The prevalence of liver function abnormalities in pediatric Celiac disease patients and its relation with intestinal biopsy findings

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Abstract

Abnormal liver function tests and liver damage are seen frequently with celiac disease. However, the pathogenesis of liver functions abnormality is not clearly understood. The aim of this study was to determine the frequency of abnormal liver functions in children with celiac disease and its relation with anthropometric measurements and severity of intestinal damage.

Patients and methods : **Twenty seven patients with celiac disease were included in the study. Anthropometric and laboratory examinations and intestinal biopsies were performed in all the patients. Mucosal lesions were classified according to the Marsh classification. Villous area, crypt height and mitotic count were measured morphometrically for all biopsy samples.**

Results : **The mean age of patients was 6 ± 5 years on admission. Alanine aminotransferase levels were normal (group 1) or elevated (group 2) in 20 and 7 patients, respectively. The mean alanine aminotransferase levels were 22.0 ± 7.2 in group 1 and 70.5 ± 31.1 U/L in group 2 patients, ($p < 0.001$). Complaints, mean age, height for age, weight for height, serum albumin level, villous area, crypt height and mitotic count were not significantly different between the two groups. Similarly, the ratio of Marsh classification was not significantly different between the two groups. All patients were given a gluten-free diet. Serum aminotransferase values returned to normal after 7.4 ± 2.7 months of a gluten-free diet.**

Conclusion : **Abnormalities of liver functions are frequently seen in paediatric celiac disease patients. These abnormalities are not correlated with malnutrition and severity of intestinal mucosal lesions. Liver enzyme activities return to normal levels in a few months after gluten-free diet** (Acta gastroenterol. belg., **2005**, 68, **424-427**).

Key words : Celiac disease, aminotransferase, child, Marsh, morphometry.

Introduction

Alterations of liver function tests and damage such as fatty liver, primary sclerosing cholangitis, chronic hepatitis, primary biliary cirrhosis and autoimmune hepatitis have been described in association with or as a presenting feature of celiac disease (1-4). In different studies, moderate elevation of serum aminotransferase levels is common in untreated celiac disease, occurring in 15-55% of patients (1,4-6).

The exact mechanism responsible for abnormal liver functions in celiac disease is still unknown. Liver damage caused by increased intestinal permeability, resulting in the arrival of toxins or antigens at the liver through the portal circulation is one of the most popular hypotheses (7,8). In addition, malnutrition is a well-known cause of liver damage (9). A shared inherited predisposition for some autoimmune liver diseases and celiac disease occurs in patients who posses certain HLA class II molecules and haplotypes (HLA DR3 and HLA DR4) (10,11). In the literature, there are only few studies evaluating the pathogenesis of abnormal liver functions in children with celiac disease (6,7). The aim of this study was to determine the frequency of abnormal liver enzymes in children with celiac disease and its relation with anthropometric measurements and severity of intestinal damage.

Patients and methods

The study population consisted of 27 patients who were admitted to Dokuz Eylül University Medical Faculty Paediatric Gastroenterology, Hepatology and Nutrition Department with different complaints, and diagnosed as celiac disease according to revised ESPGAN criteria (12). Physical examinations and anthropometric measurements of all patients were performed. Malnutrition was evaluated according to Waterlow classification. Chronic and acute malnutrition were defined as height for age below 95% of standard height, and weight for height below 90% of ideal weight, respectively (13,14). Hepatomegaly was defined as a liver edge greater than 2 cm below the right costal margin (15).

Complete blood count, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), albumin, prothrombin time and immunoglobulin A (IgA) were measured for all patients. Alanine aminotransferase normal limits were presumed as 5-45 U/L (group 1) (16). Patients with ALT levels above 45 U/L were classified as group 2. Minimum values of normal serum albumin ranges were 3.9 g/dL and 4 g/dL in younger and older than 5 years old patients, respectively (16). Measured viral markers, serum iron, ferritin, alpha 1-antitrypsin levels, serum copper and coeruloplasmin levels (5/7 patients), autoantibodies against nuclear, smooth muscle, liver and kidney microsomal type-1 antigens (3/7 patients) were screened if the patients' ALT levels were above the accepted values. Abdominal ultrasonography was performed in all patients with elevated ALT levels. Immunoglobulin A anti-endomysium and antigliadin antibodies (IgG and

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IgA) of all patients were measured. Intestinal biopsies were performed in all patients with both anti-endomysium and antigliadin antibodies positive. Duodenal biopsy was also performed in five patients whose celiac disease was suspected on the basis of clinical and laboratory findings, despite antigliadin antibodies positive but anti-endomysium antibody negative. Two of these patients were younger than 2 years old, and two of the others had IgA deficiency.

Histopathological evaluation and morphometric analysis

All biopsies were taken endoscopically from two different sites of distal duodenum and were fixed overnight in 10% buffered formalin. After processing and proper orientation of the specimens, the tissues were embedded in paraffin and cut into 5 μm sections at multiple levels (usually 10 to 12 levels) and stained with haematoxylin and eosin. The severities of intestinal lesions were graded according to the Marsh classification (17). Morphometric analysis was performed by one pathologist (E.O.) with no prior knowledge of the clinical data, using a computer assisted image analyzer system consisting of a microscope (Labophot-2, Nikon, Tokyo, Japan) equipped with a high-resolution video camera (VKC220E, Hitachi, Tokyo, Japan). The images were processed using an IBM-compatible personal computer, high-resolution video monitor and image analysis software (BS 200Docu Version 2.0, BAB Imaging Systems, Ankara, Turkey). Briefly, the images were grabbed with the video camera at ×10 magnification. Ten different sections covering at least 4 villi determined for the analysis were viewed on the monitor and outlined by drawing. The villous area (mm^2) was expressed as mean area ± SD. In addition, crypt height and mitotic count in 10 crypts at ten different sections were also determined. Mitotic count was expressed as mean mitotic counts per crypt and crypt height (μm) was expressed as mean crypt height ± SD.

Statistical analyses

Statistical analysis was performed using the Statistical Package of Social Science (SPSS), Version 11.0 (SPSS, Inc., Chicago, IL). Chi-square and Fischer's exact tests were used for comparing group ratios. Kruskal-Wallis test was used for comparing group averages. Advanced analysis of the groups was performed by Mann-Whitney U-test. Pearson's correlation coefficients were employed to evaluate correlations between Marsh classification and continuous (villous area, crypt height and mitotic count) variables. A p value less than 0.05 was considered as statistically significant.

Results

The median age of patients was 4.0 years (mean 6.07 ± 5.0 years, range 11 months-17 years) on admission. Seventeen patients (63.0%) were female.

Sixteen patients (59.3%) were admitted with diarrhoea, 10 (37.0%) for short stature and one (3.7%) for pallor and fatigue. In addition to main complaints, some of patients also had abdominal distension, weight loss or poor weight gain and muscle weakness. Any patient admitted with abnormal liver function test of unknown origin or findings of chronic liver disease such as ascites or jaundice. Hepatomegaly was found in four patients on admission.

The mean serum ALT level was 34.6 ± 27.0 U/L (range 10.4-138.1 U/L) on admission. Elevated ALT levels detected in 7 (26.9%) patients of group 2. Hypoalbuminemia, elevations of GGT and ALP were detected in 7 (26.9%), 2 (7.4%) and 2 (7.4%) patients, respectively. Rickets was diagnosed in two patients with elevated ALP levels according to physical examination, serum calcium and phosphorus levels, and radiographic findings. Serum bilirubin levels were within normal ranges in all cases. No patients had received any hepatotoxic drugs previously. Liver parenchyma was normal and steatosis wasn't detected on ultrasonographic examination. All laboratory tests performed to eliminate chronic hepatic diseases due to metabolic, infectious and immunologic aetiologies were negative. No child had abnormal glucose level or thyroid function. All the patients but one had normal complete blood count.

Histopathological findings of intestinal biopsies were determined as Marsh 1 in 5 (18.5%) and Marsh 3 for 22 (81.5%) patients. Six (22.2%) patients were classified as Marsh 3a, 8 (29.6%) as Marsh 3b, and 8 (29.6%) as Marsh 3c. There were no patients with histopathological findings in Marsh class 0, 2 and 4.

The mean villous area, mitotic count and crypt height were 0.022 ± 0.018 mm^2 (range 0.0-0.056), 5.1 ± 2.4 (1.0-8.0) and 260 ± 94.4 μm (50.6-352.5), respectively. There was significant difference in villous area, mitotic count and crypt height between the different histopathological groups ($p < 0.001$).

The mean ALT levels were 22.0 ± 7.2 in group 1 and 70.5±31.1 U/L in group 2 ($p < 0.001$). There was no statistically significant difference in percentages of complaints, the means of age, height for age, weight for height, serum albumin level, villous area, crypt height and mitotic count between the two groups (Table I). Similarly, the ratio of Marsh classification was not significantly different between the two groups.

All patients were given a gluten-free diet. Serum aminotransferase values returned to normal after 7.4 ± 2.7 months (range 2-11 months) of a gluten-free diet (Table II). Serum ALP and GGT levels also improved after treatment.

Discussion

In this study, 26% of paediatric celiac disease patients had elevated ALT levels. Hepatomegaly was found in four of these patients on admission. Typically, only AST and/or ALT elevations are present, whereas ALP increas-

Table I. — **Anthropometric, biochemical and morphometric features of patients with normal and elevated alanine aminotransferase levels**

	ALT normal (Group 1) n = 20	ALT elevated (Group 2) n = 7	p value**
Age (year)*	6 ± 5	4 ± 3	NS
Height for age*	90.2 ± 7.3	85.9 ± 5.4	NS
Weight for height*	88.1 ± 10.0	92.2 ± 11.7	NS
Main complaints n (%) : **Diarrhea** **Short stature-anemia**	 11 (55.0%) 9 (45.0%)	 5 (71.4%) 2 (28.6%)	NS
ALT (U/L)*	22.0 ± 7.2	70.5 ± 31.1	**< 0.001**
Albumin (g/dL)*	3.9 ± 0.4	3.6 ± 0.5	NS
Marsh classification n (%) : **Marsh 1** **Marsh 3**	 4 (20.0%) 16 (80.0%)	 1 (14.3%) 6 (85.7%)	NS
Villous area (mm^2)*	0.024 ± 0.01	0.018 ± 0.01	NS
Mitotic count*	4.9 ± 2.4	5.5 ± 2.5	NS
Crypt height (µm)*	261.5 ± 90.5	255.9 ± 112.7	NS

*Mean ± SD
**NS : Not significant

Table II. — **Liver functions of patients with elevated alanine aminotransferase levels at diagnosis and after gluten-free diet**

Patient no	Intestinal biopsy (Marsh)	At diagnosis					After gluten-free diet				
		ALT	AST	ALP	GGT	T.bil	ALT	AST	ALP	GGT	T.bil
1	1	47	49	344	20	0.9	32	34	268	24	0.4
2	3a	64	42	3084	54	0.8	39	41	650	13	0.5
3	3b	62	54	953	31	0.6	42	40	558	31	0.3
4	3c	73	65	250	32	0.9	39	44	330	29	0.7
5	3c	60	53	417	21	0.2	33	32	418	20	0.4
6	3c	55	47	503	28	0.3	40	38	304	22	0.5
7	3c	138	154	375	43	0.7	26	37	104	29	0.3

ALT : Alanine aminotransferase (normal : 5-45 U/L), AST : Aspartate aminotransferase (normal : 1-9 years : 15-55 U/L, 10-19 years : 5-45 U/L), ALP : Alkaline phosphatase (Normal : 1-9 years :145-420 U/L, 10-11 years : 130-506 U/L), GGT : -glutamyl transpeptidase (Normal : <10 years : 5-32 U/L, >10 years : 5-24 U/L), T.bil : Total bilirubin (Normal : 0.2-1 mg/dL) (16).

es are uncommon but, when present, are usually due to bone metabolism abnormalities (18). Our two patients (4 and 8 years-old) with rickets due to fat, vitamin malabsorption and malnutrition had elevated ALP levels. Their ALP levels were decreased to near normal values after treatment (Patients 2 and 3, table II). Serum bilirubin elevation is also exceedingly rare, and should prompt the search for other possible diagnoses (18). In our series, high serum bilirubin level wasn't found in any patients.

The pathogenesis of the hypertransaminasemia and liver damage in celiac disease is still unknown. Various pathogenic mechanisms have been noticed. It has been suggested that an increased intestinal permeability to toxins or antigens caused by mucosal inflammation and damage could be the cause of liver injury (8, 18). In previous studies showed that liver damage occurred not only in celiac disease, but also in patients with other pathologies such as cow milk protein intolerance and ulcerative colitis (7,19). Alternatively, these toxic substances may initiate an immunologic reaction toward liver antigens (8). In our study, two of our patients with elevated aminotransferases and celiac disease had no diarrhoea complaint, and villous area, crypt height and mitotic count were not significantly different between two groups, suggesting that malabsorption is not the only etiologic factor of celiac disease-related liver involvement. In a study by Farre et al (6), paediatric celiac disease patients with elevated aminotransferase levels were found younger than the patients within normal liver functions (2 and 5 years-old, respectively), but the authors bound this correlation to different factors that have not yet been identified. In the same study, the ratio of elevated ALT levels in typical celiac disease patients was found similar with the group of atypical (short stature, anaemia, alterations of liver functions) celiac patients (6). In another study, gender, age, main complaints and severity of intestinal lesions have been found to be similar in adult celiac patients with and without liver dysfunctions (4). Similarly, in our study, there were no significant differences between the two groups in terms of main complaints, age and severity of intestinal damage.

Liver dysfunction may be a gluten-dependent immunologically induced extraintestinal manifestation of celiac disease (20). Autoimmune diseases with celiac

disease are improved with gluten-free diet. Similarly, since the liver pathologies are improved with gluten-free diet, it may be hypothesized that ingestion of gluten can sometimes even result in liver damage in patients with celiac disease.

Malnutrition is a frequent finding of celiac disease, and only malnutrition itself of any origin can be cause of hepatic damage (9,21). In our study, height for age and weight for height percentages of patients were not significantly different between ALT abnormal and normal groups. These findings suggest that, it is difficult to attribute that malnutrition is the sole etiological factor of hepatic involvement. Similarly, the studies consisted of adult and paediatric celiac patients, body mass indexes were found similar in elevated and normal liver functions groups (4,6).

In previous studies on pathogenesis of liver abnormalities in celiac disease, severities of intestinal lesions were evaluated with histopathologic examinations and lactulose absorption tests (4-7). At diagnosis, reduction of villous area and increased mitotic count and crypt height were shown in celiac patients compared with control group, in a study using morphometric analyses (22,23). These parameters are improved with gluten-free diet. In our study, severity of intestinal damage was examined with both Marsh classification and morphometric analyses. Morphometric analyses were not different between the two groups.

In all of our patients, serum aminotransferase values returned to normal after 7.4 ± 2.7 months of a gluten-free diet. Abnormal liver functions associated with celiac disease are normalized in 3-12 months after gluten withdrawal (6,18,21). Moreover, progression of liver damage can be prevented with strict gluten-free diet and time of liver transplantation can be delayed (20,24).

In conclusion, abnormalities of liver functions are frequently seen in paediatric celiac disease patients. But the mechanism responsible for persistent hypertransaminasemia in celiac disease is still unknown. Degree of malnutrition and severity of intestinal damage are not the only important etiologic factors of hepatic abnormalities. Further studies related to pathogenesis are required.

References

1. HAGANDER B., BERG N.O., BRANDT L., NORDÉN Å., SJÖLUND K., STENSTAM M. Hepatic injury in adult coeliac disease. *Lancet*, 1977, **1** : 270-272.
2. SØRENSEN H.T., THULSTRUP A.M., BLOMQUIST P., NORGAARD B., FONAGER K., EKBOM A. Risk of primary biliary liver cirrhosis in patients with coeliac disease : Danish and Swedish cohort data. *Gut*, 1999, **44** : 736-738.
3. ARVOLA T., MUSTALAHTI K., SAHA M.T., VEHMANEN P., PARTANEN J., ASHORN M. Celiac disease, thyrotoxicosis, and autoimmune hepatitis in a child. *J Pediatr Gastroenterol Nutr*, 2002, **35** : 90-92.
4. BARDELLA M.T., FRAQUELLI M., QUATRINI M., MOLTENI N., BIANCHI P., CONTE D. Prevalence of hypertransaminasemia in adult celiac patients and effect of gluten-free diet. *Hepatology*, 1995, **22** : 833-836.
5. NOVACEK G., MIEHSLER W., WRBA F., FERENCI P., PENNER E., VOGELSANG H. Prevalence and clinical importance of hypertransaminasaemia in coeliac disease. *Eur J Gastroenterol Hepatol*, 1999, **11** : 283-288.
6. FARRE C., ESTEVE M., CURCOY A., CABRE E., ARRANZ E., AMAT L.L., GARCIA-TORNEL S. Hypertransaminasemia in pediatric celiac disease patients and its prevalence as a diagnostic clue. *Am J Gastroenterol*, 2002, **97** : 3176-3181.
7. LINDBERG T., BERG N.O., BORULF S., JACOBSON I. Liver damage in coeliac disease or other food intolerance in childhood. *Lancet*, 1978, **1** : 390-391.
8. MAGGIORE G., CAPRAI S. The liver in celiac disease. *J Pediatr Gastroenterol Nutr*, 2003, **37** : 117-119.
9. KUMARI R., RAO Y.N., TALUKDAR B., AGARWAL S., PURI R.K. Serum enzyme abnormalities in protein energy malnutrition. *Indian Pediatr*, 1993, **30** : 469-473 (Abstract).
10. STRETTEL M.D.J., DONALDSON P.T., THOMPSON L.J., SANTRACH P.J., MOORE S.B., CJAZA A.J., WILLIAMS R. Allelic basis for HLA-encoded susceptibility to type 1 autoimmune hepatitis. *Gastroenterology*, 1997, **112** : 2028-2035.
11. VOLTA U., DE FRANCESCHI L., MOLINARO N., CASSANI F., MURATORI L., LENZI M, BIANCHI F.B., CJAZA A.J. Frequency and significance of anti-gliadin and anti-endomysial antibodies in autoimmune hepatitis. *Dig Dis Sci*, 1998, **43** : 2190-2195.
12. WALKER SMITH J.A., GUANDALINI S., SCHMITZ J., SHMERLING D.H., VISAKORPI J.K. Revised criteria for diagnosis of coeliac disease. Arch Dis Child, 1990, 65 : 909-911.
13. WATERLOW J.C. Evolution of kwashiorkor and marasmus. *Lancet*, 1974, **2** : 712.
14. WATERLOW J.C. Classification and definition of protein-calorie malnutrition. *BMJ*, 1972, **3** : 566.
15. WOLF A., LAVINE J. Hepatomegaly in neonates and children. *Pediatr Rev*, 2000, **21** : 303-310.
16. NICHOLSON J.F., PESCE M.A. Reference ranges for laboratory tests and procedures. In : Behrman R.E., Kliegman R.M., Jenson H.B. (Eds). Textbook of Pediatrics (16th ed), W.B. Saunders Company, Philadelphia, 2000 : 2181-2258.
17. MARSH M.N. Gluten, major histocompatibility complex, and the small intestine : A molecular and immunobiologic approach to the spectrum of gluten sensitivity ("celiac sprue"). *Gastroenterology*, 1992, **102** : 330-354.
18. GONZÁLEZ-ABRALDES J., SÁNCHEZ-FUEYO A., BESSA X., MOITINHO E., FEU F., MAS A., ESCORSELL A., BRUGUERA M. Persistent hypertransaminasemia as the presenting feature of celiac disease. *Am J Gastroenterol*, 1999, **94** : 1095-1097.
19. BROOME U., GLAUMANN H., HELLERS G., NILSSON B., SORSTAD J., HULTCRANTZ R. Liver disease in ulcerative colitis : an epidemiological and follow up study in the county of Stockholm. *Gut*, 1994, **35** : 84-89.
20. KAUKINEN K., HAMLE L., COLLIN P., FÄRKKILÄ M., MÄKI M., VEHMANEN P., PARTANEN J., HÖCKERSTEDT K. Celiac disease in patients with severe liver disease : Gluten-free diet may reverse hepatic failure. *Gastroenterology*, 2002, **122** : 881-888.
21. DAVISON S. Coeliac disease and liver dysfunction. *Arch Dis Child*, 2002, **87** : 293-296.
22. CUMMINS A.G., THOMPSON F.M., BUTLER R.N., CASSIDY J.C., GILLIS D., LORENZETTI M., SOUTHCOTT E.K., WILSON P.C. Improvement in intestinal permeability precedes morphometric recovery of the small intestine in coeliac disease. *Clin Sci*, 2001, **100** : 379-386.
23. ENSARI A., MARSH M.N., LOFT D.E., MORGAN S., MORIARTY K. Morphometric analysis of intestinal mucosa. V. Quantitative histological and immunocytochemical studies of rectal mucosae in gluten sensitivity. *Gut*, 1993, **34** : 1225-1229.
24. NEUBERGER J. PBC and the gut : the villi atrophy, the plot thickens. *Gut*, 1999, **44** : 594-595.