Iron, folate, and vitamin B₁₂ nutrition in pregnancy: a study of 1000 women from southern India*

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As part of a WHO collaborative programme the prevalence of anaemia was studied and the serum concentrations of iron, folate, and vitamin B₁₂ were measured in 1000 pregnant women from southern India. The results of the study show a high prevalence of anaemia, resulting from iron and folate deficiency with iron deficiency predominating. Interrelationships between these nutrients and their effect on pregnancy and the fetus were investigated. The results indicate that, in comparison with populations in developed countries, there was a high prevalence of iron and vitamin B₁₂ deficiency in the community, but the state of folate nutrition was similar to that found elsewhere.

Nutritional anaemia is one of the main health problems in many tropical and subtropical countries (Joint FAO/WHO Expert Committee on Nutrition, 1958), but there are few accurate statistics of the prevalence of the various types of anaemia and even less information is available on the iron, folate, and vitamin B₁₂ nutrition of people in these regions. Pregnancy imposes extra nutritional demands on the mother and is likely to exaggerate any latent deficiency states. A study of the haematological status of women in the third trimester of pregnancy may be expected to provide a sensitive index of the nutritional status of the population at large.

Collaborative studies on the prevalence of anaemia and on the status of iron, folate, and vitamin B₁₂ nutrition in pregnant women, in different parts of the world, were therefore set up by the World Health Organization (Patwardhan, 1966; WHO Scientific Group on Nutritional Anaemias, 1968). This study of 1000 pregnant women in southern India formed part of this collaborative programme.

MATERIAL AND METHODS

The subjects studied were women in the third trimester of pregnancy who were attending the routine antenatal clinic or admitted at term to the maternity wards. Patients referred from elsewhere, because of obstetrical or medical complications, were not included. Any patient who had had medication of any sort, either in this hospital or (as far as could be ascertained) from outside sources, was excluded. Subjects were selected for study by taking the patients in order of presentation at the clinic or of admission to the wards, the number sampled each day depending only on the number of patients and specimens that could be handled. Two of the subjects so sampled were found to have congenital haemolytic anaemia and were excluded from the study. The same observer obtained a clinical and dietary history from each patient and carried out a physical examination. The economic status of the family was assessed as either “poor” (estimated income less than Rs 100 per month), “medium” (income Rs 100–300 per month), or “good” (income greater than Rs 300 per month).

A total of 492 women delivered in the hospital and the fetal birth weights were recorded.

Three groups of control subjects were studied.

(1) “Female controls”: non-pregnant women, similar in age and socioeconomic status to the pregnant group, who were attending the gynaecological department of the hospital for minor complaints not associated with menorrhagia or oligomenorrhea.

(2) “Male controls”: an attempt was made to obtain a random sample of the husbands of the women admitted to hospital for delivery. In fact, the sample was not strictly random since some would not cooperate, and the group is slightly biased towards the higher socioeconomic strata.

(3) “Ame-
ricans": these were 55 male and 58 female American Peace Corps volunteers, between the ages of 20 and 35, studied within 10 days of their arrival in India. None of the females was pregnant.

The haematological tests were performed on venous blood obtained from the antecubital vein by means of the techniques described by Dacie (1956). Haemoglobin was estimated by the cyanmethaemoglobin method with a photoelectric colorimeter, which was calibrated and checked at regular intervals against a standard cyanmethaemoglobin solution. Serum iron was estimated by the method of Ramsay (1957). The unsaturated iron-binding capacity of the serum was measured by the method of Bothwell et al. (1959) or that of Herbert et al. (1966), both methods giving comparable results. Serum folate and serum vitamin B12 were estimated by microbiological assay using Lactobacillus casei (Baker et al., 1959) and Euglena gracilis Z strain (Hatzer et al., 1956), respectively. Serum proteins were determined by the biuret method.

With the patients informed consent, specimens of bone marrow were obtained from 740 of the pregnant women. The group from which marrow was not obtained was similar to that from which marrow was available. The smears were stained with May-Grünwald-Giemsa stain and were interpreted without any knowledge of the patient or of the other haematological values. Marrow was classified as normoblastic or megaloblastic, and the megaloblastic marrow was graded I-IV depending on the severity of the morphological change, the mildest being designated grade I and the most severe grade IV (Baker, 1958). Marrow smears were also stained for iron with potassium hexacyanoferrate(II), a positive control slide being included with each set stained.

Correlations were sought between laboratory measurements and factors such as the height of the uterus, the period since the previous pregnancy, maternal parity, socioeconomic status, dietary grouping, serum albumin level, presence of oedema or toxoaemia, the season of the year, and fetal birth weight. In the following results, positive correlations are mentioned, but negative ones are not.

RESULTS

Characteristics of pregnant group

The pregnant women were between the ages of 15 and 35, mean age 25.3 years. Of their husbands, 48% worked as labourers, 14% were farmers, and the remainder worked as clerks, shopkeepers, or teachers, or at other professions. The economic status of 61% of the subjects was rated as "poor", 31% were from families rated as "medium", and 8% were from families rated as "good".

The diet of the majority of the patients was found to conform to that described in detail by Rao & Rao (1958a, 1958b). Most were vegetarian by religious belief or economic necessity, and only 7% ate animal protein more than once a month and were classed as "nonvegetarian".

The majority of pregnant women were normal on physical examination, but glositis was present in 19% stomatitis in 7% and kolobonychia in 3%. 11% of subjects had pitting oedema of the ankles and in 3% there was generalized oedema; and 8% of the women were considered to be suffering from toxoaemia of pregnancy (mild in 28 cases, moderate in 25, and severe in 30). Hookworm ova were found in the stools of 35% of the women.

Fetuses

The birth weight of the fetuses ranged from 900 g to 4 500 g with a mean of 2 606 g (SD 640). Because the women were never certain of their dates no attempt was made to distinguish between fetuses underweight because of prematurity and those underweight because of impaired fetal development.

Blood film and bone marrow

Examination of stained blood films from the pregnant women showed an apparently normal film in 34% of subjects, hypocromia in 53%, and megalocytes in 27%. Stainable marrow iron, in the form of occasional haemosiderin-containing cells, was found in only 12 subjects (2%). A normoblastic pattern of erythropoiesis was found in 40% of all marrows and 60% showed a megaloblastic pattern (31% grade I, 24% grade II, 4% grade III, and 1% grade IV). The prevalence of megaloblastosis increased from 48% in women sampled at the beginning of the third trimester to 66% in women sampled at term.

Haemoglobin and packed cell volume

The mean haemoglobin concentration of the pregnant women (104 g/litre, SD 27) was significantly lower (P<0.001) than that of the control women (122 g/litre, SD 15), which in turn was lower (P<0.001) than that of the male controls.
Fig. 1. Cumulative frequency polygon showing the haemoglobin concentration in the three groups of subjects.

Fig. 2. Cumulative frequency polygon showing the serum iron concentration in the three groups of subjects.

Fig. 3. Cumulative frequency polygon showing the percentage saturation of transferrin in the three groups of subjects.

Fig. 4. Cumulative frequency polygon showing the serum folate concentration in the three groups of subjects.
(149 g/litre, SD 17). A haemoglobin concentration of less than 100 g/litre was found in 32.7% of the pregnant women and this value was arbitrarily taken as indicating anaemia (WHO Scientific Group on Nutritional Anaemia, 1958). Haemoglobin levels below 110 g/litre were found in 57.4% and levels of less than 120 g/litre in 83% (Fig. 1).

The mean haemoglobin concentration was significantly lower in women who had hookworm (P < 0.001), in women who had had 3 or more previous pregnancies (P < 0.001), in those who had oedema (P < 0.01), and in those who had glossitis (P < 0.01). There was a correlation, significant at the 1% level, between maternal haemoglobin and fetal birth weight (r = +0.19).

**Serum iron concentration**

The mean serum iron concentrations of the American males (1.12 mg/litre, SD 0.23) and females (1.11 mg/litre, SD 0.22) were similar. The mean value in the male controls (1.0 mg/litre, SD 0.24) did not differ significantly from that in the Americans, but the mean of the female controls (0.53 mg/litre, SD 0.21) was significantly lower (P < 0.01) and that of the pregnant women (0.31 mg/litre, SD 0.12) was lower than that of the female controls (P < 0.001); 96% of the pregnant women had serum iron concentrations below 0.50 mg/litre (Fig. 2). The mean serum iron concentration was significantly lower in those pregnant women with hookworm infestation (P < 0.001), in multiparous women (P < 0.001), in those with kollonychia (P < 0.01), and in those with edema (P < 0.01). There was also a correlation, significant at the 1% level, between the serum iron concentration and the degree of anaemia (r = +0.62).

**Percentage saturation of transferrin**

The mean percentage saturation of transferrin in the American males (39.2%, SD 9.4) and females (38.4%, SD 10.5) and the male controls (38.6%, SD 11.9) was similar, but the values in the female controls were significantly lower (mean 19.1%, SD 10.3; P < 0.01) and in the pregnant women the mean value (7.2%, SD 4.9) was significantly lower than that in the female controls (P < 0.001); 96% of pregnant women had values below 16% (Fig. 3). The percentage saturation of transferrin was lower in those with hookworm (P < 0.01) and also in those with kollonychia (P < 0.001).

There were correlations, significant at the 0.1% level, between the percentage saturation of transferrin and the serum iron concentration (r = +0.86) and between the percentage saturation and the haemoglobin level (r = +0.56).

**Serum folate concentration**

The mean serum folate concentrations of the American males and females were identical (9.6 μg/litre, SD 3.1) and similar to those of the male (7.8 μg/litre, SD 3.3) and female controls (8.9 μg/litre, SD 3.9), but the mean concentration in the pregnant women (5.6 μg/litre, SD 2.5) was significantly lower than that in the female controls (P < 0.001); 73% of the women had values less than 6 μg/litre (Fig. 4). The mean serum folate concentration was lower in women sampled at term than in those sampled during the earlier part of the third trimester (P < 0.001). There was also a seasonal variation, the lowest values being found in the hot dry month of April and the highest values in the month of August (P < 0.01). Serum folate levels were also lower in women with glossitis (P < 0.001), in those with angular stomatitis (P < 0.01), and in those from the "poor" dietary group (P < 0.01).

Fig. 5. Cumulative frequency polygon showing the serum vitamin B12 concentration in the three groups of subjects.
There was a correlation, significant at the 0.1% level, between the serum folate and maternal haemoglobin concentrations \( r = 0.81 \). There was also a correlation between serum folate and serum iron concentrations significant at the 1% level \( r = 0.15 \). There was no relationship between serum folate and fetal birth weight.

**Serum vitamin B\(_9\) concentration**

The mean serum vitamin B\(_9\) concentrations of the American males and females were the same (300 ng/litre, SD 95). The mean values for the male controls (188 ng/litre, SD 69) and the female controls (205 ng/litre, SD 67) were both lower than those for the Americans (P < 0.01). The mean value for the pregnant women (199 ng/litre, SD 122) was significantly lower than that for the female controls \( (P < 0.001) \), and 52% of pregnant women had values below 140 ng/litre (Fig. 5). Patients with glossitis and those with angular stomatitis had significantly lower serum vitamin B\(_9\) concentrations \( (P < 0.01) \). There was no relationship between serum vitamin B\(_9\) concentration and the fetal birth weight.

**Discussion**

This study was of necessity based on a representative sample of women attending hospital for routine antenatal care or for delivery and is not necessarily representative of the community as a whole.

The study has demonstrated a very high prevalence of megaloblastosis, anaemia, iron deficiency, folate deficiency, and low serum vitamin B\(_9\) concentrations in women in the last trimester of pregnancy, and confirms the findings of an earlier study from this hospital (Karthigai, et al., 1964).

**Megaloblastosis**

The reported prevalence of megaloblastosis in pregnancy varies widely. The highest figure appears to be that of Roberts et al. (1963), who found megaloblastic changes in 75% of nonanaemic and 90% of anaemic pregnant women. Giles (1966) on the other hand reported a prevalence of only 3% in women who had not received nutritional supplements. The wide variations in different reports are presumably due to a number of factors including differences in interpretation of morphological changes, differences in socioeconomic status and dietary habits of the population, and different methods of case selection. It is therefore doubtful whether valid comparisons can be made between findings in different centres; nevertheless it is of interest that the prevalence of megaloblastosis in this study was lower than that reported by Roberts et al. (1963) from a developed country.

### Anaemia

The prevalence of anaemia in this population (32.7% with haemoglobin < 100 g/litre) was a little lower than that found in northern India (41.5%) by Sidhu et al. (1967) and in Trinidad by Chopra et al. (1967), but was similar to that found in a rural community in Israel where 29% of third trimester patients had a haemoglobin concentration below 100 g/litre (Rachmilewitz et al., 1966). The results of these studies are in marked contrast to experience in more developed countries where the prevalence is much lower (Kerr & Davidson, 1958; Lowenstein et al., 1966).

The women in this study with hookworm infestation had a slightly, but significantly, lower mean haemoglobin concentration than those without hookworm. Even in those women in whom hookworm was not demonstrated, however, 25% had haemoglobin values below 100 g/litre, suggesting that hookworm infestation was not a major factor in the etiology of the anaemia.

There were statistically significant correlations between the haemoglobin concentration and the serum iron concentration, the percentage saturation of transferrin, and the serum folate concentration. On the other hand there was no correlation between haemoglobin and serum vitamin B\(_9\) concentrations. This suggests that the anaemia was related chiefly to iron and folate deficiency.

### Iron nutrition

The mean serum iron concentration and percent saturation of transferrin of the pregnant group were considerably lower than those of the non-pregnant controls, and appear to be lower than any previous recorded values for a group of unselected pregnant women. Venkatachalam (1962) records a mean serum iron concentration of 0.329 mg/litre in pregnancy, but this was in a selected group of anaemic women. The high prevalence of iron deficiency in the present study was also borne out by the almost total absence of stainable marrow iron. The severity and high prevalence of iron deficiency in this series suggests that this was the main factor in the pathogenesis of the anaemia. Many of the
nonpregnant women also had low serum iron levels and a reduced percentage saturation of transferrin, showing that the reduction in the pregnant group reflected not merely the extra demands of that pregnancy, but also the poor iron status of women from this region in general.

The values for the Americans (males and females) are within the accepted normal range and indicate that the low values obtained in the pregnant and control women were not the result of laboratory error.

In spite of the severe degree of iron deficiency it is of interest that only 8% of women had koilonychia. The lower mean serum iron concentration and percentage saturation of transferrin indicate that subjects with koilonychia, as a group, were even more iron-deficient than those without this condition.

The extremely high prevalence of iron deficiency found in this study must reflect the prevalence of low iron stores in the community as a whole. The majority of control males had serum iron and percentage saturation of transferrin values approaching those accepted as normal in economically advanced countries. However, this does not necessarily mean that they had normal iron stores. In southern India bone marrow iron is rarely found even in males with megaloblastic anaemia (Baker, S. J., unpublished data, 1963) and in southern and northern India storage iron in both males and females is greatly reduced (Routh & Agarwal, 1967; WHO Scientific Group on Nutritional Anaemias, 1968). The reasons for the high prevalence of iron deficiency are not clear. The mean daily intake of iron provided by the average Indian diet has been variously estimated as being between 15 and 30 mg per day (Rao et al., 1959; Patwardhan, 1956). Iron losses from hookworm infestation obviously play a part, but iron deficiency is common even in the absence of hookworm. Studies of body iron excretion with 59Fe-labelled iron have shown that iron losses in subjects working in a hot humid environment are of the same order as are found in people in temperate regions (Green et al., 1968). It therefore appears that sweat losses cannot be accounted for an explanation for the low body iron stores. The most probable explanation is that the food iron is not readily available, either because of its chemical form (Chodos et al., 1957; Hussain et al., 1965; Edwood, 1966) or because of the presence of substances in the diet, such as phytates and phosphates that inhibit iron absorption (Foy et al., 1959; Hussain & Patwardhan, 1959; Apte & Venkatachalap, 1962).

Folate nutrition

With any microbiological assay, comparison of results between different laboratories is difficult. Nevertheless, the serum folate levels in the non-pregnant subjects in this study are close to those found in normal subjects by a number of other workers (Baker et al., 1959; Waters & Mollin, 1961; Solomons et al., 1962). The prevalence of values below 6 μg/litre in the pregnant women in this study is similar to that seen in developed countries (Ball & Giles, 1964; Lowenstein et al., 1966; Whitehead et al., 1968). The fall in serum levels with increasing duration of gestation is also similar to that observed by a number of workers (Young et al., 1959; Lowenstein et al., 1960; Lubby et al., 1961; Solomons et al., 1962; Hansen & Kleesabl-Pales, 1963; Ball & Giles, 1964; Temperley et al., 1968; Davis et al., 1969).

In a normal person deprived of dietary folate, the body stores of folate are sufficient for only about 4 months (Herbert, 1962). The low levels of folate occurring during pregnancy indicate that the increased demands outstrip the available supply from the food, rather than a pre-existent deficiency of body stores.

The significance of the correlation found between serum iron and serum folate levels is difficult to evaluate; however, it has been suggested that iron deficiency can cause secondary folate deficiency (Viral et al., 1966; Velaz et al., 1966). Channer et al. (1965) found that women who were given supplemental iron had a higher mean serum folate at term than those who were not, but the differences observed were not statistically significant, and in a later study (Chanarin & Rothman, 1971) there was no difference in the frequency of megaloblastosis in those women who received iron supplements in comparison with those who received a placebo. Metz et al. (1967) showed that postpartum patients treated with iron had significantly lower serum folate levels than did women not given iron. Further, the prevalence of low serum folate levels in the women in this study was no greater than that reported in other groups of women who were not so severely iron-deficient. Therefore the observed relationship between serum iron and serum folate levels should not necessarily be taken to indicate that one caused the other; both may be related to a third factor.

Vitamin B12 nutrition

The mean serum vitamin B12 concentrations of control males and females were significantly lower
than those in the American subjects. This difference presumably reflects the basic differences in dietary habit, the Indian controls being either vegetarian or eating much less animal protein, which results in lower body stores of vitamin B₁₂ (Baker, 1967).

The concentrations in pregnant women were signifi-
cantly lower than those in control women. This lower concentration of vitamin B₁₂ at the time of delivery was observed by many workers (Young et al., 1959; Loweinstein et al., 1960; Lubby et al., 1961; Ball & Giles, 1964; Whiteside et al., 1968). The significance of these findings in terms of maternal health is difficult to assess, although occasionally maternal B₁₂ deficiency may adversely affect the infant (Baker et al., 1962). Treatment of some cases of megaloblastic anaemia with folic acid may cause a rise in serum vitamin B₁₂ levels (Narayanan et al., 1957; Johnson et al., 1962) and it is possible that if the drop in folate levels were prevented by folate supple-
mentation the fall in serum B₁₂ levels might not be so marked.

Effects on Fetus

The observation that mothers with lower homoglo-
bolin concentrations tended to have children of lower birth weight suggests that maternal anaemia has a detrimental influence on fetal development. This did not appear to be related to deficiency of any specific nutrient since maternal serum iron, serum folate, and serum vitamin B₁₂ concentrations showed no significant correlation with fetal birth weight apart from their relationship to maternal haemoglobin. Roskowski et al. (1966) showed that the mean fetal birth weight in their control group was greater than in their group with serum iron deficiency. Whiteside et al. (1968) also found a positive correlation be-
tween the maternal serum iron levels at the 26th week and the fetal birth weight. However, neither group of workers gave any data regarding the rela-
tionship between maternal haemoglobin and birth weight, so that the apparent relationship with serum iron might only reflect changes in maternal haemo-
globin concentration. The reported effects of mater-
nal folate deficiency on fetal birth weight are con-
fllicting (Rothman, 1970). It is clear from this study that, before any effect of folate on birth weight can be established, the effect of maternal haemoglobin con-
centration must be taken into account.

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RÉSUMÉ

FER, FOLATE ET VITAMINE B₁₂ PENDANT LA GROSSESSE: ÉTUDE PORTANT SUR 1000 FEMMES EN INDE MERIDIONALE

La présente enquête, menée dans le cadre d’un pro-
gramme OMS d’études collectives des anémies nutri-
tionnelles, a porté sur 1 000 femmes enceintes, dans le sud de l’Inde.

L’examen de ce groupe a montré une forte prévalence de l’anémie, de la megaloblastose, de la carence mariale et de la carence en folate, ainsi que de faibles concen-
trations sériques de vitamine B₁₂. Les concentrations de fer sérique étaient plus faibles que celles signalées lors d’enquêtes du même genre. On a constitué une correlation franche entre les taux d’hémoglobine et les teneurs sériques en fer et en folate. Il semble que les carences en ces deux nutriments soient responsables de l’anémie, mais la fré-
quence très élevée et la gravité de la carence mariale

amènent à la considérer comme la cause principale.

Sur les 1 000 femmes examinées pendant cette étude, 492 ont accouché à l’hôpital. On a pu établir un rapport entre le taux de hémoglobine et le poids du nouveau-né à la naissance, mais l’insuffisance pondérale n’a pu être attribuée spécifiquement à la carence en fer, en folate ou en vitamine B₁₂.

D’après les auteurs, ces résultats démontrent que l’apport alimentaire de fer et de folate est insuffisant pour couvrir les besoins normaux chez une forte pro-
portion des femmes enceintes étudiées en Inde méridi-
ionale. Ils suggèrent de compléter le régime de cette fraction de la population par l’administration de fer et d’acide folique en quantités qui restent à déterminer.