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Quantitative Histological and Immunohistochemical Findings in Jejunal Biopsy Specimens in Giardiasis

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Summary. Jejunal mucosa biopsies from non-immune deficient patients with Giardia lamblia infestation were examined and showed three different groups of mucosal changes, distinguishable on morphological and immunohistochemical grounds. In three patients no morphological or immunohistochemical abnormalities were found (group A).

In five patients a normal villous architecture was seen. These biopsies had increased numbers of interepithelial lymphocytes and of immunoglobulin containing cells in the lamina propria, with a relative increase of the number of IgA and IgG containing cells (group B).

Two patients with a malabsorption syndrome due to giardiasis had marked villous atrophy, documented by morphometric measurements and large numbers of interepithelial lymphocytes and of immunoglobulin containing cells in the lamina propria, especially IgA and IgG (group C).

These findings differ considerably from those in patients with immunodeficiency or gluten sensitive enteropathy. This suggests that when villous atrophy of the jejunal mucosa is found immunohistochemistry of jejunal biopsy specimens may be helpful in the differential diagnosis between mere giardiasis and giardiasis superimposed on immunodeficiency or gluten sensitive enteropathy.

Key words: Giardiasis – Immunohistochemistry – Morphometry

Histological studies of the jejunal mucosa in giardiasis have been reported by many workers. Frequently the biopsy findings have been correlated with absorption studies (Yardley et al. 1964; Ridley and Ridley 1976; Wright and Tomkins 1978). Symptomless subjects may show no mucosal abnormalities, but in patients with symptomas, mild to subtotal villous atrophy may be seen together with a cellular infiltration of the lamina propria by lymphocytes, plasma cells and polymorphs. The surface epithelium is frequently cuboidal and may show an increased number

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of interepithelial lymphocytes. When multiple biopsies are taken, a patchy distribution of mucosal abnormalities has sometimes been noted (Ament and Rubin 1972).

In spite of the known association between giardiasis and immune deficiencies, Jones and Brown (1974) have shown that the development of giardiasis is not dependent upon a predisposing immune deficiency and can be found in patients with normal serum concentrations of IgA, IgG, IgM and IgE. Patients with giardiasis show a spectrum of morphological abnormalities in jejunal biopsies, and elevated levels of intestinal fluid IgG have also been described in these patients (Jones and Brown 1974).

To see whether the jejunal mucosa of patients with normal immunity and with giardiasis shows typical morphological and immunopathological changes, we have investigated biopsy specimens of ten non immune deficient infested patients. From morphological and immunohistochemical criteria, three different groups of patients could be distinguished.

The immunohistochemical findings in these jejunal biopsy specimens suggest that immunohistochemistry may be helpful in differentiating villous atrophy in simple giardiasis from villous atrophy in giardiasis superimposed on immunodeficiency or gluten sensitive enteropathy.

Patients

Jejunal biopsy specimens from nine consecutive patients with giardiasis were studied, together with a biopsy specimen of an asymptomatic carrier of Giardia lamblia, a family member of one of our patients. None of the individuals we studied had an immune deficiency. All nine patients had symptoms of poorly localized abdominal discomfort and distension, borborygmi, flatulence and frequent loose stools. Two of them had an overt malabsorption syndrome with steatorrhoea, severe weight loss, anaemia and depressed serum folate levels. The other seven patients had only mild diarrhoea without signs of malabsorption. Absorption was assessed by urinary excretion of D-xylose after 50 g oral load and daily faecal fat output on a diet containing 80 g fat.

In the two patients with the malabsorption syndrome we repeated the small-intestinal biopsy half a year after adequate treatment with metronidazole (ten days 500 mg t.d.s.).

The control group consisted of ten adults who had a jejunal biopsy done as a healthy volunteer or as a healthy family member of a patient with gluten-sensitive enteropathy in connection with a family study (Rosekrans et al. 1978).

The jejunal biopsy findings in the patients with giardiasis were also compared with the morphometrical and immunopathological observations made on jejunal biopsy specimens from 15 patients (juvenile and adult) with gluten-sensitive enteropathy in the active phase on a gluten-containing diet.

Methods

Peroral biopsies of the proximal jejunal mucosa were performed under radiological control, using a hydraulic multiple biopsy capsule. The biopsy specimens were taken distal to the ligament of Treitz. Under a dissecting microscope the biopsy specimen was orientated, with a minimum of handling, on a mesh to which it adheared. After fixation in a sublimate-formaldehyde mixture for three hours the tissue sample was embedded in paraplast. Tissue sections were cut 4 µm thick, perpendicular to the luminal surface and mounted on glass slides. Sections were stained with haematoxylin and eosin (HE) and specifically for IgA, IgG, IgM and IgE heavy chains using an indirect peroxidase staining as described earlier (Rosekrans et al. 1980). The specificity of the antisera and the control staining procedures have already been reported (Rosekrans et al. 1980).

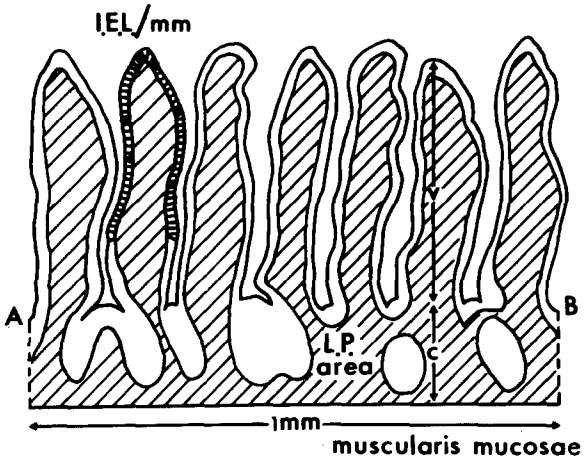

Fig. 1. Morphometry of jejunal biopsies: *V/C*, villous: crypt ratio; *IEL*, number of interepithelial lymphocytes per mm surface epithelium; *AB*, length of surface epithelium per mm extended muscularis mucosae

The stained slides were used for morphometric study (Rosekrans et al. 1980, 1981). Images of the HE and immunoperoxidase stained sections were projected with a standard magnification of 200 × on graph paper and drawings were made. Morphometric analysis of the drawn figures was made on a graphic tablet (Tektronix) interfaced with a laboratory computer (PDP 11–10), Digital Equipment Corp. Maynard, USA).

The following variables were measured (Fig. 1):

1. Mean villous to crypt ratio, that is the linear measurement of five villous heights, divided by the corresponding crypt depths (V/C).
2. Length of surface epithelium per millimeter extended muscularis mucosae (SA).
3. Number of interepithelial lymphocytes per millimeter surface epithelium (IEL).

The slides stained for IgA, IgG, IgM and IgE were used for counting the immunoglobulin-containing cells per millimeter extended muscularis mucosae in three consecutive sections. This is comparable with a mucosal tissue unit of 1 mm width and a thickness of 4 μm (Brandtzaeg et al. 1974).

Results

In all patients we detected Giardia Lamblia trophozoites in the jejunal juice aspirated during the biopsy procedure. It was also possible to demonstrate the parasite in the histological sections in the lumen or the crypts in all patients (Fig. 2).

Using morphological criteria we could divide the biopsy specimens in three different groups (Table 1):

A. Biopsy specimens with normal architecture of villi and crypts, with normal numbers of interepithelial lymphocytes and no increase of the number of immunoglobulin-containing cells in the lamina propria (No 1, 3 and 5).

B. Biopsy specimens with normal architecture of villi and crypts, but with increased numbers of interepithelial lymphocytes and increased numbers of immunoglobulin-containing cells in the lamina propria (No 4, 6, 7, 9 and 10).

C. Biopsy specimens with distinct villous atrophy, cuboidal epithelial changes, heavily infiltrated with lymphocytes, and increased numbers of immunoglobulin-containing cells in the lamina propria (No 2 and 8).

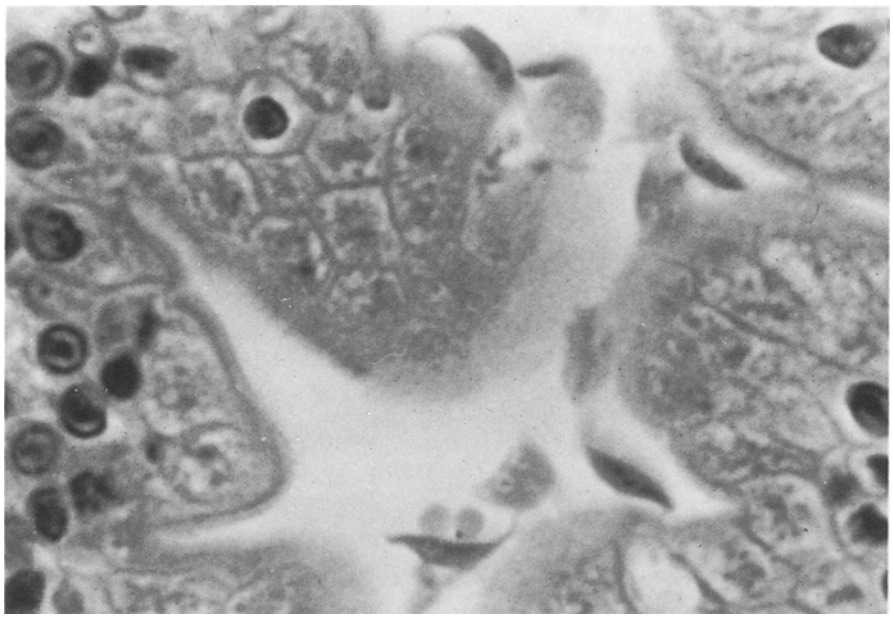

Fig. 2. Detail of jejunal mucosa of patient 3. Many giardia lambliae are present in the crypt

Table 1. Morphometric and immunohistochemical findings in ten patients with giardiasis

	No	V/C	S.A.	I.E.L.	IgA	IgG	IgM	IgE
A	1	2.03	5.38	21	87	12	19	0
	3	3.01	6.90	15	60	14	29	1
	5	2.10	5.78	16	87	26	35	0
B	4	3.00	6.02	34	186	48	35	0
	6	2.37	5.58	32	142	45	39	0
	7	3.12	6.85	27	121	51	26	2
	9	3.55	6.39	29	146	28	31	0
	10[a]	3.08	5.42	26	138	23	18	1
C	2[b]	0.71	2.73	69	196	50	45	1
	8[b]	1.01	3.04	50	178	35	39	1
Healthy controls								
	N = 10	2.88	6.07	19	87	14	23	0.5
	(± sd)	(± 0.52)	(± 0.58)	(± 4)	(± 19)	(± 6)	(± 7)	(± 0.7)
Gluten-sensitive enteropathy (active phase)								
	N = 15	0.12	1.20	72	169	25	86	1.5
	(± sd)	(± 0.14)	(± 0.16)	(± 19)	(± 70)	(± 7)	(± 39)	(± 1.1)

V/C = Villous: crypt ratio; S.A. = length of surface epithelium per mm muscularis mucosae; I.E.L. = number of interepithelial lymphocytes per mm surface epithelium; IgA, IgG, IgM and IgE = number of immunoglobulin-containing cells per mm muscularis mucosae; A, B and C = different groups of histological abnormalities in jejunal biopsy specimens

[a] Asymptomatic carrier of Giardia lamblia parasites, son of patient No 9

[b] Patients with malabsorption syndrome

Table 2. Morphometric and immunohistochemical findings in two patients with malabsorption due to giardiasis

No	V/C	S.A.	I.E.L.	IgA	IgG	IgM	IgE
2a	0.71	2.73	69	196	50	45	1
2b	2.67	5.24	21	131	38	54	1
8a	1.01	3.04	50	178	35	39	1
8b	3.45	6.89	17	103	22	35	0

a = before treatment, b = six months after metronidazole treatment

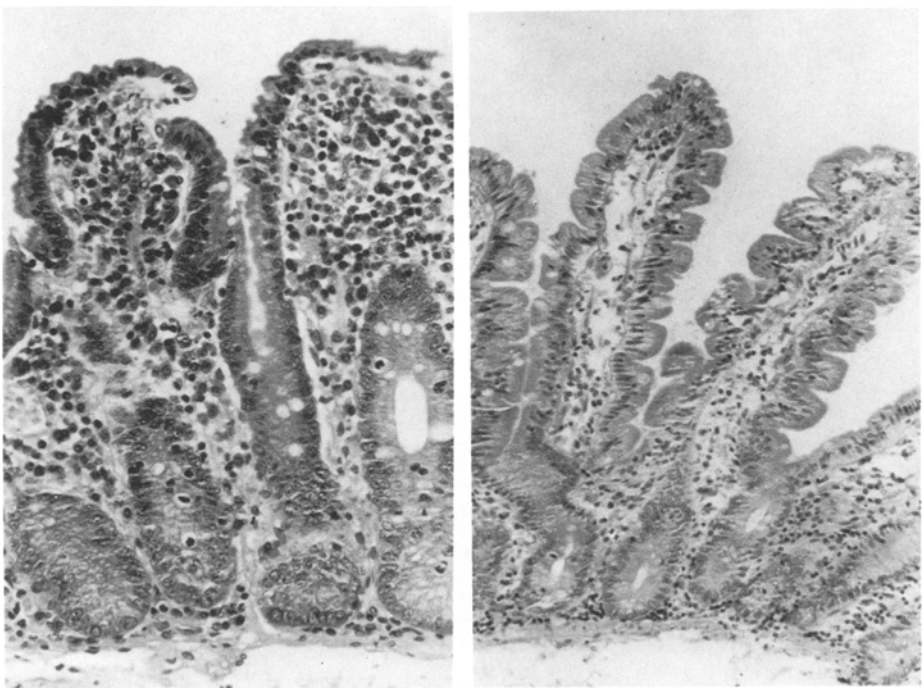

Fig. 3. a Jejunal mucosa of patient 8 before treatment (×180). **b** Jejunal mucosa of patient 8 after metronidazole treatment (×100)

On clinical grounds we could not find any difference in the patients of group A and group B with an exception of No 10, who was a healthy carrier of Giardia lamblia parasites, detected in a family examination.

The biopsy specimens of group B showed an increased number of immunoglobulin-containing cells per millimetre extended muscularis mucosae. In most of these jejunal biopsies the number of IgG-containing cells was, in contrast to the findings in the jejunal mucosa of healthy individuals, higher than the number of IgM-containing cells.

The two patients of group C whose jejunal biopsy specimens showed distinct villous atrophy, both had a malabsorption syndrome. The number of

immunoglobulin-containing cells was markedly increased. After treatment with metronidazole we could not detect Giardia lamblia trophozoites anymore in the jejunal juice. The jejunal biopsy specimens, taken half a year after adequate treatment, showed no morphological abnormalities (Table 2). The pattern of immunoglobulin-containing cells in the jejunal mucosa of patient no. 2 also showed an increase of the number of IgA, IgG and IgM-containing cells after treatment (Table II). The jejunal mucosa biopsies of patient no. 8 are shown in Fig. 3a and 3b.

In all patients the number of IgE-containing cells was comparable with that of healthy controls.

In contrast to patients with villous atrophy due to giardiasis (group C), patients with active gluten-sensitive enteropathy showed more severe villous atrophy as expressed by a lower V/C ratio and a shorter length of surface epithelium per millimeter extended muscularis mucosae. In particular, the marked increase in the number of IgM-containing cells in the jejunal mucosa in GSE patients contrasted with the findings in giardiasis.

Discussion

The present study shows that in small-intestinal mucosal biopsies from non-immune deficient patients with giardiasis, three distinct morphological groups can be separated using morphometric and immunohistochemical techniques. There are patients with Giardia lamblia infestation without abnormalities in the jejunal mucosa (group A). In patients with morphological abnormalities (group B and C) all patients showed an elevated number of interepithelial lymphocytes and an increased number of immunoglobulin-containing cells in the lamina propria. There was a marked rise in the number of IgA-containing cells and most patients also showed an absolute and relative increase in the number of IgG-containing cells.

Patients of group C differed from patients of group B by the presence of villous atrophy in the jejunal biopsy specimens. Clinically the patients of group C had symptomas different from patients of group A and B: both patients had a malabsorption syndrome.

Why the jejunal mucosa can show such a varied picture from a normal appearance to flat with a marked infiltrate in giardiasis, is unknown. The contributions of parasitic factors and host immune reactivity remain undefined.

Wright and Tomkins (1978) have shown a positive correlation between surface epithelial area and D-xylose excretion in giardiasis. Ferguson et al. (1976) reported an increased number of interepithelial lymphocytes in children with diarrhoea due to giardiasis. A marked increase in the number of IgG-containing cells was found in a giardiasis patient by Blenkinsopp et al. (1978). This finding and our observations correlate with the study of Jones and Brown (1974), who found a high level of IgG in the intestinal juice in giardiasis.

The histological picture of the jejunal mucosa in patients with malabsorption due to giardiasis resembles gluten-sensitive sprue. In our experience however, an important difference is the relatively high number of IgG-containing cells in giardiasis in comparison with the large number of IgM-containing cells in gluten-sensitive enteropathy (Brandtzaeg and Baklien 1976; Scott et al. 1980; Rosekrans et al. 1981). Perhaps this difference in immunoglobulin subclasses will differentiate

patients with villous atrophy due to giardiasis alone from patients with villous atrophy in giardiasis superimposed on immune dificiency or gluten sensitive enteropathy.

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