean age, 22.9 ± 3.3 years) were enrolled in the study. One hundred thirty-nine (49.1%) were between 18 and 22 years of age. The majority (81.9%) belonged to the lower middle and middle classes, and 31.8% were illiterate. Blood was collected from 266 women; 17 women refused because of fear or cultural reasons. The characteristics of these women were similar to those of the women who consented to provide blood samples.

We found that 67.7%, 26.3%, and 74.1% of the women were deficient in iron, folate, and vitamin B_{12} , respectively. We also analyzed the data to determine the concomitant prevalence of iron, folate, and vitamin B_{12} deficiency. We found that 48.9% of the women were deficient in both iron and vitamin B_{12} , and 16.2% were deficient in iron, folate, and vitamin B_{12} (**table 1**).

Caloric intake could be assessed in 225 women. Seventy percent of the women were vegetarians. The mean intake was 1,527 kcal/day, which is only 70.2% of the recommended daily allowance (RDA) of 2,175 kcal/day. We found that 59.9% of the women were consuming less than 75% of the RDA, indicating an overall low food intake.

Discussion

In the present study, 67.7% of the pregnant women had iron deficiency, as indicated by low serum ferritin levels. An earlier hospital-based study reported that 56% of pregnant women had low serum ferritin levels [15]. Another hospital-based study in Delhi reported low serum ferritin levels among 56.6% of pregnant women [16]. The variation in iron stores among these studies could be due to differences in the methods used for estimation of serum ferritin, differences in the

TABLE 1. Distribution of low stores of iron, folate, and vitamin B_{12} among 266 pregnant women

Nutrient	No. (%) of women
Iron (serum ferritin < 12 ng/mL)	180 (67.7)
Folate (serum folate < 3 ng/mL)	70 (26.3)
Vitamin B_{12} (serum vitamin B_{12} < 200 pg/mL)	197 (74.1)
Iron and folate	51 (19.1)
Iron and vitamin B_{12}	130 (48.9)
Folate and vitamin B_{12}	58 (21.8)
Iron, folate, and vitamin B_{12}	43 (16.2)

duration of pregnancy of the study subjects, variation in dietary intake, or a combination of these factors.

In the present study, 26.3% of the women had folate deficiency. The findings of our study are similar to those of studies conducted earlier in India, which reported a prevalence of folate deficiency in the range of 21% to 30% [17–19].

Almost 74.1% of the women in the present study had vitamin B₁₂ deficiency. Limited data are available from India on the prevalence of vitamin B₁₂ deficiency among pregnant women. Vitamin B₁₂ is available from foods of animal origin (liver, kidney, eggs, fish, and milk) [20]. The high prevalence of vitamin B₁₂ deficiency could possibly be due to the low dietary intake of vitamin B₁₂ [21].

The majority of the women in our study had low levels of serum ferritin and vitamin B₁₂. The high prevalence of these deficiencies could be predominantly due to low dietary intake of the nutrients, as indicated by the low energy intake of the women. It has been well documented that rural pregnant women in Haryana State, India, have a significantly lower food intake than India's recommended dietary intakes [22].

We are not aware of any studies in the published

literature that document the prevalence of concomitant deficiencies of iron, folate, and vitamin B₁₂ among pregnant women, and hence we could not compare our results with those of other similar studies.

The present study found a high prevalence of iron, folate, and vitamin B₁₂ deficiency among pregnant women in the area studied. The findings of our study have relevance to the development of strategies for the prevention of deficiencies of these nutrients among pregnant women, 30% to 90% of whom have low hemoglobin levels, indicative of poor iron stores and resulting in anemia [23, 24]. Our findings suggest that in addition to iron and folate deficiencies, vitamin B₁₂ deficiencies may play an important role in the causation of anemia.

Acknowledgments

We acknowledge the financial support provided by the Director General, Indian Council of Medical Research, New Delhi. The infrastructure facilities provided by the Director, All India Institute of Medical Sciences, are also acknowledged.

References

1. Rajaratnam J, Abel R, Ganesan C, Jayaseelan SA. Maternal anemia: A persistent problem in rural Tamil Nadu. *Natl Med J India* 2000;13:242–5.
2. Scholl TO, Johnson WG. Folic acid: Influence on the outcome of pregnancy. *Am J Clin Nutr* 2000;71(5 suppl):1295S–303S.
3. Iyengar L, Rajalakshmi K. Effect of folic acid supplement on birth weights of infants. *Am J Obstet Gynecol* 1975;122:332–6.
4. Neggers YH, Goldenberg RL, Tamura T, Cliver SP, Hoffman HJ. The relationship between maternal dietary intake and infant birthweight. *Acta Obstet Gynecol Scand Suppl* 1997;165:71–5.
5. Muthayya S, Kurpad AV, Duggan CP, Bosch RJ, Dwarakanath P, Mhaskar A, Mhaskar R, Thomas A, Vaz M, Bhat S, Fawzi WW. Low maternal vitamin B₁₂ status is associated with intrauterine growth retardation in urban South Indians. *Eur J Clin Nutr* 2006;60:791–801.
6. Yajnik CS, Deshpande SS, Panchanadikar AV, Naik SS, Deshpande JA, Coyaji KJ, Fall C, Refsum H. Maternal total homocysteine concentration and neonatal size in India. *Asia Pac J Clin Nutr* 2005;14:179–81.
7. Parikh U. *Manual of the socio-economic status scale (rural)*. Delhi, India: Manasayan, 1981.
8. Forman DT, Parker SL. The measurement and interpretation of serum ferritin. *Ann Clin Lab Sci* 1980;10:345–50.
9. World Health Organization. *Iron deficiency anemia, assessment, prevention, and control: A guide for program managers*. Geneva: WHO, 1999:87–99.
10. Chanarin I. *The assay and concentration of folate in blood and other tissues: The megaloblastic anemias*. Oxford, UK: Blackwell Scientific Publications, 1969:306–36.
11. Kelleher BP, Broin SD. Microbiological assay for vitamin B₁₂ performed in 96-well microtitre plates. *J Clin Pathol* 1991;44:592–5.
12. Babior BM, Bunn HF. Megaloblastic anemias. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL, eds. *Harrison's principles of internal medicine*, 14th ed. New York: McGraw-Hill, 1998:653–8.
13. Thimmayamma BVS. *A handbook of schedules and guidelines in socio-economic and diet surveys*. Hyderabad: National Institute of Nutrition–Indian Council of Medical Research Press, 1987.
14. Gopalan C, Ramasastri BV. *Nutritive value of Indian foods*. Hyderabad: National Institute of Nutrition–Indian Council of Medical Research Press, 2002: 156–9.
15. Reddaiah VP, Prasanna Raj P, Ramachandran K, Nath LM, Sood SK, Madan N, Rusia U. Supplementary iron dose in pregnancy anemia prophylaxis. *Indian J Pediatr* 1989;56:109–14.
16. Madan N, Rusia U, Manocha A, Sood SK. Detection of iron deficiency in pregnancy by haemoglobin, serum ferritin and protoporphyrin/haem ratio. *Indian J Med Res* 1988;87:246–51.
17. Iyengar L. Folic acid requirements of Indian pregnant women. *Am J Obstet Gynecol* 1971;111:13–6.
18. Chandrashekhkar U, Malathi D, Hemalatha KPA. Availability of folic acid from the diets of a group of expectant and non-expectant women in Coimbatore city. *Indian J Nutr Dietet* 1980;17:363–9.

19. Vijyalakshmi P, Shobhana R. Impact of iron and folic acid supplementation on expectant mothers and their off springs. *Indian J Nutr Dietet* 1982;19:363–9.
20. Abrol P. Vitamin B complex deficiencies. In: Gupte S, ed. *Textbook of pediatric nutrition*. New Delhi: Peepee Publishers and Distributors, 2006:10–208.
21. Antony AC. Prevalence of cobalamin (vitamin B-12) and folate deficiency in India—audi alteram partem. *Am J Clin Nutr* 2001;74:157–9.
22. Jood S, Bishnoi S, Khetarpaul N. Nutritional status of rural pregnant women of Haryana State, Northern India. *Nutr Health* 2002;16:121–31.
23. Toteja GS, Singh P, Dhillon BS, Saxena BN, Ahmed FU, Singh RP, Prakash B, Vijayaraghavan K, Singh Y, Rauf A, Sarma UC, Gandhi S, Behl L, Mukherjee K, Swami SS, Meru V, Chandra P, Chandrawati, Mohan U. Prevalence of anemia among pregnant women and adolescent girls in 16 districts of India. *Food Nutr Bull* 2006;27:311–5.
24. International Institute for Population Sciences (IIPS) and ORC Macro. *National Family Health Survey (NFHS-2), 1998–99: India*. Mumbai: IIPS, 2000.