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Nutritional anaemia in pregnant Beninese women: consequences on the haematological profile of the newborn

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1. An assessment of iron and folic acid status, blood thick film and haemoglobin (Hb) electrophoresis was performed on 126 pregnant women (and their newborn infants) and in ninety-five menstruating women in Cotonou (Benin).

2. Anaemia (according to the World Health Organization (1972)) was observed in 55% of pregnant women and in 39% of menstruating women.

3. Fe-deficiency was defined as a low serum ferritin concentration (12 μg/l or less), combined with a low transferrin saturation (less than 16%) or a high erythrocyte protoporphyrin level (more than 3 μg/g Hb), or both. A moderate elevation in the serum ferritin concentration (between 13 and 50 μg/l), associated with a low transferrin saturation or a high erythrocyte protoporphyrin level, or both, indicated Fe-deficiency in an inflammatory context. Fe-deficiency was present in 73% of pregnant women and in 41% of menstruating women.

4. Folate deficiency (defined as erythrocyte folate below 160 μg/l) was observed in 45% of pregnant women.

5. In pregnant women, anaemia was associated with Fe-deficiency in 83% of cases and with folate deficiency in 48% of cases.

6. Haemoglobinopathies were mainly heterozygous and did not seem to contribute significantly to anaemia. Intensity of malaria was not related to Hb level, but *Plasmodium falciparum* was found in 99% of subjects.

7. Hb concentration and mean corpuscular volume were significantly lower in babies born of Fe-deficient mothers than in babies born of Fe-sufficient mothers. Hb concentration in newborn infants was positively correlated with maternal serum ferritin.

Although nutritional anaemia is prevalent worldwide, it is particularly common in developing countries (DeMaeyer & Adiels-Tegman, 1985). Pregnant women are among the groups most at risk of developing nutritional anaemia (World Health Organization, 1972). Pregnancy imposes a substantial burden on the maternal haematopoietic system because of the need for augmented erythropoiesis in the face of an expanding plasma volume and obligatory placental transfer of the two most important micronutrients involved, iron and folic acid. Fe-deficiency is classically described as by far the most important aetiological factor of anaemia in pregnancy (Baker & DeMaeyer, 1979). However, and particularly in

Table 1. *Demographic and anthropometric data of Beninese mothers and newborn infants* (n *126*)

	Mean	SD	Range
Mothers			
Age (years)	25·0	5·6	16–38
Weight (kg)	58·8	10·1	38–88
Height (m)	1·585	0·067	1·38–1·74
Parity (births)	1·6 (1·0*)	1·9	0–9
Newborn infants			
Birth weight (g)	3067	403	1700–4220
Length (m)	0·492	0·022	0·41–0·54
Head circumference (mm)	341	22	280–580
Gestational age (weeks)	39·0	1·3	32–43

* Median.

a tropical context, other causes of anaemia are possible: other types of nutritional deficiency (mainly folic acid deficiency); inflammatory syndromes and infections (including malaria, bacterial and viral infections); and sickle cell diseases. Moreover, most of these can also affect the significance of the different biochemical indicators of Fe status (Hercberg & Galán, 1985). These interfering factors, very common in Africa, might be responsible for false-positive or false-negative results in the diagnosis of Fe-deficiency and Fe-deficiency anaemia. Difficulties in defining the Fe status and in recognizing Fe-deficiency could explain the conflicting results of previous studies concerning the influence of the maternal Fe balance on fetal Fe status. The use of a number of laboratory measurements leads to improved accuracy in Fe status assessment and allows separation of Fe-deficient from Fe-sufficient subjects.

The purpose of the present investigation was (1) to specify the relative contribution of the aetiological factors to anaemia in a group of pregnant women in Benin, and (2) to determine the consequences of Fe-deficiency in terms of the haematological profile of newborn infants.

MATERIALS AND METHODS

Subjects

The sample was composed of 126 pregnant women (and later their newborn infants) from the two public maternity wards in Cotonou (Benin). Maternal age, weight, height and parity, and birth weight, length and gestational age of the newborn infants are listed in Table 1. Of the women, 42% were nulliparous and 20% had three children or more. All women had normal pregnancies and deliveries. None received Fe or folic acid supplementation, nor drugs likely to modify their Fe or folic acid status during the pregnancy in question. Ninety-five menstruating women, mean age 27·3 (SD 5·8) years, chosen randomly from a district of Cotonou were studied as a control group for biological values. Informed consent was obtained from each subject. The survey was performed at the end of the dry season.

Biochemical methods

Maternal venous blood sampling (10 ml) was carried out on each mother during the first stage of labour. In newborn infants, blood (450 μl) was collected by heel prick within the first day of life. Blood samples were collected by venipuncture in the control group.

Several assays were performed within the 12 h following blood collection: haemoglobin (Hb) concentration, erythrocyte indices and leucocyte counts using a Coulter Counter (Coultronic, Margency, France) model S; packed cell volume using microcentrifugation; erythrocyte protoporphyrin using an automatic model 5 haematofluorometer (Aviv Biomedical; Analys, Namur, Belgium), and Hb electrophoresis using cellulose acetate (Helena Corp.; Helena France, St Leu-La-Forêt). Blood thick films were read after coloration by the May-Grünwald Giemsa technique.

Serum fractions were collected by centrifugation, frozen at $-20°$ and sent to France in dry ice for different assays: serum Fe concentration was measured by a colorimetric method using ferrozine (Giovanello *et al.* 1968); transferrin by an automated immunoturbidimetric technique; and total Fe-binding capacity was calculated (Vernet-Nyssen *et al.* 1983). The serum ferritin level was determined in duplicate using an enzyme-linked immunosorbent assay (Voller & de Savigny, 1981). Whole blood and serum folate concentrations were estimated by microbiological assay using *Lactobacillus casei* (Hoffbrand *et al.* 1966). Erythrocyte folate was calculated using packed cell volume. Serum and erythrocyte folate concentrations were not measured in the control group and serum folate concentration was not measured in newborn infants.

The Coulter Counter was calibrated each week by the Coulter 4 C control; the haematofluorometer was calibrated daily with standards supplied by the manufacturer. Serum ferritin was standardized using international references (NIBSC, London).

Statistical methods

Statistical analysis was carried out using Student's t test, χ^2 test and Pearson's correlation coefficient, r. Since serum ferritin, and erythrocyte and serum folate concentrations approached a log normal distribution, a log transformation of these variables was used for all calculations. Results are presented in original units.

Definitions

Fe-deficiency was defined as a low serum ferritin level (12 μg/l or less), combined with a low transferrin saturation coefficient (less than 16%) or a high erythrocyte protoporphyrin level (above 3 μg/g of haemoglobin), or both. Fe depletion was considered when a low serum ferritin level was not associated with abnormalities of other variables of Fe status. An inflammatory syndrome was suggested by a high serum ferritin level (above 50 μg/l), associated with a low transferrin saturation coefficient or a high erythrocyte protoporphyrin concentration, or both. The existence of a low transferrin saturation coefficient or a high erythrocyte protoporphyrin concentration, or both, with a moderate serum ferritin level (between 13 and 50 μg/l) indicated Fe-deficiency associated with inflammation. Subjects which had no abnormal values for the three independent indicators of Fe status were considered as 'Fe-sufficient'.

RESULTS

Maternal Fe status

Means, with their standard deviations (SD), and percentage of abnormal values for the indicators of Fe status of pregnant women and the control group of non-pregnant women are given in Table 2.

Anaemia, according to the World Health Organization (1972), was observed in sixty-nine (55%) pregnant women and in thirty-seven (39%) menstruating women. The percentage of Fe-sufficient, Fe-depleted and Fe-deficient (with or without inflammatory syndrome) subjects among pregnant women and the control group are shown in Table 3. Fe-deficiency

Table 2. *Measurements of iron status in pregnant and non-pregnant Beninese women*

	Mean	SD	Range	Values below normal* (%)
Pregnant women (*n* 126)				
Haemoglobin (Hb; g/l)	105	16	59–133	55*
Mean corpuscular volume (fl)	82·1	9·1	59–114	41†
Percent saturation of transferrin	13·2	8·4	3–77	73‡
Erythrocyte protoporphyrin (μg/g Hb)	4·1	2·2	1·6–15·0	59§
Serum ferritin (μg/l)	37 17·8‖	60	2–450	41¶
Non-pregnant women (*n* 95)				
Haemoglobin (Hb; g/l)	121	12	78–140	39*
Mean corpuscular volume (fl)	84·2	6·9	59–99	22†
Percent saturation of transferrin	18·1	5·8	3–44	43‡
Erythrocyte protoporphyrin (μg/g Hb)	3·0	1·2	1·3–12·1	38§
Serum ferritin (μg/l)	64 40·1‖	63	1–330	13¶

* Lower limits of normal for haemoglobin concentrations (g/l) were 110 for pregnant women, 120 for non-pregnant women.
† Lower limit of normal for mean corpuscular volume, 80 fl.
‡ Lower limit of normal for percentage saturation of transferrin, 16.
§ Upper limit of normal for erythrocyte protoporphyrin level, 3 μg/g Hb.
¶ Lower limit of normal for serum ferritin concentrations, 12 μg/l.
‖ Geometric mean.

Table 3. *Iron status and inflammatory syndrome in pregnant and non-pregnant Beninese women*

Group	Biochemical indices	Percent of pregnant women	Percent of menstruating women
Fe-sufficiency	SF > 12 μg/l and TS ⩾ 16% and EP ⩽ 3 μg/g Hb	11	39
Fe-depletion	SF ⩽ 12 μg/l	3	1
Fe-deficiency	SF ⩽ 12 μg/l, combined with TS < 16% or EP > 3 μ/g/Hb, or both	38·5	14
Fe-deficiency and inflammatory syndromes	SF between 13 and 50 μg/l, combined with TS < 16% or EP > 3 μg/g Hb, or both	34	25
Inflammatory syndrome	SF > 50 μg/l, combined with TS < 16% or EP > 3 μg/g Hb, or both	13·5	21

SF, serum ferritin; TS, transferrin saturation; EP, erythrocyte protoporphyrin.

Table 4. *Measurements of folate status in pregnant Beninese women* (n *126*)

	Mean	SD	Geometric mean	Value below normal (%)
Eythrocyte folate (μg/l)	182·0	98·6	157	45*
Serum folate (μg/l)	3·3	5·6	2·3	65†

* Lower limit of normal for erythrocyte folate, 160 μg/l.
† Lower limit of normal for serum folate, 3 μg/l.

was observed in ninety-one (73%) pregnant women and in thirty-nine (41%) menstruating women. Fe-deficiency was present in 83% of anaemic pregnant women and in 43% of anaemic menstruating women. Prevalences of Fe-deficiency anaemia were respectively, 45% in pregnant women and 17% in menstruating women.

In pregnant women, Hb was correlated positively with packed cell volume (r 0·79, $P < 0·001$) and negatively with erythrocyte protoporphyrin (r −0·53, $P < 0·001$). Erythrocyte protoporphyrin was negatively correlated with mean corpuscular volume (r −0·21, $P < 0·02$) and positively correlated with leucocyte count (r 0·31, $P < 0·001$). Serum ferritin was correlated with transferrin saturation (r 0·38, $P < 0·001$) and leucocyte count (r 0·28, $P < 0·001$).

Maternal folate status

Means, with their standard deviations, and percentage of abnormal values for erythrocyte and serum folate concentrations, are shown in Table 4. Erythrocyte folate values were correlated with serum folate values (r 0·56, $P < 0·001$). Folate deficiency (defined as an erythrocyte folate level below 160 μg/l) was observed in fifty-seven (45%) pregnant women and anaemia was associated with folate deficiency in thirty-three (26%) cases. Fe-deficiency was associated with folate deficiency in forty-four (35%) pregnant women.

Finally, 83% of cases of anaemia were associated with Fe-deficiency and 48% were associated with folate deficiency. Fe and folic acid deficiencies were associated with each other in 26% of anaemic women.

Types of Hb, and malarial infestation of mothers

Hb was type AA in ninety-four (75%) pregnant women, type AS in twenty (16%) cases, type AC in ten (8%) cases and type SC in two (1%) cases. Percentages were similar in the control group of non-pregnant women. Homozygous sickle cell disease was not observed. The frequency of anaemia was not significantly different among the three main Hb types.

Plasmodium falciparum was found in blood thick film in 99% of pregnant women (and in 97% of the control group). Only 7% of pregnant women had moderate or high malarial infestation density (density of five trophozoites or more per microscopic field). Mean haemoglobin, frequency of anaemia, Fe and folic acid status were not related to the density of malarial infection.

Newborn Fe and folic acid status

Mean values, with their standard deviations, of the haematological indices of newborn babies are shown in Table 5. Apart from the total Fe-binding capacity, the means of the various measurements were significantly higher in the newborn infants than in their mothers ($P < 0·05$). No baby had a Hb level lower than 110g/l or a serum ferritin concentration of less than 12 μg/l. Twelve (10%) had a Hb level less than 150 g/l and 27% had a transferrin

Table 5. *Haematological indices for newborn Beninese infants* (n *126*)

	Mean	SD	Range
Haemoglobin (g/l)	174	22	(113–230)
Packed cell volume (%)	56·0	6·7	(39–76)
Mean corpuscular volume (fl)	103·2	7·8	(76–149)
Percent saturation of transferrin	19·1	9·0	(4·4–52·1)
Erythrocyte protoporphyrin (μg/g Hb)	4·7	2·2	(1·6–12·8)
Serum ferritin (μg/l)	394 256*	582	(40–5000)
Erythrocyte folate (μg/l)	420 398*	125	(166–990)

* Geometric mean.

Table 6. *Measurements of iron status of newborn infants from Fe-sufficient and Fe-deficient Beninese mothers and from Beninese mothers with inflammatory syndrome*
(Mean values with their standard deviations)

	Newborn from Fe-sufficient mothers (*n* 14)		Newborn from Fe-deficient mothers (*n* 48)		Newborn from mothers with inflammatory syndrome (*n* 17)	
	Mean	SD	Mean	SD	Mean	SD
Haemoglobin (Hb; g/l)	183	23	166*	20	178	16
Mean corpuscular volume (fl)	108·2	13·2	102·1*	8·0	106·7	4·1
Percentage saturation of transferrin	20·2	11·1	16·2	8·0	20·0	14·3
Erythrocyte protoporphyrin (μg/g Hb)	3·7	1·2	4·9	2·4	4·4	1·5
Serum ferritin (μg/l)†	308		263		331	

* Mean value was significantly different from that for newborn infants from Fe-sufficient mothers ($P < 0·05$).
† Geometric mean.

saturation of less than 16%. Erythrocyte protoporphyrin level was higher than 3 μg/g Hb in 86% of cases. Hb, serum Fe, transferrin saturation, erythrocyte protoporphyrin and serum ferritin were not significantly different for babies born of anaemic and non-anaemic mothers.

Among babies, Hb levels were negatively correlated only with erythrocyte protoporphyrin (r −0·26, $P < 0·01$). Hb concentration in newborn infants was positively correlated with maternal serum ferritin (r 0·26, $P < 0·01$). Measurements of Fe status of newborn infants from Fe-sufficient mothers (without inflammatory syndromes), Fe-deficient mothers and mothers with inflammatory syndrome (without Fe-deficiency) are shown in Table 6. Hb concentrations and mean corpuscular volume were significantly lower in newborn infants from Fe-deficient mothers ($P < 0·05$). Erythrocyte folate in newborn infants was not related to maternal folate status. No differences were observed between the haematopoietic profiles of babies born of folate-deficient mothers and those born of normal mothers. Duration of gestation and birth weight were not related to maternal anaemia, nor to indicators of Fe or folic acid status.

DISCUSSION

The results of the present study show that the frequency of anaemia (World Health Organization, 1972) is very high in pregnant women and in menstruating women. These results are in agreement with values from other studies carried out in Western Africa (Ojo, 1965; Simic, 1973; Murray *et al.* 1978). Factors affecting Hb concentration are multiple in Africa; Fleming (1982) suggested that malaria could lower the mean Hb concentration in the population by 20 g/l, causing profound anaemia in some subjects. In our study, chronic malarial infection was very common. The intensity of malarial parasitaemia was not related to Hb concentration, but only a few women had a high malarial infestation level. Sickle cell disease is also known to affect the Hb level (Fleming, 1982), but homozygous sickle cell diseases were not observed in our study. Haemoglobinopathies were mainly heterozygous and did not seem to contribute significantly to anaemia.

Fe-deficiency and folate deficiency were very common. However, the diagnosis of these deficiencies is difficult in a tropical context and requires an adequate, acceptable definition. Several methods of measuring Fe status are available (Cook *et al*, 1976; Cook & Finch, 1979; Cook, 1982). Serum ferritin is considered as the most sensitive indicator of body Fe stores. Many studies with normal subjects, patients with Fe-deficiency and Fe overload have shown that the circulating ferritin concentration is directly proportional to the body level of Fe stores, as determined by phlebotomy, Fe absorption, and histological and biochemical determinations (Addison *et al.* 1972; Walters *et al.* 1973; Lipschitz *et al.* 1974; Bezwoda *et al.* 1979). Serum Fe level, transferrin saturation coefficent and erythrocyte protoporphyrin concentration are useful in indicating the adequacy of the Fe supply to the erythroid marrow (Cook, 1982). Anaemia and microcytosis reflect a final stage of Fe-deficiency and are useful for gauging the severity of Fe-deficiency.

The use of each of these indicators is problematic in the tropical context because of the frequency of confounding factors, particularly inflammatory syndromes and chronic infections which may be responsible of misclassification of the Fe status of subjects (Hecberg & Galán, 1985). The most important problem in assessing Fe status in an African population is distinguishing chronic inflammation from Fe-deficiency. Inflammation is responsible for a decrease in the percentage of transferrin saturation, an increase in the erythrocyte protoporphyrin concentration and a very high elevation of the serum ferritin level (Dallman *et al.* 1979; Blake *et al.* 1981). The decrease in the percentage transferrin saturation is explained by a decreasing serum Fe level linked to immobilization of Fe in the reticulo-endothelial system during inflammation (Lipschitz *et al.* 1971, Elin *et al.* 1977). It was suggested that the rise in the serum ferritin level was caused by an augmentation of ferritin synthesis rather than by a release of ferritin from inflammatory cells (Kojnin & Hershko, 1977). Thus, the use of transferrin saturation or erythrocyte protoporphyrin concentration, or both, as indicators of Fe status may cause an incorrect diagnosis of Fe-deficiency in populations presenting inflammatory syndromes. Conversely, the use of serum ferritin could lead to a diagnosis of Fe-sufficiency in Fe-depleted subjects, and an underestimation of the prevalence of Fe-deficiency. The usual approach of separating normal from Fe-deficient subjects on the basis of only one criterion inevitably involves errors in the diagnosis of both normal and Fe-deficient subjects.

In inflammation, the rise in the serum ferritin level is usually very high, particularly compared with the modifications in transferrin saturation or the erythrocyte protoporphyrin level. A moderate rise in the serum ferritin concentration, combined with a low-percentage transferrin saturation or a high erythrocyte protoporphyrin level, or both, suggests Fe-deficiency in an inflammatory context (Hillmans & Finch, 1985; Blake *et al.* 1981). This is consistent with Fleming's (1982) work in Nigeria. He observed that most women and

children with markedly reduced Fe stores, as assessed by an estimate of stainable Fe in the bone marrow, had serum ferritin values below 50 μg/l, while only a few of those with adequate Fe stores had serum ferritin values below this level. Using this approach, we found that Fe-deficiency and Fe-deficiency anaemia occurred in 73 and 45% of pregnant women respectively. The high frequency and the severity of Fe deficiency at the end of pregnancy may be explained by the low availability of dietary Fe intake and by the low level of Fe stores in women at the beginning of the pregnancy. In our control group 41% of menstruating women were Fe-deficient.

For assessment of folate status, erythrocyte folate is currently accepted as being the best test of tissue folate depletion which can be performed on the peripheral blood (Hoffbrand *et al.* 1966). Plasma folate level is considered to reflect the amount of folate absorbed during the recent period (Sauberlich *et al.* 1974). Moreover, plasma folate may be low in pregnancy even in the absence of tissue folate deficiency (Avery & Ladger, 1970). Using erythrocyte folate as a criterion to diagnosis folate deficiency, we found 45% of pregnant women to be folate deficient.

Although it is clear that the transfer of Fe and folate from mother to fetus is an active process, it is not clear whether the fetus takes up optimal amounts of Fe and folate, or whether it takes up amounts proportional to levels available in mothers who are Fe- or folate-deficient, or both. In our study no differences were found in haematological profiles of babies born of anaemic and non-anaemic mothers, nor were differences observed according to the folate status of the mothers. However, differences were found according to the Fe status of the mothers when the Fe status was assessed by a number of indicators. Results in the literature on the relation between maternal and newborn Fe status are contradictory. Some authors have found such a relation (Fenton *et al.* 1977; Kelly *et al.* 1978; Puolakka *et al.* 1980) while others have not (Rios *et al.* 1975; Hussain *et al.* 1977; Van Eyjk *et al.* 1978; Gebre-Medhin & Birgegard, 1981). These discrepancies may be explained by the different criteria used to define Fe-deficiency. In most of the studies, the mother's Fe status was defined by one or two biochemical measurements; this did not permit a valid classification of Fe-deficiency.

Results obtained in the present study suggest that Fe-deficiency is very common in pregnant women in Benin and represents the main cause of anaemia. The effect of the mother's Fe status on the haematological profile of the newborn infants was revealed when strict criteria, based on a combination of several independent biochemical indicators, were used to define Fe-deficiency.

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