

The validity of commonly used haematological indices in the detection of iron deficiency in pregnancy

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Summary

Rationale: The haemoglobin concentration (Hb) has limitations in the diagnosis of iron deficiency in pregnancy.

Objective: The objective of this study was to assess the validity of the accepted cut off points of the other commonly measured haematological indices in either detecting or excluding iron deficiency as determined by the serum ferritin (SF) assay.

Design: A cross sectional study was carried out on two groups of women in the second and third trimesters of pregnancy.

Setting: University Antenatal Clinic, Faculty of Medicine and Kyoto Medical Centre Galle.

Method: The Hb, haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell count (RBCC) were estimated by an automated haematology analyser and compared with the SF levels measured by immunoradiometric assay, in 156 women in the second trimester (T₂) and 47 women in the third trimester (T₃) of pregnancy. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy and the Kappa statistic were calculated for the accepted cut off points for diagnosis of anaemia, for each haematological index, using a SF < 12 µg/L as the diagnostic criterion for iron deficiency.

Results: The Hct, MCV and MCHC had a high specificity (96-100%) but a very low sensitivity (10-38%). Only the MCH had a high sensitivity (92% in T₂, 86% in T₃) but it had a low specificity (21-24%). The MCHC had the best accuracy (71%

in T₃) but its accuracy in T₂ was only 64%. Only the MCHC in T₃ showed some agreement with SF (Kappa 0.41, p=0.00).

Conclusion: A single haematological index *per se* has a poor ability of detecting or excluding iron deficiency in pregnancy. Although the best index is the MCHC, its accuracy is only 64% in the second trimester. Therefore several indices should be evaluated before deciding on a diagnosis of iron deficiency and subsequent supplementation or therapy during pregnancy.

Key words: haematological indices, iron deficiency, pregnancy, validity.

Introduction

The haemoglobin concentration (Hb) is poorly correlated to the iron stores of a subject as the Hb is maintained within normal limits until the iron stores are depleted, and therefore is of limited value in the detection of iron deficiency (1,2,3,4). Iron stores are usually assessed by serum ferritin (SF) assays (1,2,3). The Hb is also reduced by folate and vitamin B₁₂ deficiencies. The only known cause for a low SF level is iron deficiency (1,2,3). A low Hb associated with low SF levels is due to iron deficiency anaemia. The objective of this study was to assess the validity of the accepted cut off points of the other commonly measured haematological indices in either detecting or excluding iron deficiency, as determined by the SF assay.

Methods

A cross sectional study was carried out on two groups of women in the second and third trimesters of pregnancy. The criteria for selection

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of subjects was that they should have a period of gestation (POG) either between 14-24 weeks (group A) or between 28-40 weeks (group B).

From the pregnant women presenting themselves at the university antenatal clinic in Galle, 203 consecutive women who fulfilled the selection criteria were recruited for the study. Of these women, 156 women (group A) were in the second trimester (T_2 , POG 14 - 24 weeks) and 47 women (group B) were in the third trimester (T_3 , POG 28 - 40 weeks). Assuming the prevalence of iron deficiency to be approximately 70% (4), 323 subjects would be required to tolerate a maximum sampling error of 5% (5). However the actual sample had to be curtailed to 203 subjects on account of the limited availability of the SF assay. Therefore, the possible sampling error in this study is approximately 6.5%.

The potential benefits of the study were explained and informed consent was obtained from all the subjects. Ethical approval for the study was obtained from the Faculty of Medicine, Galle.

During venepuncture for the other routine antenatal investigations an additional 3 ml and 4 ml of mixed venous blood was taken and divided into 1 ml aliquots in group A and group B respectively. One aliquot in each group was collected into a bottle containing 0.1ml of ethylene diamine tetra acetic acid and transported to the Kyoto Medical Centre in Galle, where the Hb, haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and red blood cell count (RBCC) were estimated using an automated haematology analyser (Sysmex K-1000, TOA Medical Electronics Co. Ltd. Japan).

Another aliquot of blood from each group was transported to the Faculty of Medicine, Galle where SF was estimated by immunoradiometric assay technique using IRMA Ferritin kits (Diagnostic Products Corporation, Los Angeles) which detects SF levels of $0.1\mu\text{g/L}$ or more. The balance blood was used for another part of this study programme.

Anaemia was defined as Hb $<110\text{ g/L}$ during pregnancy (1-3, 6-8). Iron deficiency was defined

as SF $<12\mu\text{g/L}$ (1,2,3). A Hct $<32\%$, MCV $<80\text{ fl}$, MCH $<30\text{pg/L}$, MCHC $<30\%$ and RBCC $<4.1\text{ million/mm}^3$ were considered as being low and indicative of iron deficiency (1,2,3,6,9,10).

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for the accepted cut off points for each haematological index using a SF $<12\mu\text{g/L}$ as the diagnostic criterion for iron deficiency (11). The agreement between each haematological index and the SF assay in detecting or excluding iron deficiency was measured using the Kappa Statistic (12). Each haematological index was converted to a categorical variable using the accepted cut off point for this purpose. For the purpose of this study SF was considered as the 'gold standard' although it too has its limitations (6, 13-15).

Results

The distribution of the basic characteristics of the subjects are shown in Tables 1 and 2.

Table 1

**The basic characteristics in Group A
(n=156, T_2)**

Characteristic	Mean	SD.	95%CI
Age (yrs)	26.3	6.1	14 - 43
Parity	1.9		
POG. (Weeks) - T_2	19.4	2.9	14 - 24

Mean = Arithmetic mean S.D = Standard deviation
CI = Confidence interval POG = Period of gestation

Table 2

The basic characteristics in Group B (n=47, T_3)

Characteristic	Mean	SD.	95%CI
Age (yrs)	26.3	5.6	15 - 38
Parity	2		
POG. (Weeks) - T_3	35	2.5	28 - 40

See Table 1 for meaning of abbreviations.

Iron deficiency (SF<12 µg/L) was found in 62 (40%) of subjects in group A (n=156, T₂) and 22 (46%) in group B (n=47, T₂).

The distribution of the haematological indices obtained by the autoanalyser and the SF values obtained by immunoradiometric assay are shown in Tables 3 and 4.

Table 3
Haematological indices and SF values in Group A (n=156, T₂)

	Mean	SD.	95% CI	CV
Hb (g/L)	11.4	1.2	11.2 - 11.6	0.11
Hct (%)	36.1	3	35.6 - 36.6	0.08
MCV (fl)	89.5	5.3	88.7 - 90.3	0.06
MCH (pg/L)	28.3	3.1	27.8 - 28.8	0.11
MCHC (%)	31.7	0.2	31.3 - 32.1	0.01
RBCC (million/mm ³)	4.2	1.7	3.9 - 4.4	0.40
SF (µg/L)	19.7	16.5	17.1 - 22.3	0.84

CV = Coefficient of variation; See Table 1 and text for the meaning of other abbreviations.

Table 4
Haematological indices and SF values in Group B (n=47, T₂)

	Mean	SD.	95% CI	CV
Hb (g/L)	12	1.4	11.6 - 12.4	0.12
Hct (%)	39.9	7.2	37.7 - 42.0	0.18
MCV (fl)	89.1	6.6	87.1 - 91.1	0.07
MCH (pg/L)	32.6	3.7	22.6 - 42.6	0.11
MCHC (%)	30.9	1.6	30.4 - 31.4	0.05
RBCC (million/mm ³)	4.4	0.6	4.2 - 4.6	0.14
SF (µg/L)	16.5	13.7	12.5 - 20.6	0.83

See Table 1 and text for the meaning of abbreviations.

The overall accuracy of detecting or excluding iron deficiency was poor in all indices with MCHC in T₃ having the highest accuracy (71%). The accuracy of Hb in detecting or excluding iron deficiency was 65 % in T₂ and 67% in T₃. The MCH was only index which had a good sensitivity (92% in T₂ and 86% in T₃) but it had a poor specificity. Other than the MCH and RBCC, all the other

indices had good specificities. MCV had the best specificity (98%) in T₂ and MCHC had a 100% specificity in T₃. Except for the MCHC which had a moderate agreement (Kappa 0.41, p=0.00) none of the other indices had any agreement with the SF levels in the detection and exclusion of iron deficiency (Tables 5 and 6).

Table 5

The Kappa statistic and the validity of each haematological index in detecting or excluding iron deficiency in the subjects in group A (n=156, T₂)

		Sen.	Spec.	PPV	NPV	Acc.	Kappa	p
Hb	< 110 g/l	0.48	0.77	0.58	0.69	0.65	0.25	0.001
Hct	< 32 %	0.18	0.97	0.79	0.63	0.65	0.10	0.03
MCV	< 80 fl	0.10	0.98	0.75	0.62	0.62	-0.02	0.99
MCH	< 30 pg/l	0.92	0.21	0.44	0.80	0.50	-0.07	0.99
MCHC	< 30 %	0.14	0.97	0.75	0.62	0.64	-0.03	0.99
RBCC	< 4.1 million /mm ³	0.63	0.47	0.44	0.66	0.53	-1.28	0.99

Sen. = Sensitivity Spec. = Specificity PPV = Positive predictive value

NPV = Negative predictive value Acc. = Accuracy

See text for the meaning of other abbreviations.

Table 6

The Kappa statistic and the validity of each haematological index in detecting or excluding iron deficiency in the subjects in group B (n=47, T₃)

	Sen.	Spec.	PPV	NPV	Acc.	Kappa	p
Hb < 110 g/l	0.38	0.92	0.80	0.64	0.67	-0.12	0.49
Hct < 32 %	0.10	0.96	0.67	0.56	0.57	-0.01	0.58
MCV < 80 fl	0.19	0.96	0.80	0.58	0.61	0.16	0.05
MCH < 30 pg/l	0.86	0.24	0.49	0.67	0.52	0.08	0.22
MCHC < 30 %	0.38	1.0	1.0	0.66	0.71	0.41	0.00
RBCC < 4.1 million /mm ³	0.17	0.68	0.33	0.46	0.43	0.02	0.44

See Table 5 for meaning of abbreviations.

Discussion

The level of SF has been recommended as a suitable index to detect iron deficiency (1,2,3,6). However the SF is subject to biological day to day variations (13). It has also been shown that assessment of serum transferrin receptors, in addition of SF improves the detection of iron deficiency during pregnancy (14,15). However, both SF and serum transferrin receptors are not available in clinical practice and the clinician has to depend on a 'full blood count' which estimates the common haematological indices Hb, Hct, MCV, MCHC and RBCC in diagnosing iron deficiency.

The accuracy of detecting or excluding iron deficiency was low for all the readily available indices. As expected the RBCC was the least accurate (43% in T₃). Although an MCHC of <30% indicated that iron deficiency was present in all these cases (PPV 100%), a MCHC of <30% was found in only 38% of subjects who had iron deficiency (sensitivity 38%). The MCHC had an accuracy of only 64% in T₂ and 71% in T₃.

A MCH of <30 pg/L could detect 92% and 86% of subjects with iron deficiency in T₂ and T₃ respectively (sensitivity 92% in T₂ and 86% in T₃) and 80% of the subjects who had an MCH of <30 pg/L probably did not have iron deficiency (NPV 80%). However 76-79% of subjects who did not have iron deficiency were considered to have iron deficiency by this index (specificity 24-21%). Although the accepted standard cut off points for Hct and MCV could correctly identify 96-98% of subjects who did not have iron deficiency (specificities 96-98%) they failed to detect iron deficiency in 81-90% of those who actually had iron deficiency (sensitivities 10-19%).

Although the accepted standard cut off points for Hb could identify 77-92% of those who did not have iron deficiency (specificity 77-92%), 52-62% of those who had iron deficiency were not detected (sensitivity 48-38%). In a study done by us earlier we found that the Hb measured by the cyanmethaemoglobin method had a sensitivity of 63% and a specificity of 58% in a group of subjects in whom the prevalence of iron deficiency anaemia was 69% (4). We have also shown that if

a sample of blood from one subject was sent for estimation of Hb to three different laboratories the results could be significantly different from each other (16).

Results of haematological investigations should be interpreted with caution. Multiple indices should be evaluated. The best index to exclude iron deficiency in both T₂ and T₃ appears to be a MCH >30pg/L (NPV 80% and 67% respectively). The best index to detect iron deficiency appears to be a MCHC <30% (PPV 100%) in T₃ and the Hct in T₂ (PPV 79%). Therefore the combined use of these indices should improve the accuracy of the diagnosis of iron deficiency in pregnancy.

Conclusions

A single haematological index *per se* has a poor ability of detecting or excluding iron deficiency in pregnancy. The MCH had good sensitivity but poor specificity. The MCV and Hct have good specificity but poor sensitivity. Although the best index is the MCHC, its accuracy is only 64% in the second trimester. Therefore several indices should be evaluated before deciding on iron supplementation or therapy during pregnancy.

References

1. International Nutritional Anaemia Consultative Group. Iron deficiency in women. Washington. D.C. Nutrition Foundation, 1985; p 21-36.
2. Warren G.T. Comparison of tests for diagnosis of iron depletion in pregnancy. American Journal of Obstetrics & Gynaecology 1988; 159: 1132-1134.
3. Puolakka J., Janne O., Pakarinen, Vihko R. Serum ferritin in the diagnosis of anaemia during pregnancy. Acta Obstetrica et Gynaecologica Scandinavia Supplement 1980; 95: 57-63.
4. Goonewardene M., Seekkuge J., Liyanage C. Iron Stores and its correlation to haemoglobin levels in pregnant women attending an antenatal clinic. Ceylon Medical Journal 1995; 40: 67-69.
5. Vanghan J.P, Morrow R.H. Manual of Epidemiology for District Health Management, WHO Publication Geneva 1989: p175-178.
6. Earl R., Woteki C.E, Eds. Iron Deficiency anaemia: recommended guidelines for the prevention, detection, and managements among US Children and women of childbearing age. Washington DC. National Academy Press 1993.
7. Dacie J.V., Lewis S.M. Practical Haematology 8th Ed. Edinburgh. Churchill Livingstone, 1994: p 669.
8. Bunn H.F. Pathophysiology of the anaemias. In: Harrison's Principles of Internal Medicine Wilson JD, Fauci AS, Braunwald E, Isselbacher KJ *et al* Eds. 13th Ed. Vol 2. New York: McGraw Hill Inc, 1997; p1717-1726.
9. Chard T., Lilford R.J. How useful is a test? J. Studd Ed. Progress in Obstetrics & Gynaecology. 1986; 6: 3-15.
10. Armitage P., Berry G. Kappa measure of agreement. Statistical Methods in Medical Research, Oxford. Blackwell Scientific, 1994: p443-447.
11. Borel M.J., Smith S.M., Derr Janice, Beard J.L. Day to day variation in iron status indices in healthy men and women. American Journal of Clinical Nutrition 1991; 54: 729-735.
12. Carriga M.T., Skikne B.S., Finley B., Cutler, Cook J.D. Serum transferrin receptors for the detection of iron deficiency in pregnancy. American Journal of Clinical Nutrition 1991; 54: 1077-1081.
13. Cook J.D., Skikne B.S., Baynes M.D. Serum transferrin receptors. Annual Reviews in Medicine 1993; 44: 63-74.
14. Goonewardene I.M.R., Fernando R.J., Liyanage C., Somasiri K.G. The agreement between haematological indices obtained by different methods. Galle Medical Journal 2000; 2: 13-17.