# PROTEIN DEFICIENCY AND PREGNANCY ANAEMIAS

by

D. Sharma

I. Khosla

and

Y. N. Gupta

Protein deficiency was reported to cause normocytic normochromic anaemia in rhesus monkeys (Sood *et al.*, 1965). Adams *et al.*, (1967) were impressed by the similarity between the anaemia in protein deficient rhesus monkeys and anaemia in kwashiorkor. Ghitis *et al.*, (1963) and Allen & Dean (1965) were also of the opinion that protein deficiency was an important cause of anaemia in kwashiorkor. The role of protein deficiency in the etiology of anaemia has also been studied and discussed by many other workers (Whiple *et al.*, 1940; Cartwright & Wintrobe, 1948; Lucas & Obten, 1954; Woodruff, 1955; Aschkenasy, 1957; Bethard, *et al.*, 1958; Waterlow, *et al.*, 1960; Latham & Stare, 1967; Alvarado & Viteri, 1970; Barnes, 1970; Kader & Rahman, 1972; Araya & Tagle, 1973).

Pregnancy anaemias in India were reported to be often associated with marked hypoproteinaemia (Upadhyay, 1971). Poverty, ignorance and local zymotic factors in India often lead to multiple nutritional deficiencies, including gross protein deficiency. What is the contribution of hypoproteinaemia in the causation of pregnancy anaemias? It is not possible to provide an answer to this question through direct experimentation. However, it was considered interesting to see if haematologic parameters bear any statistical correlation with serum protein values in pregnancy anaemia. The present study was, therefore, carried out to evaluate this correlation.

*Department of Obstetrics and Gynaecology and Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005.*

### *Material and Method*

Ninety-four women attending an antenatal clinic and having a haemoglobin value of 8 g.% or less were admitted to the wards for a detailed haematologic evaluation and serum protein measurements. Of these 94, two attended in the first trimester, 14 in the second trimester, 44 in the third trimester of pregnancy and 34 in the early puerperal period.

After a thorough clinical examination the following investigations were done— (1) Routine urinalysis and microscopic examination of stools. (2) Bone marrow biopsy (sternal puncture). (3) Haemoglobin per cent, R.B.C. count, reticulocyte count and haematocrit (venous blood). (4) Plasma iron. (5) Total serum proteins and serum albumin and globulins.

Plasma iron was determined by the Ramsay's (1958) technique. Serum proteins were estimated by biuret method. Techniques described by Dacie and Lewis (1966) were used for haemogram and marrow biopsy. Cases were classified as megaloblastic or normoblastic anaemia on the basis of the type of erythropoiesis seen in the bone marrow smears (Montgomery, 1965).

For comparing means, the Students "t" and for assessing correlations, Pearson's product moment co-efficient, were computed (Bailey, 1959).

*Results*

Urinalysis revealed no abnormality in any patient. Microscopic examination of the stools showed a high incidence of worm infestations (Table I). Hookworm infestation was present in 47.0 per cent of cases. Many patients had multiple infestations. Cysts of entamoeba histolytica were seen in the stools of 12 (13.0%) patients.

Fifty-four (57.4%) patients showed a megaloblastic erythropoiesis and 40 (42.6%) a normoblastic marrow reaction. There was no significant difference in the cellularity of the bone marrow in the two groups.

Important clinical signs and symptoms are listed in Table II. All patients complained of weakness. Of the 80 (85.1%) patients who had oedema, only 34 had signs of concomitant congestive heart failure which could have partly contributed to the development of oedema. None of the remaining cases with general

TABLE I

*Incidence of Worm Infestation and Amoebiasis*

| Infestation | Incidence in megaloblastic group (54 cases) | Incidence in normoblastic group (40 cases) | Incidence in the entire series |
|---|---|---|---|
| Hookworm | 28 (51.9) | 16 (40.0) | 44 (46.8) |
| Roundworm | 12 (22.2) | 12 (30.0) | 24 (25.5) |
| Threadworm | 2 ( 3.7) | 2 ( 5.0) | 4 ( 4.3) |
| Entamoeba histolytica | 2 ( 3.7) | 10 (25.0) | 12 (12.8) |

Figures in parantheses indicate percentage.

TABLE II

*Important Signs and Symptoms and Their Incidence*

| Signs and symptoms | Incidence in megaloblastic group (54 cases) | Incidence in normoblastic group (40 cases) | Incidence in series as a whole |
|---|---|---|---|
| 1. Oedema (including cases under category 5 below) | 50 (92.6) | 30 (75.0) | 80 (85.1) |
| 2. Pigmentation of skin | 28 (51.9) | 16 (40.0) | 44 (46.8) |
| 3. Glossitis and stomatitis | 24 (44.4) | 12 (30.0) | 36 (38.3) |
| 4. Hepatomegaly (without congestive heart failure) | 10 (18.5) | 8 (20.0) | 18 (19.1) |
| 5. Congestive heart failure | 24 (44.4) | 10 (25.0) | 34 (36.2) |
| 6. Weakness | 54 (100 ) | 40 (100 ) | 94 (100 ) |

Figures in parantheses indicate percentages.

Incidences in the megaloblastic and normoblastic groups were not significantly different ($p>0.05$).

oedema had any other disease, except hypoproteinaemia which could give rise to oedema. Fairly high incidence of skin pigmentation, glossitis and stomatitis and hepatomegaly were further indications of a marked degree of malnutrition in these patients. There was no significant difference in the incidences of various signs and symptoms in the megaloblastic and normoblastic groups.

*Haemogram, Plasma Iron and Serum Proteins*

The incidence of normocytic, macrocytic, microcytic hypochromic and dimorphic peripheral pictures in the megaloblastic and the normoblastic groups is shown in Table III. The presence of a microcytic hypochromic peripheral picture in patients with megaloblastic bone marrow reaction was remarkable.

The mean values of haemoglobin concentration, R.B.C. count, reticulocyte count, haematocrit, mean cell volume, mean cell haemoglobin content, plasma iron, total serum proteins, serum albumin and serum globulins observed in patients with megaloblastic and normoblastic anaemias are shown in Table IV.

The mean haemoglobin percentage, the mean R.B.C. count and the mean packed cell volume (haematocrit) in the megaloblastic group were significantly lower than corresponding means in the normoblastic group. The megaloblastic group had a significantly higher mean plasma iron level as compared to the other group. The mean serum albumin level in the megaloblastic group was significantly lower than the mean serum albumin level in the normoblastic group.

*Correlation Between Serum Proteins and Haematologic Parameters*

In the megaloblastic group, the only significant correlation seen was that between the serum iron and the total serum protein levels ($r = 0.488$, $P<0.01$).

The statistically significant correlations amongst various haematologic parameters, serum iron and serum protein values in the normoblastic group are shown in Table V. All significant correlations were positive.

TABLE III

*Peripheral Blood Pictures in Megaloblastic and Normoblastic Anaemias*

| Peripheral blood picture | Incidence in megaloblastic group (54 cases) | Incidence in normoblastic group (40 cases) |
|---|---|---|
| Normocytic | 0 (00.0) | 12 (30.0) |
| Macrocytic | 18 (33.3) | 0 (00.0) |
| Microcytic hypochromic | 8 (14.8) | 8 (20.0) |
| Dimorphic | 28 (51.9) | 20 (50.0) |

*Discussion*

Low protein intake and hypoproteinaemia retard haemoglobin regeneration even in the presence of excess iron (Whipple *et al.*, 1940; Marks, *et al.*, 1963; Keele and Neil, 1970). Quality of protein consumed is also important, proteins from animal sources being most effective in haemoglobin regeneration (Keele and Neil, 1970). Protein deficiency interferes with absorption of iron from the intestines (Sood *et al.*, 1967). Even the transport of iron internally released from red cell de-

TABLE IV

*Mean (± S E) Haematilogic Values, Plasma Iron, Total Serum Proteins, Serum Albumin and Serum Globulins*

| Type of anaemia and number of cases | Haemoglobin (g%) | Red cell count ($x10^6$/ul) | Reticulocyte count (% of the red cell count) | Haematocrit (Vol. %) | Mean red cell volume ($u^3$ p) | Mean haemoglobin (pg) | Mean haemoglobin concentration (g%) | Plasma iron (ug%) | Total serum proteins (g%) | Serum albumin (g%) | Serum globulins (g%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Megaloblastic (54) | 4.5* ±0.21 | 1.6* ±0.11 | 0.98* ±0.19 | 11.3* ±0.80 | 98.6 ±2.9 | 29.5 ±1.2 | 30.0 ±0.8 | 85.0 ±17.1 | 5.0 ±0.14 | 2.4* ±0.08 | 2.6 ±0.09 |
| Normoblastic (40) | 5.6 ±0.47 | 2.2 ±0.17 | 1.2 ±0.2 | 20.1 ±1.38 | 92.6 ±3.6 | 25.7 ±1.2 | 27.8 ±1.2 | 58.2 ± 7.3 | 5.4 ±0.18 | 2.7 ±0.14 | 2.6 ±0.13 |

* Significantly higher than the corresponding mean in the other group (p 0.05).

TABLE V

*Significant Correlation in the Normoblastic Group*

| Correlation between | r | p |
|---|---|---|
| Haemoglobin and plasma iron | + 0.580 | 0.01 |
| R.B.C. count and plasma iron | + 0.492 | 0.05 |
| Haematocrit and Plasma iron | + 0.494 | 0.05 |
| Haemoglobin and Serum albumin | + 0.739 | 0.001 |
| R.B.C. count and total serum proteins | + 0.524 | 0.02 |
| R.B.C. count and serum albumin | + 0.706 | 0.001 |
| Haematocrit and serum albumin | + 0.704 | 0.001 |
| Mean cell haemoglobin content and total serum proteins | + 0.537 | 0.02 |
| Mean cell haemoglobin concentration and serum albumin | + 0.768 | 0.001 |
| Plasma iron and serum albumin | + 0.483 | 0.05 |

struction in the reticulo-endothelial system is depressed as is evident from the results of a study in rats recently reported by Araya and Tagle (1973) who demonstrated low levels of non-haeme iron in the blood and an increase in iron storage in the liver. Thus, protein deficiency seems to interfere with (i) absorption of iron from the intestines, (ii) transport of internally released iron from the reticulo-endothelial system and (iii) biosynthesis of haemoglobin even when adequate iron in available.

On the basis of the role of protein in haemopoiesis discussed above, it is evident that protein lack is likely to produce a clinical picture of iron deficiency anaemia. The nature of anaemia in protein deficient animals reported by Cartwright and Wintrobe (1948), Waterlow *et al.*, (1960) and Sood *et al.*, (1965) was normocytic normochromic. Aschkenasy (1957) reported microcytic hypochromic anaemia in protein deficiency. Reduced erythropoiesis has also been reported in protein malnutrition (Bethard *et al.*, 1958). Coward and Whitehead (1972) who found anaemia to be a feature of the final stages of kwashiorkor postulated lack of aminoacid substrates may depress synthesis of erythropoietin.

The positive correlations (some of them very strong) between haematologic parameters and serum protein values in normoblastic pregnancy anaemic reported here support the hypothesis that protein deficiency plays a role in the causation of anaemia in the normoblastic group. Although it is not strictly valid to conclude a cause and effect relationship from correlation data alone, the strong positive correlations between serum proteins and haematologic parameters cannot be outright dismissed as fortuitous.

The lack of correlation between haematologic parameters and serum protein values in megaloblastic anaemia is probably due to the fact that in this condition, deficiency of cyanocobalamine and petroylmonoglutamic acid depresses erythropoiesis at a stage when early and intermediate erythroblasts are dividing. It is after this stage that a programmed sequence of m-RNA and protein synthesis is required for red blood cell maturation and haemoglobinization. It is, as though, the lack of consumers which prevented the scarcity of a commodity from coming into prominence. However, even in the megaloblastic group, the serum iron levels showed a significant positive correlation with the total serum proteins. This is consistent with the role of protein in the absorption and transport of iron.

*Summary*

1. Haemoglobin concentrations, red blood cell counts haematocrit and serum iron levels were measured in women suffering from pregnancy anaemias. Total serum proteins, serum albumin and serum globulin were also determined.

2. Statistical correlations between haematologic parameters and serum protein levels were assessed separately in cases of megaloblastic and normoblastic anaemias of pregnancy.

3. Many haematologic parameters in normoblastic anaemia showed strong positive correlations with serum protein values, lending support to the hypothesis that hypoproteinaemia was also one of the determinants of the anaemic state.

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