Transthyretin values correlate with mucosal recovery in patients with coeliac disease taking a gluten free diet

S A McMillan, W Dickey, J P Douglas, D F Hughes

Abstract

***Aims*—To assess changes in indicators of nutrition and iron deficiency as possible non-invasive markers of mucosal recovery in patients with coeliac disease on a gluten free diet.**

***Methods*—Concentrations of transthyretin, retinol binding protein, soluble transferrin receptor, IgA anti-gliadin, and IgA anti-transglutaminase, and titres of IgA anti-endomysial antibody were measured in 36 newly diagnosed adult patients with coeliac disease and duodenal villous atrophy before (T0) and after one year (T1) on a gluten free diet. Duodenal biopsies taken at T0 and T1 were compared and graded as no improvement (no change in initial grade of villous atrophy) or improvement.**

***Results*—Twenty two patients showed histological improvement and 14 showed no improvement. Transthyretin values increased in all patients with mucosal improvement and decreased in all patients showing no improvement. However, transthyretin values did not correlate with the degree of villous atrophy at T0 and T1 when assessed separately. Changes in retinol binding protein and soluble transferrin receptor values did not correlate with mucosal improvement. Coeliac disease associated antibodies (to gliadin, endomysium, and transglutaminase) decreased in most patients between T0 and T1, irrespective of mucosal recovery.**

***Conclusions*—Serial but not single measurements of transthyretin may be used as a non-invasive test to monitor mucosal recovery and therefore reduce the need for, or frequency of, follow up biopsies in treated patients with coeliac disease.**

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Coeliac disease is characterised histologically by small bowel mucosal villous atrophy. Compliance with a gluten free diet usually results in the disappearance of clinical symptoms and mucosal recovery.[1] Effectiveness of the gluten free diet is most reliably assessed by follow up small bowel biopsy; however, less invasive methods would be useful. The disappearance of serum autoantibodies, such as anti-endomysial antibodies (EmA) and anti-gliadin antibodies (AGA), appears to indicate reduced gluten exposure and not to predict mucosal recovery.[2–4] Dynamic tests of small bowel permeability, such as the lactulose-mannitol and D-xylose absorption tests, may be useful in this context,[5 6] but serological indicators of malnutrition and specific deficiencies would be more convenient. Malnutrition and iron deficiency are common features in untreated patients with coeliac disease. Transthyretin (prealbumin) acts as a binding protein for thyroxin, a carrier protein for retinol binding protein (RBP), which in turn is the transport protein for vitamin A. Concentrations of transthyretin and RBP are both greatly reduced in malabsorption and, because they both have short half lives (approximately two days), they are suitable for monitoring nutritional status.[7–9] The serum soluble transferrin receptor (sTfR) concentration falls early in iron deficiency[10 11] and, unlike ferritin, can discriminate between iron deficiency anaemia and anaemia of chronic disease, especially in patients with concurrent chronic disease, because it is not affected by acute phase reactions.[12] The measurement of sTfR is also a marker of undernutrition.[13] We assessed these nutritional indicators as possible markers of mucosal recovery in patients with coeliac disease by correlating their values with duodenal histology before and after 12 months on a gluten free diet.

Methods

SUBJECTS

Thirty six adult patients (26 women, 10 men; age range, 26–76 years) were studied. All patients were diagnosed as having coeliac disease on the basis of villous atrophy (VA; partial, subtotal, or total) with crypt hyperplasia and a raised intraepithelial lymphocyte count and were commenced on a gluten free diet. All had follow up biopsies 12 months later (± 2 months). Serum samples were obtained at the time of initial biopsy (T0) and at re-biopsy (T1). Patients were assessed by hospital dieticians for compliance with the diet at the 12 month point, using food diaries.

As a control group, serum samples were obtained from 66 patients (40 women, 26 men; age range, 25–71 years) who presented with diarrhoea or anaemia and underwent duodenal biopsy. All had inflammatory bowel disease as a cause of diarrhoea and colon cancer as a cause of anaemia excluded by appropriate investigations. They were all EmA negative and had no histological features (raised intraepithelial lymphocytes, crypt hyperplasia, or villous atrophy) suggestive of coeliac disease.

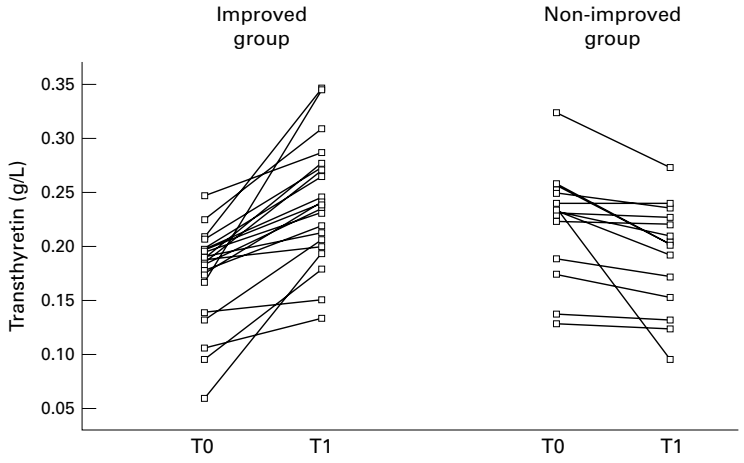

Figure 1 Changes in transthyretin values between the time of the initial biopsy (T0) and the repeat biopsy (T1) in the patients with coeliac disease.

SEROLOGICAL MARKERS

Serum samples obtained at T0 and T1 were assayed for transthyretin, RPB, and sTfR by nephelometry (Dade Behring, Marburg, Germany). The intrabatch variations for transthyretin, RPB, and sTfR were 1.9–3.1%, 2.2–3.9%, and 1.4–2.1%, respectively, and the interbatch variations were 1.4–2.9%, 3.1–4.7%, and 0.8–1.2%, respectively. The reference ranges (2.5 to 97.5 centile) of transthyretin, RBP, and sTfR were as follows: transthyretin, 0.2–0.4 g/litre (higher values were seen in men in midlife compared with women); RBP, 0.03–0.06 g/litre, and sTfR, 0.83–1.76 mg/litre.

IgA EmA were detected by indirect immunofluorescence using primate oesophagus (Bio-Diagnostics, Upton upon Severn, UK) as substrate, with a titre of 1/5 taken as positive. All positive sera were titrated until the endpoint was reached. IgA AGA (Labmaster, Turku, Finland) and IgA anti-transglutaminase antibodies (TGA; Inova Diagnostics, San Diego, California, USA) were measured by commercial enzyme linked immunosorbent assay (ELISA). The reference range for IgA AGA was 0–100 EU/ml and for IgA TGA 0–20 EU/ml. Total IgA was also measured in all patients to exclude deficiency. No patient was IgA deficient.

SMALL BOWEL BIOPSIES

Three biopsy specimens were taken from the second part of the duodenum with standard forceps during upper gastrointestinal endoscopy and carefully orientated and mounted on filter paper before immersion in formalin. They were assessed (by DFH) using the criteria of Marsh[14] for evidence of excess intraepithelial lymphocytes (Marsh type I: lymphocytes > 30/100 enterocytes), crypt hyperplasia (Marsh type II), and VA (Marsh type III). When present, VA was classified as partial, subtotal, or total (PVA, STVA, and TVA, respectively) as described by Rostami *et al*.[15]

COMPARISON OF BIOPSIES AND SEROLOGICAL MARKERS

Biopsies at T0 and T1 were compared and graded as no improvement (no change in initial grade of villous atrophy) or improvement (change from T/STVA to PVA or no VA, or from PVA to no VA) without prior knowledge of serological marker results. Concentrations and titres of transthyretin, RPB, sTfR, and coeliac disease associated antibodies were also compared between T0 and T1 and correlated with the degree of mucosal atrophy.

Table 1 Changes in duodenal histology between the initial biopsy (T0) and repeat biopsy (T1) in the 36 patients with coeliac disease adhering to a gluten free diet

Change in duodenal histology	*Number of patients*
Group A: improvement in histology	
STVA to PVA	8
PVA to no VA (Marsh type I)	4
STVA to no VA (Marsh type I)	3
PVA to normal	3
STVA to normal	4
	n = 22
Group B: no improvement in histology	
STVA to STVA	8
PVA to PVA	2
TVA to TVA	2
STVA to TVA	2
	n = 14

PVA, partial villous atrophy; STVA, subtotal villous atrophy; TVA, total villous atrophy; VA, villous atrophy.

STATISTICS

One way analysis of variance and the Wilcoxon signed rank test were used to compare serological marker values and antibody titres. Pearson's correlation coefficient was used to correlate the degree of VA with serological markers at T0 and T1. Analyses were done using an SPSS statistical software package. A p value of < 0.05 was considered to be significant.

Results

In the 36 patients studied the histology at T0 was TVA in two patients, STVA in 25, and PVA in nine. Twenty two patients showed an improvement in duodenal histology between biopsies taken at T0 and T1. No improvement was seen in 14 patients. In the improved group comparison of the T0 and T1 biopsies showed a change from STVA to PVA or no VA, or from PVA to no VA. In the patients with no improvement the biopsy histology after one year on a gluten free diet remained the same or in two cases worsened from STVA to TVA (table 1).

COMPARISON OF SEROLOGICAL MARKER VALUES BETWEEN INITIAL AND REPEAT BIOPSY

Transthyretin concentrations increased in all patients showing mucosal improvement and decreased in all patients showing no improvement (fig 1; table 2). Transthyretin values were significantly higher in the improved group than in the not improved group (0.24 *v* 0.19 g/litre; p = 0.01; table 3). sTfR values decreased in all patients showing mucosal improvement and increased in half of the non-improved group. There was no consistent trend in RBP values for either the improved or non-improved group. No significant difference was seen between mean sTfR (1.1 *v* 1.4 g/litre; p = 0.23) or RPB (0.04 *v* 0.03 mg/litre; p = 0.37) values in the improved and non-improved groups (table 3).

Table 2 Change in marker values after one year on a gluten free diet in patients showing histological improvement (group A) or no improvement (group B)

	Group A (n = 22) Number of patients showing changes in marker values between T0 and T1			*Group B (n = 14) Number of patients showing changes in marker values between T0 and T1*		
Marker	*Increase*	*Decrease*	*No change*	*Increase*	*Decrease*	*No change*
TTR	22	0	0	0	14	0
sTfR	0	22	0	7	2	5
RBP	9	4	9	5	4	5
IgA AGA	0	19	3	0	12	2
IgA TGA	0	17	5	0	10	4
IgA EmA	0	18	4	0	10	4

IgA AGA, antigliadin antibody; IgA EmA, endomysial antibody; IgA TGA, transglutaminase antibody; TTR, transthyretin; RBP, retinol binding protein; sTfR, soluble transferrin receptor.

IgA AGA and IgA TGA values fell or remained the same in both the improvement and non-improvement groups of patients between initial and repeat biopsy. Similarly, titres of IgA EmA fell or remained the same in both patient groups (table 2). Five patients in the improved group and three in the non-improved group were IgA EmA negative at T0.

SEROLOGICAL MARKER VALUES IN PATIENTS WITH COELIAC DISEASE AND CONTROLS

There was a significant difference between the mean concentration of transthyretin in the control group and patients with coeliac disease patients at initial diagnosis (0.23 *v* 0.19 g/litre; p = 0.009). However, there was considerable overlap in the range of transthyretin values in these patients groups, with all groups having patients with concentrations both below and within the reference range (table 3). No significant difference was seen between RBP and sTfR values between the patients with and without coeliac disease and between patients either showing mucosal recovery or no recovery.

The coeliac disease associated antibodies showed good sensitivity and specificity between the patients with coeliac disease and the control group. However, after the introduction of a gluten free diet, most of these antibody values fell or remained the same in patients with and without mucosal improvement (table 3). Five patients in the improved group and three in the non-improved group were IgA EmA negative at T0. In the improved group, the initial titres (apart from the five IgA EmA negative patients) ranged from 10 to 320. In all these patients, the titres fell to low values (titre 20) or were negative. In the non-improved group (apart from the three IgA EmA negative patients), the initial titres ranged from 10 to 80 and again in all patients the IgA EmA titres became negative after one year.

CORRELATION BETWEEN SEROLOGICAL MARKER VALUES AND HISTOLOGICAL FINDINGS

There was no significant correlation between the grade of VA (ST/T *v* PVA) and concentrations of transthyretin, RBP, sTfR, IgA AGA, and IgA TGA or the titre of IgA EMA at the time of initial biopsy. A small correlation was seen between VA grade at T1 and transthyretin, sTfR, and IgA AGA values ($r = 0.40$, $p = 0.016$; $r = 0.429$, $p = 0.009$; $r = 0.401$, $p = 0.015$, respectively), with higher transthyretin values found in patients with PVA compared with S/TVA and lower amounts of sTfR and IgA AGA found also in patients with PVA compared with S/TVA.

Discussion

We are aware of only one previous study of transthyretin in coeliac disease.[16] Reifen *et al* demonstrated significantly lower transthyretin values in untreated children with coeliac disease compared with both controls and with children with coeliac disease who were on a gluten free diet.[16] Our study confirms that the nutritional marker transthyretin can be used to assess mucosal recovery in patients with coeliac disease who were on a gluten free diet. Patients with an improved duodenal histology after one year on a gluten free diet all showed an increase in transthyretin values, whereas patients with coeliac disease who did not show any improvement had a decrease in transthyretin values. In the improved group, the percentage increase between T0 and T1 ranged from 7% to 229%, and in the non-improved group the percentage decrease ranged from 4% to 50%. Because the interbatch variation for transthyretin was 1.4–2.9%, we can regard transthyretin values in those patients with low percentage increases or decreases to be valid.

There were also significant differences between the mean transthyretin value in the control group and the patients with coeliac disease at T0 and those showing recovery on a gluten free diet. Although our study was on an adult population, our results generally agree with those of Reifen *et al*,[16] who also showed that mean transthyretin concentrations were low in children diagnosed as having coeliac disease who were eating a gluten containing diet, and differed significantly from mean values found both in children with coeliac disease who were on a gluten free diet and healthy school

Table 3 Comparisons of mean values and ranges of serological markers in control subjects and patients with coeliac disease (CD) at initial biopsy and at follow up biopsy (T1)

	Mean value and range of serological markers in							
	Control subjects (n = 66)		*CD at initial biopsy (n = 36)*		*CD at T1: group A (n = 22)*		*CD at T1: group B (n = 14)*	
Marker	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*
TTR	0.23 g/l	(0.09–0.38)	0.19 g/l	(0.10–0.32)	0.24 g/l	(0.13–0.35)	0.19 g/l	(0.15–0.27)
sTfR	1.47 g/l	(0.74–4.17)	1.46 g/l	(0.88–2.72)	1.1 g/l	(0.80–1.69)	1.4 g/l	(1–2)
RBP	0.06 mg/l	(0.01–0.38)	0.03 mg/l	(0.01–0.07)	0.04 mg/l	(0.02–0.07)	0.03 mg/l	(0.02–0.05)
IgA AGA	40 EU/ml	(11–205)	115 EU/ml	(18–465)	33 EU/ml	(8–171)	77 EU/ml	(17–234)
IgA TGA	4 EU/ml	(1–22)	67.2 EU/ml	(5–271)	25 EU/ml	(3–121)	36 EU/ml	(1–112)

Group A, improved; group B, non-improved.
AGA, antigliadin antibody; RBP, retinol binding protein; sTfR, soluble transferrin receptor; TTR, transthyretin.

children. However, in our patients there was a considerable overlap in the ranges of transthyretin values seen in the patient groups, with samples from patients in each group having transthyretin values falling within and below the reference range. Therefore, the use of a single point measurement of transthyretin is unsuitable as a diagnostic marker for coeliac disease or for differentiating between improvers and non-improvers.

Comparison of RBP and sTfR values at initial and follow up biopsy did not discriminate between patients showing improvement and no improvement. As with transthyretin values, RBP and sTfR concentrations showed a large overlap between the patients groups and therefore their single point measurement was not considered to be of diagnostic value.

Our study confirms earlier findings that coeliac disease associated antibodies fall in a high proportion of patients on a gluten free diet regardless of repeat biopsy outcome. Antibody status appears to reflect gluten exclusion rather than mucosal recovery,[2–4] and may therefore aid in the assessment of dietary compliance. In our study group, eight of our patients with coeliac disease were IgA EmA negative at the time of diagnosis, raising the possibility of incorrect diagnosis of coeliac disease. False negative EmA is well recognised in patients with coeliac disease.[15 17] Such patients respond similarly to a gluten free diet in terms of clinical and histological improvement.

Our study was only carried out over a one year period. Because some patients with coeliac disease take several years to show histological recovery on a gluten free diet it is important to continue this study until there is histological improvement. In addition, other parameters of nutritional status (such as haemaglobin, albumin, ferritin, weight, body mass index, etc) and detailed information on compliance would be helpful.

Dynamic tests of intestinal permeability have to date shown most promise in assessing response to treatment. Uil *et al* showed that the lactulose-mannitol sugar absorption test correlated closely with the degree of VA on treatment.[5] The D-xylose test may be similarly valuable.[6] However, a blood test such as described above offers advantages in terms of convenience.

In conclusion, the serial measurement of the nutritional marker transthyretin may be of value as a relatively non-invasive test for determining which patients need follow up biopsies and at what stage, and as an indicator of refractory coeliac disease.

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