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Relationship of anaemia and hypoproteinaemia to the functional and structural changes in the small bowel in hookworm disease

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A variable proportion of patients with hookworm disease have functional and structural changes of the small bowel leading to the clinical features of malabsorption and nutritional deficiencies (Sheehy, Meroney, Cox, and Soler, 1962; Salem and Truelove, 1964; Chaudhuri and Saha, 1964; Tandon, Das, Saraya, and Deo, 1966a; Burman, 1966; Chuttani, Puri, and Misra, 1967). This observation has great significance for investigators in India and other tropical countries where hookworm infection and idiopathic tropical malabsorption are prevalent. These patients usually belong to a low socio-economic class, are poorly nourished, and have moderate to severe anaemia. Hence three factors, namely, the parasite (Sheehy *et al*, 1962), severe anaemia, and hypoalbuminaemia (Layrisse, Blumenfeld, Carbonell, Desenne, and Roche, 1964) have been considered for the study of the pathogenesis of malabsorption in hookworm disease.

SELECTION OF CASES AND METHOD OF STUDY

One hundred and four patients (88 males and 16 females), all with hookworm ova in their stools were selected for study. The age range was 13 to 59 years. None had any associated systemic disease. Haematological studies included haemoglobin, packed cell volume, blood smear, and bone marrow examinations (Dacie, 1956). Total serum proteins and albumin and globulin were estimated (King and Wootton, 1956). The D-xylose absorption test was carried out in 83 cases with a 5 g dose (Santini, Sheehy, and Martinez-de Jesus, 1961) and urinary estimation was done by the method of Roe and Rice (1948). A fat balance test was done in 82 patients by putting them on a 100 g fat diet for seven days and collecting the 72-hour faeces on the fifth, sixth, and seventh day for fat estimation (King and Wootton, 1956). An intestinal biopsy was obtained, using Rubin's multipurpose biopsy tube, from about 12 cm beyond the ligament of Trietz, under fluoroscopic control. Specimens were properly orientated; serial sections were cut and stained according to standard histological techniques. Adequate intestinal biopsy specimens were studied in 76 cases. The normals for different parameters were established and have been reported earlier (Tandon, Magotra, Saraya, and Ramalingaswami, 1968).

Anaemia was classified as severe if the haemoglobin level was 5 g% or less, moderate if it was between 5·1 and 10 g%, and mild if it was 10·1 g% to 12 g%. Similarly, an arbitrary classification of the severity of hypoalbuminaemia was performed. A serum albumin level less than 2·75 g% was classified as severe, 2·76 to 3·25 g% as moderate, 3·26 to 3·75 g% as mild, and above this level was considered normal. A diagnosis of iron deficiency was based on (1) a variable degree of microcytosis, hypochromia, and the presence of target cells in the peripheral smear, and (2) normoblastic hyperplasia, poor haemoglobinization of red cell precursors, and the absence of haemosiderin in the bone marrow smear. B_{12} and/or folic acid deficiency was diagnosed if a peripheral smear showed macropolycytes, macrovalocytes with or without leucopenia and thrombocytopenia, and the bone marrow showed the presence of giant myelocytes, metamyelocytes, and large band forms with normoblastic or complete megalopoietic haemopoiesis. Urinary D-xylose excretion, of less than 1 g in five hours on a 5 g dose, and faecal fat of more than 5 g/24 hours on a fat balance test were considered as indications of malabsorption. Villus histology was considered abnormal if the villus: crypt ratio had become less than 3:1; if the epithelial cells were abnormal and the lamina propria was infiltrated with chronic inflammatory cells. Minor and non-specific changes like blunting or branching of the villi with or without a slight increase in the cellular infiltration of the lamina propria were not considered unequivocal abnormalities as similar findings had been noted by Magotra in healthy control subjects (Magotra, 1966).

RESULTS

The clinical features studied were anaemia, weakness, palpitation, dyspnoea, and pallor. Diarrhoea and

oedema of the feet were noted in 27% and 19% of the patients respectively. However, none of the patients gave a history of passing large, bulky, and frothy stools, which could be considered characteristic of steatorrhoea.

The distribution of anaemia is presented in Table I. Severe anaemia was noted in 30·7%, moderate in 27·9%, and mild in 41·3% of the cases. Severe hypoalbuminaemia was noted in 15·5%, moderate in 18·5%, mild in 37·8%, and normal albumin levels were recorded in the remaining 28·2% of the cases (Table II).

TABLE I

DISTRIBUTION OF HAEMOGLOBIN LEVELS IN 104 PATIENTS WITH HOOKWORM DISEASE

Haemoglobin Level (g%)	*Percentage Distribution*
5·0	30·7
5·1 to 10·00	28·8
10·1 to 12·00	41·3

TABLE II

SERUM ALBUMIN DISTRIBUTION IN 103 PATIENTS WITH HOOKWORM DISEASE

Serum Albumin Level (g%)	*Percentage Distribution*
<2·75 or less	15·5
2·76 to 3·25	18·5
3·26 to 3·75	37·8
+3·76	28·2

TABLE III

PERCENTAGE DISTRIBUTION OF D-XYLOSE, FAT BALANCE TEST, AND INTESTINAL BIOPSY ABNORMALITIES IN HOOKWORM DISEASE

Laboratory Test	*Percentage Abnormal*
D-xylose absorption test[1]	28·9
Fat balance test[2]	31·7
Intestinal biopsy[3]	11·8

[1]Done in 83 cases.
[2]Done in 82 cases.
[3]Done in 76 cases.

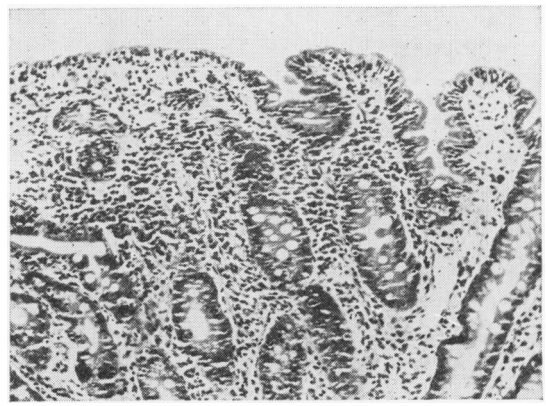

FIG. 1. *Subtotal villous atrophy in the jejunum. The villi are blunted and fused over wide areas. Lining epithelial cells are abnormal and the lamina propria is infiltrated with chronic inflammatory cells. Haematoxylin and eosin × 135.*

Table III presents the results of intestinal function and structure studies. D-xylose absorption was abnormal in 28·9% and fat balance tests recorded steatorrhoea in 31·7% of the cases. Definite intestinal histological changes of villous atrophy (Fig. 1) were recorded in 11·8% of the patients.

INTESTINAL FUNCTION AND STRUCTURE STUDY IN RELATION TO ANAEMIA Table IV presents the relationship of haemoglobin concentration with D-xylose absorption. Normal D-xylose absorption was observed in 56%, 74%, and 83% of the patients with severe, moderate, and mild anaemia respectively. These differences do not attain statistical significance ($\chi^2_{(2)} = 5{\cdot}21$, $P = 0{\cdot}05$), but suggest a weak association between the severity of anaemia and poor D-xylose absorption.

The relationship of fat absorption to haemoglobin concentration is presented in Table V. There is no evidence of any association between the severity of anaemia and the fat absorption. Fifty-nine per cent of the patients with severe anaemia had normal fat

TABLE IV

HAEMOGLOBIN CONCENTRATION AND D-XYLOSE ABSORPTION IN PATIENTS WITH HOOKWORM INFESTATION[1]

Haemoglobin (g%)	*No. of Patients*	*Urinary D-xylose Excretion (g)*[1]						*Mean D-xylose Excretion*
		Less than 0·50	*0·50—*	*1·00—*	*1·50—*	*2·00 or More*	*1·00 or More*	
Less than 5·00	27	4	8	8	4	3	15 (56%)	1·14
5·1–10·00	27	1	6	7	6	7	20 (74%)	1·54
10·1 or more	29	1	4	13	5	6	24 (83%)	1·56
Total	83	6	18	28	15	16	59 71%	

[1]D-xylose absorption test was done with a 5 g dose.

TABLE V

ASSOCIATION OF HAEMOGLOBIN CONCENTRATION WITH FAECAL FAT IN PATIENTS WITH HOOKWORM INFESTATION[1]

Haemoglobin (g%)	No. of Patients	Gram(s) of Faecal Fat/24 Hours[1]					Mean Faecal Fat/24 Hours
		Less than 1·00	1·0–	5·0–	9·00 or More	Less than 5·0	
Less than 5·0	27	2	14	9	2	16 (59%)	4·46
5·1–10·0	25	4	16	3	2	20 (80%)	3·55
10·1 or more	30	0	20	9	1	20 (67%)	4·20
Total	82	6	50	21	5	56 (68%)	

[1]On a fat balance test.

TABLE VI

SERUM ALBUMIN LEVELS AND D-XYLOSE ABSORPTION IN PATIENTS WITH HOOKWORM INFESTATION

Serum Albumin Level	No. of Patients	Urinary D-xylose Excretion (g)						Mean D-xylose Excretion %
		0·50	0·50–	1·00–	1·50–	2·00 or More	1·00 or More	
2·75	14	3	6	3	2	0	5 (36%)[1]	0·85
2·76–	17	1	6	5	3	2	10 (59%)[1]	1·18
3·26–	32	2	5	9	8	8	25 (78%)[1]	1·67
3·76 or more	19	0	1	10	2	6	18 (95%)[1]	1·68
Total	82	6	18	27	15	16	58 71%	

[1]Percentages based on fewer than 25 observations.

TABLE VII

ASSOCIATION OF SERUM ALBUMIN LEVEL WITH FAECAL FAT IN PATIENTS WITH HOOKWORM INFESTATION[1]

Serum Albumin Level	No. of Patients	Gram(s) of Faecal Fat/24 Hours						Mean Faecal Fat/24 Hours
		1·00	1·0–	3·0–	5·0–	9 or Above	Less than 5·00	
2·75	14	0	2	3	4	5	5 (36%)[2]	6·70
2·76–	18	2	6	2	6	2	10 (56%)[2]	4·58
3·26–	30	2	13	10	3	2	25 (83%)	3·36
3·76 or more	18	1	11	2	3	1	14 (78%)[2]	3·16
Total	80	5	32	17	16	10	54 68	

[1]On a fat balance test.
[2]Percentages based on fewer than 25 observations.

balance tests compared with 80% of the moderate and 67% of the mild anaemia group. Intestinal biopsy did not reveal any association between the severity of anaemia and morphological change. Normal appearances were noted in 92% of the severe anaemia, 81% of the moderate anaemia, and 93% of the mild anaemia group respectively.

INTESTINAL FUNCTION AND STRUCTURE STUDY IN RELATION TO HYPOALBUMINAEMIA Table VI presents the analysis of association of serum albumin levels with the D-xylose absorption test. Normal D-xylose absorption was observed in 36% of the patients with serum albumin levels of 2·75 g% or less, in 59% with the levels between 2·76 and 3·25 g%, and in 78% with levels between 3·26 and 3·75 g%, 95% with levels of 3·76 g% or more. These differences are statistically significant ($\chi^2_{(3)} = 15{\cdot}21$, $P < 0{\cdot}01$). The mean D-xylose excretion levels in the urine presented in the last column of Table VI also bear out this observation. Table VII presents the association of a faecal fat excretion on a fat balance test and the serum albumin levels. There is an increase in the percentage of patients with normal faecal fat excretion with the increasing levels of albumin up to 3·25 g%, but above this level of albumin there is no increase in the percentage of the patients with normal faecal fat excretion. The changes in faecal fat excretion in relation to hypoalbuminaemia are statistically highly significant ($\chi^2_{(2)} = 11{\cdot}75$, $P < 0{\cdot}01$). The relationship of decreasing mean levels of faecal fat with the increasing levels of mean serum albumin presented in the last column of Table VII also reflects this association. Intestinal biopsy was normal in 77% of cases with albumin levels of less than 2·75 g% compared with 91% of the patients with albumin levels higher than 2·75 g%. This difference

TABLE VIII

MEAN URINARY D-XYLOSE EXCRETION ACCORDING TO HAEMOGLOBIN CONCENTRATION AND SERUM ALBUMIN LEVEL IN PATIENTS WITH HOOKWORM INFESTATION

Haemoglobin (g%)	*No. of Patients*	*Serum Albumin Level*		
		2·75	*2·76–*	*3·26 or More*
5·0	26	0·69 (9)[1]	1·08 (9)[1]	1·62 (8)[1]
5·1–10·0	27	1·14 (5)[1]	1·35 (5)[1]	1·81 (17)[1]
10·1 or more	29	(0)[1]	1·20 (3)[1]	1·60 (26)[1]

[1]The figures enclosed in parentheses indicate the number of observations.

TABLE IX

IRON, FOLIC ACID, AND B_{12} DEFICIENCIES IN RELATION TO D-XYLOSE ABSORPTION AND FAT BALANCE TEST

Type of Deficiency	*Total No. of Patients*	*Urinary D-xylose (g)*		*Mean Urinary D-xylose (%)*	*Total Patients*	*Faecal Fat (g/24 hr)*		*Mean Faecal Fat (24 Hours)*
		1·0	*1·0 or More*			*5·0*	*5·0 or More*	
No deficiency	14	3	11 (79%)[1]	1·38	14	8 (57%)[1]	6	4·21
Iron deficiency	25	8	17 (68%)	1·74	23	18 (78%)[1]	5	3·72
B_{12} and folic acid deficiency	11	2	9 (82%)[1]	1·36	11	8 (73%)[1]	3	4·07
Iron B_{12} and folic acid deficiency	33	11	22 (67%)	1·26	34	22 (65%)	12	4·29

[1]Percentages based on fewer than 25 observations.

was not statistically significant but it did suggest a weak association between low serum albumin levels and abnormal intestinal biopsies. Since D-xylose absorption showed a weak association with the haemoglobin concentration (Table IV) and a statistically significant association with the serum albumin level (Table VI) a cross analysis of the three parameters was done. Table VIII indicates that for any given level of haemoglobin concentration the mean D-xylose excretion in the urine increases with the rising serum albumin level. However, for a given serum albumin level, it is seen that the D-xylose absorption does not show any association with the increasing haemoglobin concentration of the blood. Table IX presents the association of D-xylose and fat absorption tests with the iron, B_{12}, and folic acid deficiency as indicated by changes in bone marrow and peripheral smear. It is apparent that neither the fat balance test nor the D-xylose excretion test bear any association with a specific deficiency of iron, B_{12}, or folic acid.

DISCUSSION

Results of the present study confirm our preliminary observations (Tandon Iyenger, Deo, and Saraya, 1966b) and the reports of other investigators (Chaudhuri and Saha, 1964; Burman, 1966; Chuttani *et al*, 1967) on intestinal functional and structural abnormalities in patients with hookworm infestation. Functional tests used in this study are more frequently abnormal (28·9% for the D-xylose absorption test and 31·7% for the fat balance test) than unequivocal morphological changes in the intestinal villi (Fig. 1) which were observed only in 11·8% of cases. However, there are contradictory reports in the literature (Gilles, Williams, and Ball, 1964; Banwell, Marsden, Blackman, Leonard, and Hutt, 1967) for which one possible explanation is suggested in the discussion of this report.

Anaemia is the most prominent feature of patients with hookworm infestation and it was noted in all the cases of the present series. However, analysis of Tables IV and V clearly indicates that the severity of anaemia has no association with the intestinal functional test abnormalities and the same is true for morphological changes. A weak association with the D-xylose absorption test, as suggested by Table IV ($P = 0·05$), is possibly due to low serum albumin levels noted with low haemoglobin levels (Saraya, Ramachandran, and Tandon, 1968). This is confirmed by a cross analysis of the data (see Table VIII) where for a fixed haemoglobin level, D-xylose absorption improved with the rising serum albumin level while the reverse was not true. Thus, it is reasonable to infer that 'anaemia' in hookworm disease does not contribute to the intestinal abnormalities. This is further supported by the observations of Magotra (1966) and Rawson and Rosenthal (1960) demonstrating almost normal function and morphology of small bowel in iron-deficiency anaemia in adults. There was no association of D-xylose and fat malabsorption with the evidence of vitamin B_{12} and folic acid deficiencies in the bone marrow

(Table IX). This is of interest with reference to the reportedly high incidence of folic acid and/or B_{12} deficiency in 'tropical sprue', the commonest cause of the malabsorption syndrome in the tropics (Jeejeebhoy, Desai, Noronha, Antia, and Parekh, 1966; Baker, 1966).

Twenty-four per cent of the cases in the present series had moderate to severe hypoalbuminaemia. Darke (1959) reported that nitrogen absorption was diminished in heavy hookworm infestation but dietary deficiency of protein was a major factor in the pathogenesis of malabsorption and anaemia. Blackman, Marsden, Banwell, and Craggs (1965), in a study of albumin metabolism in hookworm disease, found increased faecal loss and decreased synthesis of albumin in patients with hookworm infection, which was manifested as hypoalbuminaemia, if the added drain on the albumin pool was not compensated for by an adequate diet. Low albumin levels in patients with hookworm infection thus seem to be due to multiple factors, of which possibly less protein in diets is the most important. Abnormalities of D-xylose absorption and the fat balance test showed a statistically significant correlation with the hypoalbuminaemia ($P < 0.01$) in the present study. A suggestion of a similar trend was noted with the morphological changes in intestinal villi. How far intestinal changes contributed to the pathogenesis of hypoalbuminaemia in these patients cannot be answered. The following pertinent observations reported in the literature would indirectly support the possibility that hypoalbuminaemia could be a major contributory factor in the causation of intestinal changes in hookworm disease. Reversal of malabsorption in hookworm disease on a high protein diet, without any additional vitamin, antibiotic, or dewormification therapy has been demonstrated in a small number of patients (Mayoral, Tripathy, Garcia, and Ghitis, 1966). Defective absorption of fat and D-xylose and villous atrophy have been reported in hypoproteinaemia associated with malnutrition in adults (Tandon *et al*, 1968; Mayoral, Tripathy, Garcia, and Ghitis, 1967). Intestinal abnormalities have been recorded in kwashiorkor, a disease due to severe protein malnutrition in children (Stanfield, Hutt, and Tunnicliffe, 1965; Trowell, Davies, and Dean, 1954). Experimental studies in monkeys have shown that villous atrophy and defective absorption of nutrients occurred in protein-depleted animals (Ramalingaswami, 1964).

It is suggested that malnutrition associated with protein deficiency leads to hypoalbuminaemia which is possibly a primary factor in the pathogenesis of intestinal dysfunction in patients with hookworm infestation. The protein nutritional status of the patients with hookworm infestation may be one of the important factors causing the variable incidence of intestinal abnormalities. In India, 'tropical sprue', an idiopathic type of malabsorption syndrome, is fairly prevalent (Jeejeebhoy *et al*, 1966; Baker, 1966). Whether protein malnutrition simply acts as a precipitating factor for the malabsorption syndrome in a population having a background of an unknown defect of tropical sprue, or whether it has a more direct role in the pathogenesis of villous atrophy and intestinal enzyme deficiency with consequences of malabsorption needs to be studied further. However, it is to be noted that although the association of hypoalbuminaemia with abnormalities in the absorption test was highly significant there were a small number of patients with normal serum albumin levels or only a mild hypoalbuminaemia who poorly absorbed d-xylose and had steatorrhoea (Tables VI and VII). This would suggest that some other factor or factors contribute to the occurrence of malabsorption in a small proportion of these patients.

SUMMARY

Diarrhoea and ankle oedema were found in 27% and 19% of patients with hookworm disease. D-xylose absorption was abnormal in 28·9% and fat balance tests revealed steatorrhoea in 31·7%. Intestinal biopsy showed significant histological abnormalities in 11·8%. Intestinal functional or structural changes had no association with the severity of anaemia, or the deficiency of iron, vitamin B_{12}, and folic acid. A statistically significant association was noted between the absorption test abnormalities and the severity of hypoalbuminaemia. A weak association of intestinal biopsy changes with hypoalbuminaemia was also recorded. It is suggested that malnutrition associated with protein deficiency is the chief cause of hypoalbuminaemia in hookworm disease.

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