Lactase, sucrase-isomaltase, and carbamoyl phosphate synthase I expression in human intestine
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Chapter 5

Pediatric Duodenal Biopsies: Mucosal Morphology and Glycohydrolase Expression do not Change Along the Duodenum

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Chapter 5

Epidermal-Derivative Biology: Mucosal Morphology and Chylophagocytes Expression do not Change Along the Duodenum
Pediatric Duodenal Biopsies: Mucosal Morphology and Glycohydrolase Expression do not Change Along the Duodenum

Abstract

Duodenal mucosal biopsies are routinely taken for diagnosis in children with complaints of the upper gastrointestinal tract. Surprisingly, little is known about the usefulness of proximal duodenal versus distal duodenal biopsies for routine diagnostic purposes. This study evaluated the comparability of proximal and distal duodenal biopsies, with respect to mucosal morphology as well as glycohydrolase expression as indicator of intestinal epithelial function. Methods: Specimens obtained in duodenal endoscopic biopsies from 64 children, ranging in age from three months to eighteen years with normal or affected mucosa, were studied. Biopsies were performed in anatomically defined regions in the bulbus duodeni (the very proximal part of the duodenum) and distally of the papilla of Vater (distal of the pancreatic duct). Biopsy specimens either were paraformaldehyde-fixed for histological examination and immunohistochemical evaluation or were homogenized to isolate RNA. Crypt/villus morphology was assessed as is routinely determined by pathologists. In addition, several aspects of lactase and sucrase-isomaltase expression as paradigms of intestinal brush-border enzymes were assessed: localization at the cellular level, semi-quantitative immunohistochemistry, and quantitative measurement of the mRNA levels of the respective brush-border glycohydrolases. Results: As anticipated, there was a wide interpatient variation in mucosal morphology and expression of lactase and sucrase-isomaltase. Nonetheless, the consistent finding was that in each patient, measurements of morphology, lactase, and sucrase-isomaltase gene expression were very similar between samples observed in the proximal and distal biopsies. Conclusion: Biopsies in either location in the duodenum are equally suitable for diagnostic workup of patients suspected of mucosal abnormalities affecting morphology or small intestinal brush-border glycohydrolase activities.

Introduction

The intestinal mucosa is made up by a single-layer epithelium consisting of various specialized cell types and underlying connective tissue. Many common and widespread diseases affect the morphology and the functions of the intestinal mucosa. Since this mucosa is pivotal in the digestion and absorption of nutrients, gastrointestinal endoscopy is widely valued for its diagnostic merit in gastrointestinal disease.

Diagnosis of upper gastrointestinal disease is generally based on a combination of clinical observations, laboratory tests and in particular: endoscopic findings. In children, endoscopic examination has become a valuable, and often indispensable, procedure in the diagnosis of upper gastrointestinal disease (Black et
Mucosal biopsies taken during endoscopic sessions provide reliable information about the severity of gastrointestinal disease (Black et al., 1988; Dandalides et al., 1989). Over the years, duodenal biopsies have widely replaced jejunal biopsies in the diagnosis of upper gastrointestinal disease (Dandalides et al., 1989, Newcomer and McGill, 1966 1967; Saverymuttu et al., 1991; Oderda et al., 1993; Smith et al., 1989; Schmitz-Moorman et al., 1984; Forget et al., 1985). The major advantage of performing duodenal biopsies guided by endoscope, over performing jejunal biopsies with the Crosby capsule is that the procedure is less distressing to the patients. Jejunal biopsies often require X-ray survey or a capsule mounted on a gastroscope, considerably increasing both the risk of tissue damage and the time required to perform the procedure. Moreover, jejunal biopsies are taken without visual contact with the tissue, whereas duodenoscopy enables visual survey of the mucosa before and after the taking of the biopsy specimen.

There is surprisingly little literature describing differences along the cephalo caudal axis of the small intestine as a determining factor in the outcome of the diagnosis obtained via intestinal biopsy. Duodenal biopsies seem to be as suitable for diagnosis as jejunal biopsies (Dandalides et al., 1989, Newcomer and McGill, 1966 1967; Saverymuttu et al., 1991; Oderda et al., 1993; Smith et al., 1989; Schmitz-Moorman et al., 1984). However, along the duodenum, the effect of the location where the biopsy is taken on the outcome of the diagnosis is uncertain. Especially in children, in whom the intestinal mucosa is particularly important for growth and development, the data on this potentially important issue are very scarce.

Here, we describe the morphology of the duodenal mucosa and the expression of the two most important small intestinal brush-border glycohydrolases, lactase and sucrase-isomaltase (SI) in pediatric duodenal biopsies. We studied 64 children from various ethnic origins, ranging in age from three months to eighteen years with normal or affected duodenal mucosae. The object was to compare expression patterns of lactase and SI, along with study of mucosal morphology, in the proximal and distal duodenal mucosae of children, to compare the usefulness of specimens from the two locations for diagnostic workup.

Materials and methods

Patients and ethical considerations

A prospective study was done on biopsy specimens obtained with endoscopic forceps from the duodenal mucosae of 64 pediatric patients with upper gastrointestinal symptoms. Indications for endoscopic examination included intractable diarrhea, suspected celiac disease, Giardia lamblia or Helicobacter pylori infection, Crohn's disease, chronic abdominal pain, chronic diarrhea, failure to thrive, or oesophageal reflux. Race included 49 white, 4 Maghrebian, 4 black African, 3 Asian, 2 Turkish, and 2 Surinams. All patients were analyzed as one group. Each patient underwent gastroduodenal endoscopy and duodenal biopsy for diagnostic purposes. We obtained permission to perform four extra biopsies per patient, the number ethically considered
Pediatric Duodenal Biopsies: Mucosal Morphology and Glycohydrolase Expression

the maximum for this study. Informed consent was gained from the patients and their parents for the performance of the biopsies, and the study was conducted with the permission of the medical ethical committee of our institution.

Tissue

Mucosal specimens were obtained in biopsies performed on the proximal and distal duodenum: two in the bulbus duodeni and two distal to the papilla of Vater. In this study, the proximal and distal biopsies from one patient were designated 'paired-specimens'. Figure 1 depicts the locations of the specimens obtained, henceforth referred to as proximal and distal. Proximal and distal biopsies were made during one session and the specimens were immediately fixed in 4% (wt/vol) paraformaldehyde in PBS, embedded in paraffin, sectioned and used in immunohistochemistry or immediately homogenized to isolate RNA. Because we were able to obtain only one specimen for quantitative, biochemical measurements, we chose to quantify mRNA levels rather than enzyme or protein levels. This allowed us to measure gene expression very sensitively, and to probe identical RNA samples sequentially with the different cDNA probes representing the various gene products of interest (lactase, SI, GAPDH, and CPS, as described below under 'messenger RNA quantification'). The biopsies taken for diagnostic purposes, which were reviewed by the pathologist as part of normal diagnostic workup, were taken at the distal site in the duodenum and were used as reference for the morphologic scoring of the distal specimens in this study.

Figure 1. Schematic drawing of the human upper gastrointestinal tract indicating the locations of the biopsy sites. In the text these are referred to as proximal (P) or distal (D) biopsy.

Antibodies

We used ascites fluid containing antihuman lactase monoclonal antibody HBB 1/90/34/74 and two ascites fluids containing antihuman SI monoclonal antibodies (HBB 2/614/88 and HBB 2/219/20), respectively. Results obtained by either antihuman SI antibody were indistinguishable. These antibodies have been described to recognize the enzyme precursors as well as the mature forms of these brush-border enzymes (Hauri et al., 1985). Three independent antihuman lactase
monoclonal antibodies prepared from the hybridoma supernatant (mLacl, mLac4, and mLac5) were used in some experiments (Maiuri et al., 1991). Furthermore, we used ascites containing antirat lactase monoclonal antibody (Quaroni and Isselbacher, 1985) as a negative control in immunohistochemical studies.

**Morphology**

The morphology of the duodenal mucosa, in particular the crypt/villus ratio, was assessed at least twice by independent and blinded observers for each specimen obtained, on at least four villi and crypts, using light microscopy at low magnification (100x). We assigned scores as follows: 0, for total villus atrophy; 1, for moderate villus atrophy (villus/crypt ratio < 1); 2, for mild villus atrophy; 3, for normal morphology, according to established criteria (Mercer et al., 1990).

**Immunohistochemical analysis**

Seven-micrometer thick sections from formaldehyde-fixed, and paraffin-embedded specimens were deparaffinated, incubated for 30 min in 3% H2O2, in PBS to inactivate endogenous peroxidase activity, and for 30 min in a solution of 10 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, 0.25% gelatin, 0.05% Tween-20, to prevent background staining. The sections were incubated subsequently overnight with adequate dilutions in PBS of the primary antibodies, 1.5 h with rabbit-anti-mouse serum (1:7500, Dako, Glostrup, Denmark), 1.5 h with goat-anti-rabbit serum (1:1000, Dako) and with rabbit peroxidase-anti-peroxidase (1:1000, Dako). All incubations were done at room temperature. Anti-lactase (HBB1/90/34/74) and anti-SI (and HBB2/219/88) monoclonal antibodies were diluted 1:1000 from ascites (Hauri et al., 1985). Further details were as described earlier (Van Beers et al., 1995). Absence or presence of immunohistochemical staining was determined using light microscopy at high magnification (400x). We have discriminated four localizations of immunohistochemical staining; 1) intracellular or 2) brush-border staining of crypt enterocytes and 3) intracellular or 4) brush-border staining of villus enterocytes. Furthermore, we distinguished four semi-quantitative classes of staining intensity: 0, absence of staining; 1, very weak, patchy staining; 2, evenly distributed, moderate staining; and 3, evenly distributed dark staining. Semi-quantitative measurements of staining intensities was only assessed in specimens that had been immunostained at least in triplicate in independent experiments. All sections were judged at least twice by two independent and blinded observers.

**Cloning of a partial human CPS complementary DNA**

Using the reverse transcriptase-polymerase chain reaction technique we have amplified 1394 nt of the 3' end of the human CPSI gene (Haraguchi et al., 1991). Polymerase chain reaction primers were designed as indicated hereafter; Forward: 3' AATTTGTTGAAGGGGCC 5', Reverse: 5' GGAATTCTGCCCTGTTAAAGTGTCC 3'. Reverse transcriptase-polymerase chain reaction was done in a final volume of 20 μl containing 1x reverse transcription-buffer (Boehringer Mannheim) 4 mM of dATP, dCTP, dTTP and dGTP each, 50 nM of each primer, 2 U RNasin (Promega), 2 U
reverse transcriptase M-MuLV (Boehringer Mannheim), and 1 µg of human jejunal RNA isolated essentially as described by Chirgwin et al. (1979). Amplification consisted of 30 consecutive rounds of 1 min 94°C, 1 min 55°C, 2.5 min 72°C and finally 7 min at 72°C. The resulting polymerase chain reaction product was digested with Apal and EcoRI restriction enzymes and ligated into pBluescript (Stratagene). Nucleotide sequencing confirmed the identity of the CPS cDNA fragment.

**Messenger RNA quantification**

RNA was isolated from individual biopsy specimens using the RNeasy protocol (Qiagen, Chatsworth, CA). mRNA quantification was performed on spot blots. In short, between 100 and 500 ng of RNA was spotted onto negatively charged Qiabranse nitrocellulose (Qiagen) using a vacuum-operated BioRad 96 wells spot blot system (BioRad, Richmond, CA). The amount of messenger RNA isolated from each specimen was quantified using specific DNA probes synthesized using random hexamer primers, incorporating α-32P-dATP to at least 1x10^8 cpm/µg (Sambrook et al., 1989). Lactase probes were synthesized from the 6.3-kb full-length human lactase cDNA (Nairn et al., 1991), SI probes from 428 bp of a human SI clone (Van Beers et al., 1995), probes transcribed from 1394 bp of a CPS cDNA clone synthesized from human jejunal RNA (as described earlier), and GAPDH from 1 kb of human GAPDH cDNA (Tokunaga et al., 1987). RNA from each biopsy was blotted onto Qiabranse filters, dried and baked at 80°C for 2 h. Prehybridization was performed at 65°C for 1 h in (7% sodium dodecyl sulphate (SDS), 0.1 mM EDTA, 0.5 M Na2HPO4 pH 7.2). Hybridization was performed for 17 h at 65°C. Filters were washed at 65°C twice 20 min in 6xSSC, 0.1% SDS, once 20 min in 3xSSC, 0.1% SDS and once 20 min in 0.1xSSC, 0.1% SDS. Autoradiographs were prepared using PhosphorImage screens (Molecular Dynamics), that were exposed to hybridized membranes for 48 hours and quantified in a PhosphorImager using ImageQuant software (Molecular Dynamics). Membranes were stripped of adherent probe by washing at 80°C in 0.1% SDS for several hours before hybridization with each probe. Stripped blots were checked for absence of radioactivity by exposing PhosphorImager screens for 48 h. Sequentially, all the blotted RNA samples have been incubated with probes for SI mRNA, lactase mRNA, CPS mRNA, and GAPDH mRNA. Signals detected for lactase, SI, and CPS were then normalized to the housekeeping gene Gapdh.

**Results**

**Patients**

The 64 patients that we have studied ranged in age from 3 months to 18 years old (mean 7 years old, 35 boys). Diagnoses of these patients were obtained at the end of medical workup from their medical records and included esophageal reflux, *H. pylori* infection of the stomach, celiac disease, *Giardia lamblia* infestation of the small intestine, intractable diarrhea, Morbus Crohn, but also a nonclassified immunodeficiency in one child. The number of specimens per patient was restricted...
for this study for ethical reasons: Four duodenal biopsy specimens were obtained during one endoscopic session.

Duodenal mucosal morphology and variation in lactase and SI immunostaining

Two strikingly different staining phenotypes of immunodetection for lactase and SI representative of our patient population are shown in figure 2. It shows two patients that have similar amounts of immunodetectable lactase or SI in the brush-borders of their enterocytes, but additional intracellular localization of both lactase and SI was detected only in one patient. Intracellular staining, if detected, was always observed in biopsies from both sites of the duodenum and always for both the glycohydrolases simultaneously. Moreover, intracellular staining was found in all cells expressing lactase and SI on their brush-borders, in villus as well as in crypt enterocytes, whereas staining was never detected in the other cell types: goblet, enteroendocrine, or Paneth's cells. Results obtained using two distinct anti-human SI mAbs were indistinguishable. A control experiment with similarly, or less, diluted ascites obtained from a sibling mouse, containing an unrelated monoclonal antibody (Quaroni and Isselbacher, 1985), failed to stain adjacent sections (results not shown); indicating that the intracellular staining indeed represented lactase and SI. Three independent anti-human lactase monoclonal antibodies, mLac1, mLac4, and mLac5, showed no intracellular staining in any of the specimens, and lactase staining was restricted to the brush-border (not shown).

Most of the tissue samples showed that specimens obtained in proximal and distal biopsies in the same patients were very comparable in morphology and in lactase and SI phenotype. Therefore, we performed quantitative and semi-quantitative studies to judge whether specimens from proximal and distal biopsies were equally suitable for diagnostic purposes, especially for the assessment of mucosal morphology and the abundance and localization of intestinal glycohydrolases.

Quantification of lactase, sucrase-isomaltase, and CPSI mRNA

Generally, there are clear-cut correlations between the levels of the brush-border glycohydrolase activities, and their respective mRNA and protein levels (Van Beers et al., 1985). Because the genes of these glycohydrolases are considered to be primarily regulated at the transcriptional level, the mRNA levels of these enzymes are good measures of the amounts of the enzymes. Therefore, we determined the levels of lactase and SI mRNAs as isolated from individual specimens. We found a strong correlation between amounts of lactase mRNA or SI mRNA detected in proximal biopsy specimens compared to levels detected in the accompanying distal specimens (figure 3). The correlation coefficient for lactase mRNA is 0.69 and for SI mRNA 0.66. The GAPDH mRNA level was used to standardize the lactase and SI mRNA levels. The GAPDH mRNA levels are thus used to correct for both the biopsy size and possible differences in cell type composition between the biopsies. Not shown here are the absolute amounts of Gapdh mRNA amounts isolated from proximal and distal specimens. However, comparison of GAPDH mRNA values themselves between 'paired-specimens', which were not corrected for size, correlated very well (r = 0.75),
strongly indicating that processing of proximal and distal specimens, and subsequent mRNA isolation and detection was essentially identical.

We then performed an analysis of a 'control' enterocyte-specific enzyme. We measured CPS mRNA in the 'paired-specimens' and found a very high correlation coefficient for CPS mRNA values of the 'paired-specimens' ($r = 0.80$, $p = 0.0001$).

**Figure 2.** Photomicrographs of immunohistochemistry of villus enterocytes using the anti-human lactase antibody HBB 1/90/34/74 and the anti-human SI antibody HBB 2/614/88 on adjacent 7 μm thick biopsy sections from two healthy individuals (morphology score 3). The photographs of the epithelium in the panels were taken at approximately mid-villus positions, and were representative for all lactase and SI positive cells within the epithelium of the biopsy sections. Panels a and b, lactase and SI staining respectively of patient no 5 and panels c and d lactase and SI staining of patient no 8. Intracellular staining for both lactase and SI was noted in patient no 8, indicated with arrows, but not in patient no 5. Original magnification 400x.

**Figure 3.** Quantification of lactase (A) and SI mRNA (B) isolated from paired proximal (x-axis) and distal (y-axis) duodenal biopsies ($n = 43$). All mRNA values for lactase and SI were normalized to the Gapdh mRNA level detected in the same biopsy. The correlation coefficient ($r$) for lactase mRNA as found in the 'paired-specimens' is $0.69$ ($p = 0.0001$), and for SI $r = 0.66$ ($p = 0.0001$).
Comparison of duodenal mucosal morphology of 'paired-specimens'

We assessed morphology and immunohistochemical results for lactase and SI, and we compared results from 'paired-specimens'. Results were expressed as percentages concordance (i.e. identical score for 'paired-specimens') and discordance (i.e. different score for 'paired-specimens').

Figure 4 shows comparisons of the morphology of two specimens from the same patient. All biopsy sections were scored in a double-blinded fashion, the sections were assigned a number only and were observed in random order. First we determined the degree of reproducibility for two duplicate distal specimens that were independently processed and their morphology assessed. Morphology scores were concordant in 54 of 64 cases (84%), whereas 6 of the discordant scores differed by only one morphology class and only 3 scores by more than one morphology class (figure 4A). We then examined the similarity between 'paired-specimens'. Proximal versus distal morphology scores were concordant in 53 of 64 cases (83%). In 9 cases of 64 (14%) the morphology differed by one class and in only 2 of 64 cases (3%) by more than one class (figure 4B). This indicates that the percentage of identical morphology scores between two (i.e. proximal and distal) specimens are as high as between duplicate specimens both taken at the distal location.

Figure 4. Comparative morphology of two duodenal biopsies from the same patient. A. Morphology of two closely adjacent, separately processed distal biopsies D1 and D2 (n = 64). Biopsy D1 was obtained for this study, while biopsy D2 was processed and reviewed by an independent pathologist during diagnostic workup. B. Morphology of paired proximal (P) and distal (D) biopsies from the same patient (n = 64)

Comparison of immunodetection of lactase and sucrase-isomaltase in 'paired-specimens'

Similarly, we compared immunohistochemical staining patterns and intensities between the 'paired-specimens'. First, we restricted scoring to positive or negative (figure 5), then we compiled semi-quantitative data (figure 6). Despite efforts to use reproducible methods of fixation, during the 1.5 year period in which specimens were collected, some biopsies appeared to immunostain irreproducibly or aberrantly. Therefore, it was occasionally difficult to reach a proper verdict on certain aspects of the expression of lactase and/or SI within some specimens. In such a case, the score of this patient was omitted in the analysis regarding this particular aspect, explaining why the number of patients per figure deviates from 64.
A. Intracellular lactase in crypt enterocytes

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B. Brush-border lactase on crypt enterocytes

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C. Intracellular lactase in villus enterocytes

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D. Brush-border lactase on villus enterocytes

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E. Intracellular SI in crypt enterocytes

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F. Brush-border SI on crypt enterocytes

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G. Intracellular SI in villus enterocytes

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H. Brush-border SI on villus enterocytes

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Figure 5. Lactase and SI immunohistochemistry in paired proximal and distal biopsies. (Proximal, P; Distal, D). Panels A-D show results for lactase immunohistochemistry and E-H contain results of SI. We specified 4 localizations; intracellular staining in crypt enterocytes (A and E), brush-border staining of crypt enterocytes (B and F), intracellular staining in villus enterocytes (C and G), and brush-border staining of villus enterocytes (D and H). Scores were restricted to positive (+) or negative (-).

Detection of intracellular lactase in crypt enterocytes was carried out on 60 patients in whom there were crypts in both of the 'paired-specimens' (figure 5A). This resulted in 60/60 (100%) concordant scores between 'paired-specimens' with 21 pairs positive and 39 pairs negative. In brush-borders of crypt enterocytes, 56/60 (93%) of the pairs were concordant, the majority were negative (52/60) (figure 5B). Detection of intracellular lactase in villus enterocytes appeared concordant in 60/61 (98%) of the pairs (figure 5C), and lactase presence in brush-border of villus enterocytes was concordant in 56/58 (97%) of all pairs (figure 5D).

Detection of intracellular SI in crypt enterocytes showed 57/60 (95%) concordant scores between 'paired-specimens' with 41 pairs negative and 16 pairs positive (figure 5E). In brush-borders of crypt enterocytes 56/60 (93%) of the pairs were concordant, of which 44 were positive (figure 5F). Detection of intracellular SI
in villus enterocytes appeared concordant in 60/61 (98%) of the pairs (figure 5G) and
detection on the brush-border of villus enterocytes was concordant in all 59/59 (100%)
pairs (figure 5H). The observed frequencies of the positive staining for lactase or SI
varied widely according to cellular location among the patients. However, for each of
the locations separately, the 'paired-specimens' were at least 93% concordant.

Figure 5 shows that the number of discordant scores for each of the cellular
locations is very low. Nevertheless, among these sparse discordant scores, there was a
slight bias towards positivity only in the distal biopsy for the detection of lactase and
SI (i.e. distal positive, proximal negative). This may reflect the existence of slight
quantitative gradients of gene expression for both enzymes increasing towards the
distal duodenum in some patients.

As noted earlier (figure 2), both the anti-lactase monoclonal antibody and the
two independent monoclonal antibodies directed against SI appeared to detect high
levels of intracellular staining in about one third of the patients (figure 5). Despite the
very strong correlation of the intracellular staining of lactase and SI, this staining did
not correlate with sex, age, ethnic background, clinical diagnosis, or morphology score
(not shown). Moreover, within one biopsy the intracellular staining for either lactase
or SI was nearly always found in crypt as well as in villus enterocytes. This explains
the high similarity of the results in figure 5A, 5C, 5E and 5G.

Although brush-border expression of lactase is normally detected on villus
enterocytes only, 7% (4/60) of the patients have detectable lactase in the brush-border
of crypts in both specimens (figure 5B). In contrast, presence of SI in the brush-border
of crypt enterocytes is the prevalent phenotype, since it occurs in approximately 80%
(44/60) of the patients (figure 5F).

Finally, we counted the overall similarities between 'paired-specimens'. This
analysis was done for 55 patients in which we were able to determine lactase and SI in
all locations according to the scores in figure 5A through 5H. The comparisons
between proximal and distal specimens showed that 43 patients were identical on all 8
criteria, 11 patients were identical on 7 criteria and 1 patient showed similarity only on
five out of eight criteria.

Semi-quantitative evaluation of immunohistochemical staining was performed
for staining in the brush-border of villus enterocytes. Semi-quantitative measurement
of lactase was concordant in 47 of 58 (81%) of all studied cases (figure 6A). There
were only 2 of 58 (3%) patients in which we assessed higher amounts of lactase in the
proximal biopsy than in the distal biopsy, whereas 9 of 58 (16%) patients had higher
lactase amounts in the distal specimen compared to the proximal specimen. Seven out
of these 9 differed by one class and only 2 by two classes. Semi-quantitative SI
measurement was concordant in 52 of 59 (88%) of all cases studied (figure 6B). There
were only 2 of 59 (3%) patients in which the proximal specimen had higher amounts
of SI, whereas 5 of 59 (8%) had higher amounts in the distal specimen. Four of these 5
patients differed by one class and 1 patient by two classes. This further shows that at
the semi-quantitative immunohistochemical level the findings for lactase and SI from
either specimen has a high predictive value for the other specimen of the pair.
Figure 6. Semi-quantitative immunohistochemical comparison of proximal and distal lactase and SI. Comparison between semi-quantitative immunohistochemical scores of proximal (P) and distal (D) lactase (panel a, n = 58) and SI (panel b, n = 59) expression in villus-associated enterocyte brush-border. The four categories (0, 1, 2, and 3) are defined in materials and methods.

Discussion

In the majority of the patients studied, there appeared to be no major difference between 'paired-specimens' in mucosal morphology, immunohistochemical quantities of lactase and SI, patterns of lactase and SI expression, and levels of lactase, SI, and CPS mRNAs. Because the composition of this prospective study population was determined only by order in which the patients came to the clinic, this implies that the population studied may be considered representative of childhood. Therefore, proximal and distal specimens seem to be equally informative about lactase and SI expression as well as mucosal morphology.

In evaluating the usefulness of proximal versus distal duodenal biopsies, it is important to determine if gradual differences exist along the longitudinal axis of this organ with respect to the criteria that were chosen. A very small number of patients exhibited discordant presence of lactase or SI, with prevalence for higher expression in the distal biopsy. The vast majority of the children in our study showed no gradient along the length of their duodenum. In our predominantly white study population, lactase was present in most children at the mRNA level and also by immunohistochemical staining. Importantly, the amounts of lactase detected by either technique were similar along their duodenum as measured in 'paired-specimens', implicating that no proximal-to-distal gradient of lactase expression exists in the duodenum during (at least) the first 18 years of human life. This was notably different from our recent findings in the rat, which showed that lactase expression was restricted after weaning to the central part of the jejunum, generating an expression gradient in the proximal small intestine (Rings et al., 1994). Similarly, SI was present in most children studied, and expression levels measured at mRNA or SI immunodetection level were not age-dependent. In that the levels of enzyme or SI mRNA were very comparable between 'paired-specimens', there is no indication that SI displays a proximal-to-distal expression gradient in the duodenum at any time during post-natal development.
The anti-human lactase monoclonal antibody HBB 1/90/34/74 has been used in a variety of studies and shown to recognize the endoplasmic reticulum-synthesized lactase precursor as well as the mature, brush-border enzyme forms of lactase (Hauri et al., 1985; Van Beers et al., 1995; Nairn et al., 1991). Both anti-human SI monoclonal antibodies used have been observed to recognize SI precursors and mature, brush-border SI (Hauri et al., 1985). Regarding the intracellular staining of both lactase and SI, the results indicate that there is genuine detection of the corresponding antigens for several reasons. First, intracellular staining is only detected in enterocytes and absent from goblet, enteroendocrine and Paneth's cells. Second, each of the three monoclonal antibodies is directed to a peptide epitope, because they recognize (in addition to the high-mannose and complexly glycosylated forms of the enzymes) the de-N-glycosylated precursor, as shown by immunoprecipitation (Hauri et al., 1985; Van Beers et al., 1995). This implicates that these antibodies have the potency to detect antigen at the level of the endoplasmic reticulum and Golgi apparatus and at the brush-border membrane. Third, an unrelated ascites, prepared in sibling mice containing monoclonal antibody against rat lactase (Quaroni and Isselbacher, 1985), which does not cross-react with human lactase (Van Beers; unpublished results), did not show any intracellular staining when used at identical or even at higher concentrations. Thus, we conclude that immunostaining with both anti-lactase and anti-SI antibodies within the enterocytes represents authentic lactase and SI. The cellular location of the staining in the enterocytes makes it most likely that these lactase and SI molecules are present within the Golgi apparatus.

It remains to be explained why intracellular staining is present in specimens from some but not other patients, although the antigen under investigation is present in the brush-border of the biopsies in comparable amounts. The explanation of the origin of the intracellular staining for both glycohydrolases is beyond the scope of this article. In fact, this phenomenon only helped us to discriminate additional aspects of the lactase and SI phenotypes among our study population.

Our findings on the similarities between proximal and distal duodenal biopsies were based on results from a large and diverse group of children of various ages. Because there were no inclusion criteria for our prospective study, we believe that the results are representative for children up to 18 years old. Therefore, we conclude that independent from racial origin, age or disease, specimens taken in the most proximal area of the duodenum, are equally suitable as specimens taken distally of the papilla of Vater in the diagnosis of upper gastrointestinal disease in children.

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