NUTRITIONAL DEFICIENCY ANAEMIAS IN LATER MONTHS OF PREGNANCY

by

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The commonest association met with in pregnancy in all developing countries of Asia is anaemia. In India, the seriousness of its nature can be understood by the fact that it accounts for about 10 to 40% of maternal deaths in certain regions (Menon, 1965). The condition seems to be intimately connected with the socio-economic status, dietetic habits of the community and the geographical location. Preliminary results of a World Health Organisation study (taking 10.0 g.% as normal) in India show that 40% of the women are anaemic (Baker, 1966). The lack of data on nutritional deficiency anaemias in the later months of pregnancy, especially with respect to serum iron, folic acid, vitamin B_{12} and proteins initiated us to undertake this study.

Material and Methods

Control group

Thirty pregnant women in the second trimester of pregnancy were put on vitamins and iron therapy (Multivitamin 1 tab. and Fersolate 1 tab.) till the last days of the third trimester. The drugs were withdrawn one week prior to collection of blood samples.

Test group

Fifty-five pregnant women without any history of vaginal bleeding, hoemoglobin 10.0 g.% and not receiving supplementary vitamins and iron therapy were selected.

Ten ml. of blood was drawn from the anterior cubital vein with an all glass, iron-free, autoclaved syringe fitted with a stainless steel needle. All glassware was iron free and autoclaved, and chemicals were A.R. grade in the following procedures.

1. Haemoglobin: Cyanmethaemoglobin method (Crosby *et al.*, 1954).
2. Peripheral smear: Geimsa's stain.
3. Serum iron: According to Ramsay, 1958.
4. Unsaturated iron binding capacity of serum: According to Ressler and Zak, 1962.
5. Serum vitamins B_{12} and folate estimations (Mittal *et al.*, 1967).

a. Extraction of serum vitamin B_{12} (Dacie and Lewis, 1963).

b. Extraction of serum folates (Waters and Mollin, 1961).

c. The serum vitamin B_{12} assays

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were carried out with the organism lactobacillus leichmanii (Spray, 1955).

d. The serum folate assays were carried out with the organism lactobacillus casei (Waters and Mollin, 1961).

6. Serum total proteins and albumin and globulin fractions: (Meites and Faulkner, 1962).

All estimations were done on a P.M.Q. II (Zeiss) spectrophotometer.

Observations

Age and parity distributions for both control and test groups were almost similar.

Analysis of the clinical features in anaemic cases is summarised in Table 1.

TABLE 1

Analysis of clinical findings in anaemic cases

Clinical features	no.	%
Pallor	28	50.90
General weakness	24	43.60
Oedema	11	20.00
Palpitation	11	20.00
Loss of appetite	5	9.09
Glossitis	5	9.09
Diarrhoea	4	7.27
Congestive cardiac failure	3	5.45
Enlarged tender liver	3	5.45
Vomiting	2	3.60

In the control and test groups mean haemoglobin values were 10.9 $\pm$ 0.66 and 6.8 $\pm$ 2.02 g.%, respectively. Peripheral smears in the control group showed a hypochromic microcytic picture in 20% and hypersegmentation of neutrophils in 6.6%, while in the test group, 47.2% showed hypochromic microcytic and 25.4% had dimorphic smears; frank macrocytosis with hypersegmented neutrophils was observed in 3.6%.

Serum iron

In table 2, serum iron values for both the groups are presented. Only one case in the control group had serum iron of 38 μg%. The difference of means between the control and test group iron values is (values: 86.9 $\pm$ 40.1 and 48.3 $\pm$ 20.1) highly significant ($P < 0.001$). Patient with haemoglobin < 5.0 g.% had lesser mean serum iron value, the difference was statistically significant ($P < 0.01 > 0.005$). Serum iron values were less than 70 μg.% in 82.3% cases with hypochromic microcytic anaemia and 21.4% of dimorphic anaemia. Out of 11 cases with normal peripheral smear 36.3% had values < 70 μg.%. Two patients with macrocytosis and hypersegmented neutrophils showed high serum iron values of 120 and 127.7 μg.%, respectively.

In the test group, the mean serum iron value was 48.3 μg.% with a mean unsaturated iron binding capacity of 345.8 μg.%. An attempt was made to draw correlation between serum iron and total iron binding capacity, but values were so widely scattered that no significant correlation could be obtained. The percentage saturation was found to be lower than normal in the test group (Table 2).

Serum vitamin B_{12}

In the control series, only 2 women had values < 100 $\mu\mu$g./ml. One of them showed large hypersegmented neutrophils in the peripheral smear. In the test group 20 patients had values < 100 $\mu\mu$g./ml. (Table 3).

TABLE II

Mean values of serum iron, UIBC, TIBC and percentage saturation in the control and test groups

Investigations	Control			Test		
	Mean	SD±	SE	Mean	SD±	SE
Serum iron μg.%	86.9	40.10	7.30	48.3	20.10	2.70
*UIBC μg.%	211.2	142.21	25.96	297.5	13.93	1.87
**TIBC μg.%	298.1	137.80	25.17	345.8	82.70	11.10
Saturation %	37.1	23.69	4.32	15.3	10.08	1.36

*UIBC unsaturated iron binding capacity
**TIBC Total iron binding capacity

TABLE III

Mean values for serum vitamin B_{12} in control and test groups

Serum vitamin B_{12} μμg./ml.	Control n = 30	Test n = 55	'P'
Mean	189.05	105.9	0.0005
Range	62.5-675	10-381.5	
SD±	54.9	34.1	
SE	10.0	4.6	

TABLE IV

Values of serum folic acid in control and test groups

Serum folic acid mμg./ml.	Control n = 30	Test n = 55	'P'
Mean	6.23	3.14	0.0005
Range	1.56-17.0	0-17.0	
SD±	3.53	2.88	
SE	0.64	0.38	

No significant correlations were found of serum vitamin B_{12} with haemoglobin, peripheral smear and parity.

Serum folates

In the control group, 2 patients showed large and hypersegmented neutrophils in the peripheral smear, their values for serum folate were 2.6 and 3.8 μμg./ml., respectively. Thirteen patients showed values between 4-10 mug./ml. In the test group, serum folate was 0 in two patients with peripheral blood picture of dimorphic and hypochromic microcytic anaemia, respectively. About 82% of the patients showed values < 4 mμg./ml. (Table 4).

The data of table 5, demonstrated that patients with haemoglobin <5.0 g.% had lesser mean serum folate level. Serum folate levels were less than 4 mμg./ml. in all the cases which showed dimorphic and macrocytic pictures. In microcytic hypochromic group 20% had normal serum folate levels (4 mμg./ml.). No statistically significant correlation was found between parity and serum folate values.

No interrelationship was detected between serum vitamin B_{12} and serum folate levels in control and test groups. Similarly, serum iron levels were not correlated with serum folate levels.

TABLE V

Correlation between haemoglobin values and serum folic acid

Group	Hb. g.%	n	Serum folic acid			'P'
			Mean	SD±	SE	(Between)
1	5.0	11	1.91	0.31	0.09	1 & 2 =
2	5.1-8.0	30	3.11	2.37	0.43	0.005
3	8.1-9.5	14	4.02	4.50	1.20	1 & 3 =
						<0.05 >0.025

TABLE VI

Value of total serum proteins, serum albumin and serum globulin in the control and test groups

Investigations	Control			Test		
	Mean	SD±	SE	Mean	SD±	SE
Serum total proteins g.%	5.98	1.24	0.22	5.90	1.30	0.18
Serum albumin g.%	3.48	0.43	0.07	3.50	0.97	0.13
Serum globulin g.%	2.50	0.09	0.01	2.40	0.76	0.10

Total serum proteins, serum albumin and serum globulin values

Table 6 gives the mean values of total proteins, serum albumin and serum globulin in the control and test groups. The mean values in the two groups are more or less the same. There seems to be no significant difference between the protein values in the control group and pregnancy with anaemia ($P < 0.45 > 0.40$ and $< 020 > 0.15$, respectively).

Discussion

The clinical findings observed in this study are very much similar to those reported by various other workers (Mudaliar and Menon, 1942; Forshaw *et al.*, 1957 and Dass *et al.*, 1967 a and b). Three patients demonstrated features of congestive heart failure. All of them had haemoglobin values less than 5 gm.%.

The mean haemoglobin values for the control material in this group was found to be 10.9 ± 0.66 g.% which is consistent with those reported by most of the Indian workers. The mean value of the present study is lower than that reported by most of the various European workers. Our observations support the fact that the mean haemoglobin value in normal Indian pregnant women receiving supplementary vitamins and iron is lower than the reported Western figures.

On the basis of red cell and white cell morphology 47% of the cases had hypochromic microcytic anaemia, 25% had dimorphic and 7% showed changes of megaloblastic anaemia. Sidhu *et al.* (1967), reported an incidence of 38% as iron deficiency and 18% megaloblastic on peripheral smear examination. Surprisingly, around 26% of the normal control material in this study showed some degree of hypochromia indicating that there may be iron deficiency.

Dass *et al.* (1967) and Sidhu *et al.* (1967) found serum iron values less than 70 μg.% in approximately 53% of the patients in their studies. The higher incidence of 84% iron deficiency anaemia found in the present study is difficult to explain as the other studies from Delhi have reported a lower incidence. The data of the present study demonstrated that the mean serum iron values increased with rising haemoglobin levels. The significance of this findings is difficult to evaluate as serum iron always does not represent the status of the body iron stores. However, it may sugges that the degree of anaemia for iron deficiency patients may also indicate severity of iron deficiency.

The findings of the peripheral smear indicated that the majority of the microcytic hypochromic anaemia cases had low serum iron and 21.4% of the dimorphic were also iron deficient. The data of this study did show normal or slightly raised iron level for 7% of the microcytic anaemic group, but in dimorphic anaemia values were lower than 86.0 μg.%. Several workers have demonstrated in dimorphic anaemia high serum iron values and approaches normal or even subnormal values during therapy with anti-megaloblastic vitamins (Menon, 1965; Giles, 1966 and Dass *et al.*, 1967 a and b). However, the level of serum iron need not always be high as it might depend on whether the predominant deficiency is that of folic acid and/or vitamin B_{12} or of iron (Dass *et al.*, 1967 a and b).

The mean unsaturated iron binding capacity was 297.5 μg.% which was higher than the mean for the control group. The low serum iron is generally associated with a high total iron binding capacity (Mukherjee, 1953; Menon and Ramaswamy, 1955 and Dass *et al.*, 1967, a and b). In contrast, Sidhu *et al.* (1967, found that high serum iron was associated with high total iron binding capacity. The observations of the present study do not suggest any significant correlation between serum iron and total iron binding capacity in the anaemic patients. The anaemic patients also demonstrated a low saturation index for transferrin as compared to the control values. The finding of the low saturation index is strongly suggestive of iron deficiency anaemia and stands as second best criterion after the stimulation of bone marrow iron.

The data of the present study demonstrate a somewhat lower incidence of serum vitamin B_{12} levels as only 20 out of 55 anaemic subjects had values less than 100 μμg. The difference of selecting 100 μμg. and 80 μμg./ml. as the lowest serum vitamin B_{12} levels in the present study and that of studies by Sidhu *et al.* (1967), are due to the different methods used. There are no other authentic studies to indicate the incidence of serum vitamin B_{12} level in pregnancy anaemias in our country. The results of this study and those of Sidhu *et al.* (1967) warrant a high incidence of vitamin B_{12} deficiency in pregnancy anaemias in India and necessitate more studies, especially on a large group of patients with anaemia in pregnancy.

In the present study the control material showed a wide range of 1.56-17 mμg./ml. for serum folates. Only two cases had serum folate values less than 4 mμg:/ml.; indicating that 2

out of 8 cases had low serum folate levels.

Interestingly, 82% of the anaemic patients in this study had serum folate levels lower than 4 mμg./ml. (which is taken as the lower limit for normalcy). Although the figure of 4 mμg./ml. is for normal subjects and it may be that in pregnancy due to haemodilution the value is somewhat lower, Ball and Giles (1964), found a mean level of 3.6, with the lowest figure of 1.5 mμg./ml. in normal pregnancy. It is of great interest to find that the folate deficiency and iron deficiency have equal incidences.

As expected, serum vitamin B_{12} levels did not show any correlation with haemoglobin and parity. No correlation between serum vitamin B_{12} values and peripheral smear morphology was observed in the present study.

Similar to the findings that the serum iron values increased with increasing haemoglobin levels in anaemic patients it seems that for serum folate levels also a similar pattern was evident. The significance that the anaemic patients showed correlation for haemoglobin and iron and serum folate levels is of great interest, although this statement refers to the average results obtained in groups of subjects. In individual patients the result showed considerable overlap.

Serum folate levels in our study were not correlated with parity.

As found by other workers (Barua and Foll, 1960; Karthigaini *et al.*, 1964; Menon, 1965; Lowenstein, *et al* 1966, and Giles, 1966). Most of the dimorphic and macrocytic smear cases had low serum folate values while only 20% of the microcytic hypochromic group showed low serum folate values.

The serum folate and vitamin B_{12} levels did not show any correlation in this study.

The mean value for total serum proteins in this study was found to be 5.98±1.24 g.% for normal pregnancy in the third trimester. This is lower than those reported by other Indian workers except for a figure of 5.56 reported by Kulkarni *et al.* (1960).

Serum albumin showed a mean value of 3.48 ± 0.43 g.% and serum globulin, 2.50 ± 0.09. Menon *et al.* (1958), observed a value of 3.13 g.% for serum albumin in his studies with normal pregnancy. Except for this, other Indian workers have observed values less than 3 gm.% in normal pregnancy (Menon and Chandrasekharan, 1954 and Sidhu *et al.*, 1967).

Our values for total serum proteins in pregnancy with anaemia are not much different from the findings of Sidhu *et al.* (1967). There was no significant difference between the values in normal pregnancy and those in anaemia in pregnancy. Singh *et al.* (1967) in his observations reported that the socio-economic status and the dietetic habits did not affect the serum proteins qualitatively and quantitatively. Similar, to the findings of Menon *et al.* (1958), Kulkarni *et al.* (1960), Karthigaini *et al.* (1964) and Sidhu *et al.* (1967), we could not correlate the findings of serum proteins with the degree and type of anaemia.

Summary

In this study, 55 patients of anaemia in later months of pregnancy

were investigated for serum levels of iron vitamin B_{12}, folate and proteins. The mean serum iron and total iron binding capacity were 48.3 ± 20.1 and 345.8 ± 82.7 μg.%, respectively. The mean serum vitamin B_{12} and folate levels were 105.9 ± 34.1 μμg./ml. and 2.88 mμg./ml., respectively. The percentage of anaemic patients who showed deficiency of iron, vitamin B_{12} and folate in serum were 84%, 36% and 82%, respectively. As many as 38.1% had low serum levels of iron and folate and 25.4% showed low values for vitamin B_{12}, folate and iron. The serum protein levels were within normal limits.

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