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Baseline Iron Indices as Predictors of Haemoglobin Improvement in Anemic Vietnamese Women Receiving Weekly Iron-Folic Acid Supplementation and De-Worming

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Abstract

Iron deficiency anemia is a highly prevalent problem among women living in rural Vietnam. However, the utility and cutoffs of indices for diagnosing iron deficiency anemia in the public health context are ill-defined. We sought to assess the ability of iron indices to predict the haemoglobin response (HBR) to weekly iron-folic acid supplementation (WIFS) in anemic rural Vietnamese women. We compared haemoglobin, serum ferritin and soluble transferrin receptor in a cohort of 221 non-pregnant women of reproductive age before and after three months of WIFS and deworming. At baseline, anemia (Hb<120g/L) was present in 81/221(36.7%) of subjects. After 3 months, anemia prevalence fell to 58/221(26.2%), and the mean haemoglobin change was +3.5 g/L (95% CI [0.9, 6.6]). A haemoglobin response was observed in 50/75 (66.6%) of anaemic women. For this population, measurement of transferrin receptor adds little to measurement of ferritin. We propose a ferritin cut-off below 30ng/mL as a more sensitive predictor of response to WIFS.

Keywords

Iron-deficiency anemia; ferritins; Iron metabolism disorders; Vietnam; Women

Introduction

Globally it is estimated that 41.8% of pregnant and 30.2% of non-pregnant women are anaemic, with the main burden occurring in developing countries in Africa and Asia.¹ The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have reiterated the importance of anemia control at the public health level.² Iron deficiency is believed to be the main cause of anemia and iron deficiency and anemia are linked to increased maternal morbidity and mortality, impaired functional capacity in women.³

Weekly iron-folic acid supplementation (WIFS) is currently proposed for non-pregnant women as an effective, safe and practical anemia prevention strategy.⁴ Although the proportion of anemia attributable to iron deficiency varies in different settings,^{5, 6} WHO currently recommends universal iron-folic acid supplementation for all women living in populations where the prevalence of anemia is above 20%.⁷ Given that testing for iron indices is relatively expensive and requires specialised laboratory resources, WHO guidelines do not insist on specifically establishing the prevalence of iron deficiency.⁸ Other causes of anemia, including Vitamin A deficiency and genetic haemoglobinopathies, have been identified as common in South East Asian populations ^{9, 10} and it is possible that population estimates of anemia in this region may not accurately reflect the burden of iron deficiency. This may influence the proportion of recipients of universal iron supplementation who could be expected to respond to such an intervention.

A firm diagnosis of iron deficiency anemia can be established if an improvement in haemoglobin to a trial of iron occurs¹¹. Iron indices may also be used to identify iron status: ferritin is an accurate indicator of total body iron stores, especially in the absence of

inflammation 12 and is often used as an indicator of iron status in populations. However, as with all diagnostic tests, sensitivity and specificity depend on the cut-off used 13. For adults, a cut-off of <15ng/mL is recommended by WHO as an indication of iron deficiency 8. Soluble transferrin receptor (TfR) reflects tissue iron status and is an alternate index, but its role in population evaluations of iron status remains unclear 14, and reference ranges differ between manufacturer's assays 15. TfR is also elevated in patients with increased haematopoiesis as is found in the haemoglobinopathies 16. The TfR/ log (ferritin) ratio (TfR-F index) appears to closely reflect marrow iron 17. Overall, the selection of indices for use in monitoring of field iron supplementation programmes, and the thresholds to define iron depletion or repletion, remain indistinct 14.

We have implemented a universal WIFS and de-worming program for women of reproductive age in two districts of the northern mountainous province of Yen Bai, Viet Nam from May 2006. Estimates of prevalence of iron deficiency amongst women in Vietnam range from 22% to 86.3%.^{18, 19} The broad aims of this study were to determine the burden of anemia and iron deficiency in non-pregnant women of reproductive age in this region of Vietnam,²⁰ and to explore associations between various baseline variables and their effect on response to the intervention. In this paper, we describe the effect of the intervention in terms of the change in haemoglobin and iron indices from baseline to 3 months, and investigate the value of iron indices to predict whether anaemic women of reproductive age will have a haemoglobin response. The baseline survey was conducted in November 2005. The three month follow up survey was conducted in August 2006.

Methods

Study site and subjects

Yen Bai province, a mountainous, rural region of northwest Vietnam, was selected for implementation of the WIFS/de-worming program. Almost one third of communes in the area are considered to have 'very poor' socio-economic status.²¹ Malaria has been eradicated from this region and there were only two imported cases reported in Yen Bai in 2005 (Phu L, Director Malaria Control Program, Yen Bai Province, Personal Communication).

Intervention

From May 2006, a programme of weekly iron-folic acid supplementation and de-worming every 4 months for all women of reproductive age living in Yen Bai province was implemented by active distribution through the existing primary health structure. Women received packs of four ferrous sulphate/folic acid tablets (60mg/0.4mg, UNICEF, Copenhagen) each month from village health workers. Albendazole (400 mg, UNICEF, Copenhagen) was administered as observed treatment on locally designated days. Prior to implementation, two staff of each district Department of Preventive Medicine, two nurses from each commune health station and all village health workers in the two districts had been trained about the causes, treatment, prevention and health risks of anemia and hookworm infection and received promotional and educational materials for distribution to

the population. More than 52,000 women living in the district received supplementation and de-worming.

Sampling

Two districts in the province (out of a possible 7 districts) were chosen, based on their proximity to the main city and access to health services, for evaluating the programme. The total population of each district was similar. A multi-stage cluster sampling design was used to select villages, and individual women within villages. Primary sampling units (villages) were chosen using a 'probability proportional to size' random sampling method, with half the target sample of villages taken from each district. Secondary sampling units (women) were randomly selected from each village from provincial lists. Approximately 13 women were selected from each village.

Laboratory methods

Blood samples were collected from each participant. Haemoglobin was evaluated by haemoglobinometer (HemoCue AB, Angelholm, Sweden) using finger-prick blood, and 3mL of venous blood was collected into tubes containing fast clotting agent. The tubes were spun at 4000rpm for 20 minutes at room temperature, after which serum was collected and stored at -4°C and transported on dry ice to Sydney, Australia, for analysis. Serum ferritin was evaluated using a sandwich immunoassay (IEA) (Beckman Coulter Access reagents, Fullerton, CA), and TfR was estimated using Enzyme Linked Immunoassay (ELISA) (IT, Orion Diagnostica, Espoo, Finland). The ratio of transferrin receptor to log (base 10) serum ferritin (TfR-F Index) was then calculated from these results.¹⁷ Serum C-reactive protein (CRP) levels were assessed by an ELISA method (Beckman Coulter, Inc., Fullerton, CA). We chose a level of above 10mg/L to represent a state of inflammation.²²

Anemia was defined as a haemoglobin concentration less than 120g/L, and iron deficiency as a serum ferritin below 15ng/mL, in accordance with WHO recommendations for non pregnant women.⁸ TfR levels of 2.3 mg/L or above were considered to represent iron deficiency based on the manufacturer's reference interval (0.8 – 2.3 mg/L). The TfR-F Index has been previously found to provide an excellent indicator of iron stores, with depletion when the ratio exceeds 1.8, using the same kit as was used in this study.²³

Ethics

Consultation was undertaken between the project team and village, district and provincial health staff and members of the community. The subjects were educated about the project by local village health workers, following which informed consent was obtained. The project was approved by the Human Research Ethics Committees of the National Institute of Malariology, Parasitology and Entomology (Hanoi, Vietnam), The Walter and Eliza Hall Institute of Medical research (Melbourne, Australia) and Melbourne Health Research Directorate (Melbourne, Australia).

Statistical Analysis

Haemoglobin data were normally distributed. The distribution of ferritin, TfR and TfR-F index were approximately log-normal, and these data were log-transformed for subsequent

analysis. We estimated the mean within-subject haemoglobin change (haemoglobin_{3 months} – haemoglobin_{baseline}), and geometric mean within-subject ratio of ferritin and TfR ($[\text{ferritin}_{3\text{months}} / \text{ferritin}_{\text{baseline}}]$ and $[\text{TfR}_{3\text{months}} / \text{TfR}_{\text{baseline}}]$ respectively). We defined a haemoglobin response (HBR) in anaemic women as a rise in haemoglobin by at least 10g/L or resolution of anemia. Haematological data were compared in subjects with and without an HBR using the two-sample *t* test. All estimates were obtained with robust confidence intervals to allow for the sampling design. 'Receiver operator characteristic' curves (ROCs) were used to illustrate the sensitivity and specificity of ferritin at various cut-point values to predict an HBR. The area under the curve of the ROC (AUC^{ROC}) was calculated to estimate the accuracy of each index of iron stores to distinguish between responders and non responders. Statistical analysis was performed using Stata (Stata 9.2 for Windows, StataCorp LP, Texas).

Results

Of 349 subjects who had baseline haemoglobin assessments, 221 (63%) underwent repeat survey after three months of WIFS and one dose of albendazole. At baseline, the mean haemoglobin amongst these subjects was 122.5 g/L (95% CI 120.1, 124.8), the geometric mean ferritin was 28.6 ng/mL (n=210; 95% CI [24.9, 32.8]), geometric mean TfR was 1.8 mg/L (n=214; 95% CI [1.7, 1.9]) and geometric mean TfR-F Index 1.3 (n=208; 95% CI [1.2, 1.4]). CRP was <10mg/L in all but 2 subjects. Table 1 depicts the baseline prevalence of anemia and indices indicating iron deficiency amongst the overall group, the prevalence of indices indicating iron deficiency within the anaemic group, and the improvements in these indices following three months of intervention. The prevalence of iron deficiency was higher when using a ferritin cut-off of <30ng/mL compared with <15ng/mL.

After 3 months of the WIFS/de-worming intervention, the mean within-subject change in haemoglobin from baseline to 3 months was +3.5 g/L (95% CI [0.9, 6.6], $p<0.001$). In 199 women with paired ferritin data, the mean within-subject ferritin ratio ($\text{ferritin}_{3\text{months}} / \text{ferritin}_{\text{baseline}}$) was 1.33 (95% CI [1.18, 1.51], $p<0.001$), i.e. average ferritin levels increased by 33%. In 207 women with paired TfR data, the mean within-subject TfR ratio was 0.92 (95% CI [0.86, 0.99], $p=0.025$), i.e. TfR levels decreased by 8%.

An HBR was achieved in 50/75 (66.6%) subjects with baseline anemia for whom haemoglobin, ferritin and TfR data was available. Of the women with ferritin <15ng/mL and anemia, 21/26 (80.8%) attained an HBR. Table 2 shows a comparison of haemoglobin and iron indices for clinical responders and non responders at baseline and after 3 months. Amongst those individuals who were clinical responders, iron indices improved over 3 months, but there was no apparent change amongst non responders.

The AUC^{ROC} for ferritin as a test for predicting HBR was 0.68 (95% CI [0.55, 0.81]). A plot showing both sensitivity and specificity (vertical axis) versus ferritin (horizontal axis) was also used to assess various cut-point values of ferritin to predict response (Table 3 and Figure 1). The curves intersect at a ferritin cut-off of 26 ng/mL, where the sensitivity and specificity were 66.0% each respectively. The AUC^{ROC} for TfR was 0.65 (95% CI [0.51, 0.80]), and for the TfR-F Index was 0.68 (95% CI [0.55, 0.81]). Notably, a ferritin cut-off of

<30ng/mL improved sensitivity for prediction of HBR, with some loss of specificity. Similar findings were obtained with the other indices.

Discussion

We report a significant reduction in prevalence of anemia and iron deficiency and rise in mean haemoglobin in non-pregnant women of reproductive age after three months of an iron-folic acid and de-worming program. A significant response in haemoglobin occurred in two thirds of anaemic women, even though only a third had iron deficiency at baseline according to conventional iron indices (ferritin and TfR).

Given the low prevalence of iron deficiency as defined by ferritin <15ng/mL, it is surprising that so many (66%) anaemic women achieved a HBR. This suggests that the present definition of iron deficiency (ferritin of <15ng/mL) may be too specific, and that women with higher ferritin levels also benefited. Increasing the cut-off of ferritin to 30ng/mL improved the sensitivity for detection of HBR from 44 to 72%, at the expense of specificity. Others have found that up to one quarter of patients with stainable bone marrow iron have ferritin levels above 15ng/mL.²⁴ Our results and conclusions also reflect those of a previous study in which the sensitivity of ferritin as an indicator of response to iron supplementation was improved if the cut-off increased from 15ng/mL to 30ng/mL¹³.

Ferritin rose significantly in the group who achieved a HBR, but remained static in the non-response group. This latter group were more iron replete at baseline and it is likely that a cause other than iron deficiency was the explanation for the anemia in this group. It is also possible that iron replete, anaemic women may have experienced smaller improvements in physical wellbeing, and thus had poorer compliance with iron supplementation. However, the programme was delivered following intensive education of both health workers and the population and surveys were conducted which confirmed that compliance was high (Tran Tuan personal communication). Another factor may have been that intestinal iron absorption would have been down-regulated in iron replete individuals, mediated by down regulation of the luminal divalent metal transporter (DMT) protein and the iron exporter, basolateral ferroportin.²⁵

A response to the folic acid component of the supplement may have partially contributed to the high HBR rate. We do not believe this is likely as preliminary biochemistry results suggest that folate deficiency was uncommon in our subjects (T. Green, personal communication). Furthermore, the folate dose (0.4 mg weekly) was relatively low,²⁶ and there is little evidence that folic acid improves haemoglobin in non-pregnant women without megaloblastic anemia.

The area under the curve (AUC^{ROC}) reflects the overall ability of a continuous measure to discriminate between individuals with and without a disease.²⁷ In this study we used AUC^{ROC} to determine the ability of baseline iron indices to discriminate between women who would and would not have a HBR to the WIFS/deworming intervention. The AUC^{ROC} values for ferritin, TfR and TfR-F index were each below 0.7, suggesting iron indices had only a moderate diagnostic value in predicting HBR.²⁸ The AUC^{ROC} value for

determination of HBR for anaemic individuals using ferritin was superior to that for TfR, and similar to the TfR-F index, suggesting little added diagnostic benefit from assaying the baseline TfR, despite significant additional cost.

Our analysis was limited by incomplete data collection. The baseline survey had comprised 349 non-pregnant women, of whom 221 returned for follow up analysis at three months, and complete biochemical data was available for 208. It is possible that women more interested in their health, or attended by more effective health workers, may have had better adherence to the program and have been more likely to return for follow up. This may have selected for a group of women with a more robust response in haemoglobin due to better compliance. However, the baseline prevalence of anemia and iron deficiency²⁰ was similar in women who returned for follow up evaluation and those who did not. Anecdotally, village health workers reported that loss to follow up was due to reluctance of women to undergo a second venipuncture. The other limitation of our study is the absence of a control group. This would have allowed us to establish the prevalence of iron deficiency, based on the proportion of women who responded to iron supplementation compared with those receiving placebo, as previously described.²⁹ However, we did not feel it ethical to include a control arm in this study given that WIFS is an established strategy for reducing morbidity amongst women of reproductive age in developing countries.

The current WHO recommendations suggest screening for iron deficiency anemia at the population level utilising haemoglobin alone is sufficient in settings where iron data is not available.⁸ Our results suggest that although anemia was not diagnostic of iron deficiency for the individual, these recommendations were appropriate in this population as the majority of anaemic women responded to supplementation. Where iron indices are available, our results indicate that there is little additional gain from assaying TfR if ferritin is performed. However, adjusting the upper limit of ferritin for detection of iron deficiency to 30ng/mL would have a greater sensitivity for identifying anaemic subjects (and thus populations) likely to respond to iron supplementation programs.

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Figure 1.
Sensitivity and specificity of different ferritin cut-offs for detection of clinical response

Table 1

Prevalence of anaemia and indices indicating iron deficiency at baseline and after three months of WIFS and de-worming amongst the overall group (n=221) and those who were anaemic at baseline (n=81)

	Group	Baseline, n, %, [95% CI]	Three months, n, %, [95% CI]
Haemoglobin <120g/L	Overall	81/221, 36.7 [29.6, 44.3]	58/221, 26.2 [20.3, 32.2]
	Anaemic	81/81, 100.0	36/81, 44.4 [35.2, 54.0]
Ferritin <15ng/mL	Overall	44/210, 21.0 [15.0, 28.5]	25/210, 11.9 [8.7, 16.1]
	Anaemic	26/75, 34.7 [22.7, 49.0]	11/75, 14.7 [9.4, 22.2]
Ferritin <30ng/mL	Overall	96/210, 45.7 [36.8, 54.9]	75/210, 35.7 [28.4, 43.8]
	Anaemic	46/75, 61.3 [47.9, 73.2]	37/75, 49.3 [37.8, 61.0]
TfR >2.3mg/L	Overall	50/214, 23.4 [17.6, 30.3]	29/214, 13.6 [9.3, 19.3]
	Anaemia	31/78, 39.7 [28.0, 52.8]	16/78, 20.5 [13.5, 29.9]
TfR-Fe Index >1.8	Overall	43/208, 20.7 [14.9, 27.9]	29/210, 13.8 [10.1, 18.6]
	Anaemic	29/75, 38.7 [27.8, 50.8]	16/75, 21.3 [15.4, 28.7]
Haemoglobin <120g/L & Ferritin < 15ng/mL	Overall	26/210, 12.4 [8.0, 18.7]	12/210, 5.7 [3.7, 8.7]

Table 2

Comparison of baseline haemoglobin and iron indices between baseline anaemic women who achieved a haemoglobin response (of >10 g/L) to WIFS/de-worming, and those who did not.

	Haemoglobin Response, Mean [95% CI]		Non Response, Mean [95% CI]	
	Baseline	3 month	Baseline	3 month
Haemoglobin (g/L)¹	102.7 [98.8, 106.6] ⁴	124.4[121.8, 127.0] ³	107.5 [101.6, 113.5] ⁴	105.4 [100.8, 110.1]
Ferritin (ng/mL)²	16.6 [10.9, 25.5]	31.5 [23.0, 43.1] ³	30.9 [17.2, 55.5]	32.4 [21.0, 50.1]
TfR (mg/L)²	2.19 [1.96, 2.45]	1.80 [1.54, 2.04] ³	1.86 [1.45, 2.38]	1.86 [1.54, 2.25]
TfR-F Index²	2.02 [1.54, 2.65]	1.28 [1.02, 1.59] ³	1.35 [0.93, 1.96]	1.31, [1.04, 1.67]

¹Arithmetic mean

²Geometric mean

³p<0.05 for change in mean between baseline and 3 month evaluation.

⁴p>0.05 for no difference between baseline haemoglobin between HBR response and non response groups

Table 3

Sensitivity and specificity of different cut-offs for ferritin, TfR and TfR-F Index to predict a HBR response amongst anaemic subjects

Ferritin cut-off (ng/mL)	Sensitivity %	Specificity %
<15	44	80
<30	72	52
TfR (mg/L)		
>1.8	76	52
>2.3	46	68
TfR-F Index		
>1.1	76	56
>1.83	44	72