ASSESSMENT OF ANAEMIA AND IRON DEFICIENCY BY HAEMOGLOBIN AND PLASMA FERRITIN ASSAY IN RURAL ANTENATAL CASES USING FINGERPRICK SAMPLES

By

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SUMMARY

Haemoglobin (Hb) and plasma ferritin levels were measured in fingerprick capillary blood samples from 413 antenatal cases from rural and tribal areas between April 1986 to April 1988. Based on Hb levels 96% of cases were anaemic (Hb $<$ 11.0 gm), 53% of cases had moderate anaemia (Hb 8.1 to 10 gm%) and 3% had severe anaemia (Hb $\leqslant$ 8 gm%). Plasma ferritin levels indicated that only 3% of cases had adequate iron stores (ferritin $>$ 60 ng/ml), 75% of cases had moderately depleted iron stores (ferritin: 11-60 ng/ml) and a very high percentage i.e. 22% had severely depleted iron stores (ferritin $\leqslant$ 10 ng/ml). The mean Hb and ferritin levels in tribal women (24.1% of all cases) were significantly lower ($P < 0.01$) than in the non-tribal cases (Hb 9.53 $\pm$ 0.82 SD gm% vs 10.11 $\pm$ 0.71 SD gm%; ferritin 19.3 $\pm$ 12.4 SD ng/ml vs 29.4 $\pm$ 18.5 SD ng/ml).

Introduction

Anaemia is a major health hazard in developing countries (WHO 1972; De Maeyer E. and Tegman A. D., 1985) and contributes, directly or indirectly to maternal and foetal morbidity or mortality (Hingorani and Kinra, 1976; Achari *et al*, 1984). The most common cause is nutritional deficiency which may be aggravated by factors like menorrhagia, worm infestations and chronic infections. Anaemia, i.e. deficiency of haemoglobin is but one manifestation of iron deficiency. Iron deficiency per se can cause nonhaematologic clinical problems like leg pains, lethargy, mental and physical, because iron is utilized in a number of enzymatic and metabolic reactions (Dallman *et al*, 1980). The circulating level of ferritin has been shown to be the most sensitive and specific indicator of iron depletion and ferritin assay has replaced the more invasive technique of bone marrow smear (Cook *et al* 1974, Puolakka Jukka, 1980). In this study we describe the distribution of haemoglobin and plasma ferritin levels in rural and tribal pregnant women who attended the rural clinics of the Institute.

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Material and Methods

Women attending the rural clinics of the Institute, 50 kms away from the Institute over a two year period were included in the study. The antenatal check-up was conducted by the Institute's staff as part of the primary health care programme. The development of a simple technique to use capillary pinprick blood and heparinised micropipette for estimation of haemoglobin and plasma ferritin has been recently reported by us (Donde *et al*, 1988). Whole blood (300 to 400 μl) was collected in the micropipette and samples were transported to the laboratory. Hb was estimated by cyanmethaemoglobin method using Drabkin's reagent (Sigma Lab Kit) prior to separation of plasma. Plasma ferritin was estimated by radioimmunoassay (Gamma Dab, Dade). The sensitivity of this assay was 1 ng/ml. This analysis includes 413 women who attended the rural antenatal clinics between April 1986 and April 1988. Thirty six non-pregnant rural women in the same age group served as controls.

Results

The distribution of Hb and ferritin in this rural antenatal population is given in Table I. The mean Hb and ferritin levels in antenatal group as a whole were lower than in matched control cases (Hb 10.04 $\pm$ 0.76 SD vs 11.1 $\pm$ 0.52 SD; $P < 0.01$). Based on Hb levels, only 4% of pregnant rural women were healthy (Hb $>$ 11 gm%) and only 3% had adequate iron stores (ferritin $>$ 60 ng/ml). Moderate anaemia (Hb 8.1 to 10.0 gm%) was observed in 53% of antenatal cases whereas moderate depletion of iron stores (ferritin 11 to 60 ng/ml) was observed in 75% of cases. There were 12 cases (3%) of severe anaemia (Hb $\leqslant$ 8 gm%). In this group plasma ferritin in all but one was below 10 ng/ml.

Out of 18 women (4% of total) who had normal Hb level ($>$ 11 gm%) only 2 had plasma ferritin more than 60 ng/ml, 13 had plasma ferritin values between 11 and 60 ng/ml and 3 had ferritin levels below 10 ng/ml.

Table II depicts the mean Hb and ferritin levels with respect to the different periods of gestation. These levels were significantly lower at 20-28 weeks of gestation and at 36 + weeks as compared to other periods ($P < 0.01$). When the total population was considered there was no direct correlation between Hb levels and ferritin levels ($r = .092$; P—n.s.). On the other hand in anaemic women with Hb $<$ 10 gm% there was a significant correlation between Hb and ferritin in the Ist trimester and at term (36 weeks +) only $r = .455$ and 0.338 respectively; $P < 0.05$).

There was no significant correlation between Hb or plasma ferritin levels and the gravidity of the women. However Hb level in women with 4th or higher parity (n = 21) was significantly lower than that in primipara (n = 124) (Hb 9.71 $\pm$ 0.75 SD vs 10.07 $\pm$ 0.74 SD gm% $P < 0.05$). Tribal women formed 24.1% of the ante-

TABLE I

Distribution of Haemoglobin and Ferritin in 413 Rural Antenatal Cases

Haemoglobin gm%	No. (%) Women	Plasma Ferritin ng/ml	No. (%) Women
>11	18 (4)	>60	12 (3)
10.1-11	164 (40)	31-60	103 (25)
9.1-10.0	169 (41)	11-30	207 (50)
8.1-9.0	50 (12)	<10	91 (22)
⩽8	12 (3)	—	—
Total	413 (100%)	Total	413 (100%)

TABLE II

Levels of Haemoglobin and Ferritin in Relation to Weeks of Gestation

Weeks of Gestation	No. of cases	Haemoglobin gm% (Mean ± SD)	Plasma Ferritin ng/ml (Mean ± SD)	Significance
<13	28	10.38 ± 0.74	27.0 ± 17.0	N.S.
13-20	134	10.11 ± 0.89	29.37 ± 18.10	N.S.
20-28	164	9.96 ± 0.82*	25.47 ± 17.41	*P <.01
28-36	66	10.06 ± 0.74	26.89 ± 16.76	N.S.
>36	21	9.70 ± 0.55*	27.00 ± 16.83	*P <.05
Total	413	10.04 ± 0.76	27.23 ± 17.36	N.S.

natal population. The mean Hb level was significantly lower in tribals as compared to non-tribals (Hb 9.53 ± 0.82 SD gm% vs 10.11 ± 0.71 SD gm%, $P < 0.01$). Similarly plasma ferritin levels were also significantly lower in the tribals than in non-tribals (19.3 ± 12.4 SD ng/ml vs 29.4 ± 18.5 SD ng/ml; $P < 0.01$). Not a single tribal pregnant woman had Hb above 11 gm%. Seven out of 12 cases of severe anaemia (Hb < 8 gm%) were observed in the tribals.

Discussion

This study has demonstrated that anaemia and iron deficiency are still widely prevalent amongst Indian rural women. The mean haemoglobin level in antenatal cases in this study was 10.04 ± 0.76 SD gm%. This is higher than that reported from Rajasthan i.e. 9.35 gm% (Sarin *et al*, 1985). However it is significantly lower than 11.05 ± 1.85 gm% reported from Delhi (Madan *et al*, 1988). The haemoglobin levels in women from other countries are much higher: 11.86 gm% (Huisman and Aarnaudse, 1986); 12.6 gm% (Ho *et al*, 1987). Even more important is the fact that only 4% of women in this study had Hb > 11 gm% against 76% of cases from Netherlands. This indicates that a large proportion of women in India have a subnormal health status. These women are likely to suffer from leg pains, lassitude, weakness, dizziness and a number of non-specific symptoms which may prevent them from going to the health centre for immunisation, their own as well as the children's and for utilization of antenatal and family planning services.

There was no correlation between haemoglobin and ferritin levels when all the cases were pooled in one group. This is not unexpected as haemoglobin levels are influenced by iron folate, B12 etc. Since ferritin levels are more specific indicators of progressive iron depletion that haemoglobin, it is recommended that plasma ferritin should be estimated whenever possible for assessment of iron deficiency, specially in pregnancy. The mean plasma ferritin level of 25.52 ± 17.0 SD ng/ml in this study compares poorly with the minimum level of 35 ng/ml suggested by the W.H.O. Similar levels (24.2 ± 21.4 SD ng/ml) were reported by Pawashe *et al* (1987) in South Indian women. Even lower levels i.e. 14.5 ± 13.4 SD ng/ml have been reported recently by Madan *et al* (1988) in North Indian women although their Hb levels were higher. An analysis of ferritin levels in women with Hb more than 11 gm% in this study revealed that only 11% of these had adequate iron stores. Iron is essential for several metabolic and enzymatic processes in the body and iron deficiency per se can cause symptoms like decreased mental alertness or muscle fatiguability (Dallman *et al,* 1980) Ho *et al* (1987) have reported that 15.4% of normal pregnant women at term had subclinical iron deficiency. In the present study a very high percentage (22%) of women had severe iron depletion (Ferritin $\leqslant$ 10 ng/ml). Prophylactic iron therapy should therefore aim not only at normal Hb levels but also towards building up of adequate iron stores if 'Health for all—2000 AD' is the goal in the true sense. This is particularly relevant in the antenatal cases who are vulnerable to anaemia and iron deficiency. The tribal women showed a higher prevalence of anaemia and iron deficiency than the non-tribals. Prophylactic iron therapy and monitoring by Hb and ferritin levels will be particularly useful in this high risk group. Currently we are in the process of evaluating the response of Hb and ferritin levels to prophylactic oral iron therapy in antenatal cases under field conditions. Radioimmunoassay of ferritin is very expensive and attempts are being made to prepare local reagents for enzyme immunoassay of ferritin to reduce the cost per test.

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