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SERUM PROTEINS AND HEPATIC FUNCTION IN PREGNANCY WITH ANAEMIA IN NIGERIAN WOMEN

BY

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In Nigeria, as in several other parts of Africa, anaemia is a frequent complication of pregnancy (Lawson and Lister, 1955). The management of this anaemia has been greatly influenced by the view that it is the result of protein malnutrition and of nutritional liver damage (Woodruff, 1951, 1956).

There is, however, very little information available about liver function and the incidence of protein malnutrition syndrome in pregnant Nigerian women.

Significant changes take place in the serum protein pattern in severe protein deficiency in children (Anderson and Altmann, 1951; Edozien, 1960) and in adults (Peters *et al.*, 1927; Denz, 1947; Walters *et al.*, 1947), the most important findings being hypoproteinaemia and hypoalbuminaemia. The serum protein changes provide a more sensitive index of protein deficiency than haemoglobin concentration, a point clearly shown by the fact that hypoproteinaemia and hypoalbuminaemia are invariable in kwashiorkor, while anaemia is found in only a proportion of cases and severe anaemia is unusual (Trowell *et al.*, 1954; Foy and Kondi, 1958). Protein deficiency of a degree sufficient to cause severe anaemia in pregnancy should therefore be associated with highly significant differences in total protein and in albumin concentration between anaemic and non-anaemic pregnant women.

In this paper we record observations on the serum proteins and on liver functions of pregnant Nigerian women, with and without anaemia.

Materials and Methods

The pregnant subjects were Nigerian women, mostly of the Yoruba tribe, who attended the antenatal clinics at University College Hospital and Adeoyo Hospital, Ibadan, in January to December, 1958. All the non-anaemic women had haemoglobin concentration of 10 g./100 ml. (68%) or over throughout pregnancy,

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while the anaemic patients had haemoglobin levels of less than 10 g./100 ml. at the time of investigation. Some of the pregnant women, both normal and anaemic, had other complications, such as disproportion, which were not thought to have altered the blood picture. Electrophoresis of haemoglobin was done on all the pregnant women, and those with two abnormal genes have been excluded from this series.

The non-pregnant adult women were healthy volunteers, being part of a group of adults who were being used to establish normal biochemical values in Nigerians.

Haemoglobin and haematocrit were estimated by standard techniques as described by Dacie (1956). Plasma iron was determined by the method of Bothwell and Mallett (1955).

For haemoglobin electrophoresis, haemolysates on Whatman No. 3 MM. paper were subjected to a direct current of 0.34 mA/cm. at 200 volts for 15–17 hours, using barbitone buffer (pH 8.6 and ionic strength 0.05).

Marrow was aspirated from the left iliac crest and the smears were stained with May–Grünwald and Giemsa stains (Dacie, 1956).

The analytical procedures adopted in this investigation for total proteins and the protein fractions and for liver-function tests have been reported previously (Edozien, 1958a).

Haematological Data

Anaemic Subjects

The haematological data on the anaemic pregnant women are shown in Table I. There were 24 women with a severe degree of anaemia (haemoglobin of 4 g./100 ml. or less), 22 with haemoglobin between 4.1 and 7.9 g./100 ml., and 30 mildly anaemic pregnant women whose haemoglobin was between 8 and 9.9 g./100 ml. at the time of investigation.

TABLE I.—Haematological Data on Anaemic Pregnant Women

Haemoglobin (g./100 ml.)	No. of Cases Investigated	Bone-marrow Findings	
		Megaloblastic	Normoblastic
2.5–4.0 ..	24	17	7
4.1–7.9 ..	22	3	7
8.0–9.9 ..	30	—	—
Total ..	76	20	14

The mean corpuscular haemoglobin concentration was between 26% and 35%. Ten patients had a M.C.H.C. of less than 29%: in seven of these who had not previously received therapeutic iron the plasma iron level, except for Case No. 16826 (Table IV), ranged from 74 to 213 µg./100 ml. (normal values: 50–250 µg./100 ml.).

The bone-marrow was examined in 34 cases. This investigation was made in all 24 patients having haemoglobin concentration of 4 g./100 ml. or less, and 17 (70.8%) had megaloblastic erythropoiesis.

Haematological Response to Treatment

Because of the severe degree of anaemia, many patients had to have blood transfusion. In the most severely anaemic women it was necessary to give red cells without increasing the total blood volume ("exchange transfusion"), and such patients were initially treated by a simultaneous infusion of 1,000–1,500 ml. of concentrated red cells and venesection of 1,200–1,700 ml. (W. T. Fullerton, 1960, personal communication).

All patients were given ferrous sulphate, 180 mg. three times a day, as routine antenatal procedure. Malaria is believed to be a factor in the development of anaemia among pregnant women in Ibadan, and therefore all patients were given a curative dose of chloroquine followed by pyrimethamine ("daraprim"), 50 mg. weekly until delivery. Megaloblastic erythropoiesis was found in 70.8% of the patients with a haemoglobin of less than 4 g./100 ml., and this high incidence is reflected also by the finding of megaloblastic erythropoiesis in 193 (55%) of the 352 severely anaemic pregnant women admitted to University College Hospital in 1958 (Watson-Williams, 1960). Although it has been reported (Berry, 1955) that vitamin B₁₂ may be necessary in the treatment of megaloblastic anaemia of pregnancy in Nigeria, this has not been confirmed in Ibadan, and all our patients received folic acid, 10 mg. three times a day by mouth (Watson-Williams, 1959).

In Table II the response of the patients to treatment is summarized. The assessment of response in patients who had initial haemoglobin of 4 g./100 ml. or less is complicated by the fact that nearly all of them were transfused, but it is clear from the results for the other patients that response to treatment was generally satisfactory. All patients who had at least three weeks' treatment achieved a haemoglobin concentration of more than 8 g./100 ml. before delivery, and 75% reached levels above 10 g./100 ml.

Biochemical Results

Serum Proteins in Normal Pregnancy

The mean values, together with standard deviations, for the total serum proteins and the protein fractions in healthy adult women in Ibadan and in normal pregnancy are given in Table III. Compared with adult Europeans, the non-pregnant Nigerian women have lower albumin concentration and higher gamma-globulin concentration, but this is the usual pattern in symptom-free Nigerians (Edozien, 1958b). In pregnancy there is a progressive fall in the concentration of total protein and of albumin and a rise in the concentration of α_1 -, α_2 -, and β -globulins. These changes are most marked at term. There is also a drop in the gamma-globulin concentration towards the end of pregnancy. These changes are similar to those which take place in pregnant European and American women (Mack, 1955).

Serum Proteins in Pregnancy with Anaemia

Five of the 76 anaemic patients—that is, 6.6%—had total serum protein values of less than 5.5 g./100 ml. and the electrophoretic patterns in these cases resemble the pattern in severe protein malnutrition. Of these five patients, one had iron-deficiency anaemia which responded well to iron therapy, while the remaining four had megaloblastic anaemia and were successfully treated with folic acid plus iron. The anaemia in these patients was therefore due to deficiency of haemopoietic factors and not to protein deficiency. The biochemical and haematological data on these five patients are shown in Table IV.

The remaining 71 patients did not show any biochemical evidence of severe protein deficiency. The mean values for total serum proteins and the protein fractions are compared with values for normal pregnancy in Table V. The differences between the two groups of

patients are not statistically significant, and the results thus indicate that most of the anaemic patients were not grossly protein deficient.

Tests of Liver Function

The results of the liver-function tests are summarized in Table VI. Normal pregnancy does not cause significant alteration in the results of the thymol and colloidal-gold flocculation tests. Approximately 10% of the anaemic patients had abnormal sero-flocculation results. Quite clearly, the majority of the anaemic patients were free of severe liver disease, otherwise the proportion of patients having abnormal flocculation reactions would have been much greater. The small increase in the incidence of abnormal flocculation reactions in the anaemic patients as compared with the non-anaemic patients should probably be attributed to the anaemia.

There is a progressive increase in alkaline phosphatase activity. In the second trimester there is an increase of 33% as compared with non-pregnant subjects, in the third trimester there is a twofold increase; and by the end of pregnancy the mean alkaline phosphatase activity is about four times normal mean value. Increase in alkaline phosphatase activity in pregnancy was first reported in 1935 by Cayla and Fabre, and has since been confirmed by several other investigators (Meranze *et al.*, 1937; Bodansky *et al.*, 1939; Young *et al.*, 1946; Hoch *et al.*, 1948; Speert *et al.*, 1950). Our data on Nigerian women are consistent with these reports on European and American women. These changes are probably secondary to the profound alterations in the body's economy of calcium and phosphate which occurs during pregnancy because of the demands of the foetus. The differences in alkaline phosphatase level between the anaemic and non-anaemic patients are not statistically significant.

Normal pregnancy causes increased retention of bromsulphalein. Approximately 35% of the non-anaemic patients have abnormal bromsulphalein clearance. A similar tendency for the normal pregnant women to excrete bromsulphalein less rapidly than the normal non-pregnant women has been reported in America (Christhilf and Bonsnes, 1950). There was no difference in the results of the bromsulphalein retention test between the anaemic and the non-anaemic patients.

There is a slight increase in serum bilirubin concentration in the third trimester of normal pregnancy. In the second and third trimesters there is an increase in the proportion of conjugated bilirubin. The anaemic patients have a significantly higher concentration of bilirubin as compared with the non-anaemic patients, but this finding is not unexpected, because most of the anaemias were megaloblastic, which is usually associated with an increase in serum bilirubin.

Discussion and Conclusions

Our data do not support the view that anaemia of pregnancy in Nigerian women is due to deficiency of protein or to liver disease.

No significant differences in total protein concentration and in the serum protein fractions have been found between pregnant Nigerian women without anaemia and the majority of the anaemic pregnant women. Only 6.6% of our anaemic patients had biochemical evidence of severe protein deficiency, but even in these cases the anaemia was due to deficiency of iron and/or folic acid, and not to deficiency of protein. Schofield (1957)

TABLE II.—*Response to Treatment**

Initial Haemoglobin	No. of Cases	Bone-marrow		Transfusion		Final Haemoglobin (g./100 ml.) of Patients Treated for at Least Three Weeks with Iron, Folic Acid, and Antimalarials			
		Megaloblastic	Normoblastic	Exchange	Simple	> 10	9-9.9	8-8.9	Not Followed Up
2.5-4.0	19	13	6	17	1	7	1	0	0†
4.1-7.9	22	3	6	1	1	13	6	3	0
8.0-9.9	30	—	—	—	—	20	3	1	6

* Five patients summarized in Table V have been excluded. † In 11 patients pregnancy terminated within 7 days.

TABLE III.—*Serum Proteins (g./100 ml.) in Non-pregnant Women and in Normal Pregnancy*

	Non-pregnant (75)		2nd Trimester (24)				3rd Trimester (39)				Term† (17)			
	Mean	S.D.	Mean	S.D.	t*	p*	Mean	S.D.	t*	p*	Mean	S.D.	t*	p*
Total proteins ..	7.02	0.41	6.75	0.59	2.5	0.01 < p < 0.02	6.80	0.73	2.3	0.01 < p < 0.02	5.94	0.51	9.4	p < 0.01
Albumin ..	3.50	0.31	2.66	0.46	10.1	p < 0.01	2.61	0.35	13.9	p < 0.01	1.87	0.21	20.7	p < 0.01
α_1 -globulin ..	0.25	0.09	0.35	0.11	4.6	p < 0.01	0.38	0.12	6.5	p < 0.01	0.44	0.09	7.9	p < 0.01
α_2 " ..	0.51	0.13	0.78	0.18	8.0	p < 0.01	0.82	0.15	12.7	p < 0.01	0.82	0.14	8.8	p < 0.01
β - " ..	0.73	0.18	0.93	0.15	5.0	p < 0.01	0.98	0.16	7.7	p < 0.01	1.04	0.10	6.9	p < 0.01
γ - " ..	2.03	0.34	2.03	0.44	0.0	—	2.02	0.49	0.1	0.8 < p < 0.9	1.75	0.42	2.9	p < 0.01

* Test of the significance of difference from value for non-pregnant women. † Blood collected within 12 hours after delivery.

TABLE IV.—*Biochemical and Haematological Data on the Five Anaemic Patients with Total Protein <5.50 g./100 ml.*

Case No.	T.P. g./100 ml.	Alb. g./100 ml.	α_1 -glob. g./100 ml.	α_2 -glob. g./100 ml.	β -glob. g./100 ml.	γ -glob. g./100 ml.	Plasma Iron μ g./100 ml.	Hb g./100 ml.	P.C.V. %	M.C.H.C. %	Marrow	Treatment	Response
21436	5.30	1.90	0.44	0.74	0.75	1.47	—	2.6	8	32	Megalo-blastic. Hyper-plasia	Exchange transfusion, folic acid, Fe, chloro-quine, vitamins	After transfusion Hb 5.3 g./100 ml. P.C.V. 14%. Response to treatment good. Hb 10.7 g./100 ml. M.C.H.C. 32% on discharge after three weeks
22801	3.90	1.10	0.44	0.58	0.76	1.02	—	3.9	13	26	Megalo-blastic erythro-poiesis	Exchange transfusion, folic acid, Fe, chloro-quine, vitamins	Labour followed after exchange transfusion. Did well subsequently on treatment
4363	5.30	1.37	0.38	0.80	1.87	1.87	—	4.0	11	35	Megalo-blastic	Exchange transfusion, folic acid, Fe, chloro-quine, vitamins	Abortion followed after transfusion. Did well subsequently on treatment
16826	5.40	2.00	0.29	0.69	0.71	1.71	26	3.3	12	27	Normal	Chloroquine, Fe, vitamins	Good response to treatment. Three weeks after admission, Hb 8.7 g./100 ml., F.C.V. 31%, M.C.H.C. 28%
19901	4.30	1.25	0.24	0.35	0.53	1.93	—	4.0	13	30	Megalo-blastic	Delivered shortly after admission. Post-partum: exchange transfusion, folic acid, Fe, chloro-quine, vitamins	Did not return for follow-up
Mean	4.84	1.52	0.36	0.63	0.73	1.60							

TABLE V.—*Serum Proteins in Normal Pregnancy and in Pregnancy with Anaemia*

	2nd Trimester						3rd Trimester					
	Normal (24)		Anaemic (19)				Normal (39)		Anaemic (52)			
	Mean	S.D.	Mean	S.D.	t	p	Mean	S.D.	Mean	S.D.	t	p
Total protein ..	6.75	0.59	6.67	0.53	0.5	0.6 < p < 0.7	6.80	0.73	6.69	0.71	0.7	0.4 < p < 0.5
Albumin ..	2.66	0.46	2.52	0.36	1.1	0.2 < p < 0.3	2.61	0.35	2.43	0.46	1.4	0.1 < p < 0.2
α_1 -globulin ..	0.35	0.11	0.39	0.11	1.2	0.2 < p < 0.3	0.38	0.12	0.43	0.15	1.7	0.05 < p < 0.6
α_2 " ..	0.78	0.18	0.77	0.12	0.2	0.8 < p < 0.9	0.82	0.15	0.84	0.16	0.6	0.5 < p < 0.1
β - " ..	0.93	0.15	0.90	0.21	0.5	0.5 < p < 0.6	0.98	0.16	0.97	0.22	0.2	0.8 < p < 0.9
γ - " ..	2.03	0.44	2.09	0.47	0.1	0.9 < p	2.02	0.49	2.02	0.48	0.0	—

TABLE VI.—*Results of Liver-function Tests*

Test	Non-pregnant (75)	Normal Pregnancy			Pregnancy with Anaemia		
		2nd Trimester (24)	3rd Trimester (39)	Term (17)	2nd Trimester (19)	3rd Trimester (52)	Term
Thymol test: % having turbidity greater than 4 MacLagan units and flocculation greater than one plus	0	1.5	1.5	—	7.5	10.5	—
Colloidal gold: % having flocculation > +	0	1.5	2	—	9.5	12.0	—
Alkaline phosphatase: King-Armstrong units/100 ml.	7.2 ± 3.5	9.8 ± 3.7	15.0 ± 8.3	24.9 ± 9.2	11.7 ± 5.0	15.3 ± 6.3	—
B.S.P. retention: % having retention of 6% or more	0	30	37.5	—	28.5	38.5	—
Bilirubin (total): mg./100 ml. ..	0.6 ± 0.3	0.6 ± 0.3	0.8 ± 0.4	—	1.0 ± 0.5	1.0 ± 0.8	—
Conjugated bilirubin: mg./100 ml. ..	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	—	0.7 ± 0.4	0.8 ± 0.6	—
As % of total mean value ..	33	50	50	—	70	80	—

studied 11 anaemic pregnant women in Gambia and treated eight with a high-protein diet for one to three months. He noted no improvement in haemoglobin concentration and also concluded that dietary deficiency of protein is not a cause of anaemia in pregnancy in Gambia.

With the exception of the seroflocculation tests, no significant differences exist between the results of the liver-function tests in normal pregnancy and in pregnancy with anaemia. Of the anaemic patients, 10% had abnormal seroflocculation reactions, but this finding is considered to be secondary to the anaemia. It is clear from the analysis of our results that the majority of the anaemic patients must be free from severe liver disease.

On the other hand, our data show dietary deficiencies of haemopoietic factors—and in particular deficiency of folic acid—to be important in producing the anaemias of pregnancy in Nigerian women.

Summary

The serum proteins and liver function of non-pregnant Nigerian women and of pregnant women, with and without anaemia, have been investigated.

Dietary deficiency of protein and hepatic damage are not important aetiological factors in the anaemias of pregnancy in Nigerian women.

The majority of the anaemic pregnant women had megaloblastic erythropoiesis. The response of the patients to treatment with antimalarials, iron, and folic acid was satisfactory.

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CYCLICAL CUSHING'S SYNDROME

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Though Cushing's syndrome is not uncommon, the following case is most unusual, demonstrating a picture of gross cyclical endocrine activity of an abnormal pattern.

Remissions in Cushing's syndrome are well known. These may be spontaneous, as was seen in one of Cushing's original cases (Cushing, 1932, Case 16), and reference to this type of remission has been made by other workers (Thompson and Eisenhardt, 1943; Anderson and Haymaker, 1944; Plotz *et al.*, 1952; Knowlton, 1953; Crooke, 1958), though the relationship of these remissions to therapeutic measures is uncertain in some of these cases. Remissions induced by removal of the pituitary gland or by bilateral adrenalectomy are quite common, while radiotherapy directed at these sites has been used with varying success (Cushing, 1932; Thompson and Eisenhardt, 1943; Anderson and Haymaker, 1944; Heinbecker, 1944; Sosman, 1949; Cochran *et al.*, 1950; Knowlton, 1953).

Cases with spontaneous cyclical recurrence of symptoms in Cushing's syndrome, while they are stated to occur quite commonly, are rare in the literature. Several cases of this type are recorded (Thompson and Eisenhardt, 1953; Anderson and Haymaker, 1944; Sosman, 1949; Crooke, 1958; Luft, 1958), but only that reported by Anderson and Haymaker (1944) closely resembles the present case. Their patient was a 34-year-old woman who had experienced cyclical recurrence of symptoms of Cushing's syndrome during the 12 years prior to her death. Exacerbations were seen in the spring and summer, with remissions in the autumn and winter. The remissions became progressively shorter, none occurring in the last year of life. Radiotherapy to the pituitary and adrenal glands and treatment with methyltestosterone proved ineffective. Exploration revealed no evidence of either adenomatous or carcinomatous lesions of the adrenal glands, but promoted a remission lasting four months. A right adrenalectomy was then performed, with subsequent improvement, but she died two years later in left ventricular failure. Necropsy revealed hyperplasia of the zona reticularis of the remaining adrenal gland (similar to the changes found in the right adrenal gland after its removal two years previously), and a benign thymic neoplasm. The pituitary showed that in a moderate number of basophils there was cytoplasmic degranulation, vacuolization, and hyalinization. Sections of the hypothalamus showed some changes in the proportion of cell types.

Case Report

The patient was a 59-year-old woman who was 10 years post-menopausal. Her past illnesses included an attack of encephalitis in 1921.

In August, 1953, after a domestic crisis she developed transient ankle and leg oedema of four days' duration. She