Diagnostic duodenal bulb biopsies in celiac disease

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Abstract

Objective: To evaluate duodenal bulb biopsies with respect to histological changes in the diagnosis of celiac disease as compared with biopsies from second part of duodenum.

Methods: Endoscopic biopsies were obtained from 44 patients (23 females and 21 males), suspected clinically as having celiac disease. Four biopsy samples were obtained from each duodenal bulb and second part. The histological changes were reported according to Marsh's classification.

Results: The histological changes were equally similar from biopsies of duodenal bulb as compared to second part in all except four patients.

Conclusion: Mucosal specimens taken from duodenal bulb and second Part of duodenum were strongly correlated histologically.

Introduction

Celiac disease (CD) is characterized by intestinal mucosal injury resulting from immunological damage upon exposure to gluten in persons genetically predisposed to this condition which demonstrate improvement with withdrawal of gluten from the diet. However, the availability of serologic testing for (CD) and the common use of upper endoscopy has greatly complicated the definition, since these tests have identified patients who appear to have the disease but have variable degree of histopathologic changes and / or symptoms. Thus, several categories of (CD) have emerged. Whether these phenotypes are clinically useful remains to be determined[1,2]. There is strong association of (CD) with human leucocytes antigen (HLA) class 11 molecules particularly HLA DQ_2 and HLA DQ_8**.** Because up to 30% of the healthy population also caries these HLA haplotypes, their presence is not diagnostic for (CD), although their absence particularly rules it out[3].

Testing for celiac disease should be considered in the following group of patients.

- Those with gastrointestinal symptoms including chronic diarrhea, malabsorption, weight loss, and abdominal distension.
- Individuals without other explanations for signs and symptoms such as persistent elevation in serum aminotransferases, short stature, delayed puberty, iron-deficiency anemia, recurrent fetal loss, and infertility.
- Those at high risk for celiac disease including patients with type I diabetes mellitus or other autoimmune endocrinopathies, first-and second degree relatives of individuals with celiac disease, patients with Turner, Down, or Williams syndromes.
- There are other conditions in which testing may also be considered such a patients with irritable bowel syndrome, persistent apthous stomatitis, autoimmune disease, peripheral neuropathy, cerebellar ataxia, and dental enamel hypoplasia.

The mainstay for establishing the diagnosis of celiac disease remains a small intestinal biopsy showing the typical celiac enteropathy followed by clinical (and, in selected cases, histologic) remission after treatment with the gluten free diet, however, its use may be limited by patient's aversion to undergo upper GI endoscopy especially in asymptomatic patients. Until the early 1980s, the diagnostic tools for celiac disease were rudimentary at best, being based on non-specific tests. In the past 2 decades, however, a number of serologic tests have been developed (including antigliadin [AGA], antiendomysium [EMA], and human anti-tissue transglutarninase [tTG] antibody assays) that now have a definitive role in the diagnostic process. Although the AGA antibody assay has recently lost favour due to its poor specificity, new evidence presented during Digestive Disease Week Meeting 2006 in USA seems to suggest that using novel antibody tests based on synthetic gliadin-related peptides may have a much better yield for the disease[4]. An intriguing report on a new noninvasive diagnostic algorithm based on the combination of 3 serologic tests (tTG, anti-actin antibodies, and serum zonulin levels) that yields a 100% positive predictive value in patients at risk for celiac disease was also presented during this meeting[5].

Zonulin , a human protein, which regulates the permeability of the intestine is at increased level during the acute phase of celiac disease , it opens the junctions between cells allowing gluten and other allergens to pass. Once these allergens get into the immune system , they are attacked by antibodies and thus characterize the early phase of celiac disease.[5]

Duodenal or jejunal biopsies are needed to establish the diagnosis of (CD). It is widely advocated that these biopsies be taken from the distal duodenum.

This study was carried out to compare between biopsies taken from the bulb to that of second part of duodenum with respect to histological outcome in patients with signs and/or symptoms suggestive of (CD).

Patients and Methods

A total of 44 patients, 23 females age ranged 11-60 mean age 27 and 21 males age ranged 16-62 mean age 27.7 with symptoms of chronic diarrhea, flatulence, weight loss, unexplained iron deficiency anaemia, short stature and diabetes mellitus type I were selected randomly for duodenal biopsies from those attending endoscopy unit.

Giardiasis was excluded as a cause for diarrhea, by stool examination. The study was conducted in Basrah Teaching Hospital from the period November 2003-November 2005.

From each patient, four biopsy samples were obtained from first (Bulb) and four from the second part of duodenum. Duodenal biopsies were considered adequate only if properly oriented and was classified by one pathologist (who was blind of clinical features of the patients) according to Marsh criteria. Table 1.Briefly, Marsh type 0 lesion indicates normal histology, Marsh type I is characterized by increased number of intra epithelial lymphocytes in an architecturally normal small bowel mucosa, Marsh type II presents crypt hyperplasia but normal villi , finally Marsh type III is characterized by increasing degree of villous atrophy. Marsh class I – III were considered as compatible with (CD).

Unfortunately, no serological markers were available at the time of study to support the diagnosis of celiac disease.

Using duodenal histology of the second part as the gold standard, we have calculated the sensitivity and specificity of duodenal histology of the first part.

Results:

Forty-four patients were included in the study. All duodenal biopsies were deemed by the pathologist to be adequate for the evaluation of the mucosa and the villi.

As regards to histological changes of the first part of the duodenum, 26 patients had mucosal changes compatible with (CD) [Marsh I: 6, Marsh II: 12, Marsh III: 8]. 16 had a normal duodenal histology and 2 had non-specific duodenitis, while changes of the second part, 25 patients had mucosal changes compatible with (CD) [Marsh I: 8, Marsh II: 10, Marsh III: 7]. 17 had normal duodenal biopsy and 2 had non-specific duodenitis Table 2.

The details of duodenal histological changes as regards to sex of patients is seen in table 3 and 4.It is noticed that the histological changes of stage III in female group were seen in 7 (30.4%) and 6 (26%) of patients in the first and second part of the duodenum respectively, while the histological changes of stage III were seen in only 1 (4.2%) male patient from each of the first and second part of duodenum.

The histological changes according to stages of Marsh criteria seen in duodenal bulb were equally similar to that of second part in almost all patients (90.9%) except in three females and one male patient, in whom the duodenal bulb changes were in advanced stage as compared to that of the second part Table 5. The histological lesions as detected in the duodenal bulb versus second part of duodenum is seen in Table 6.The sensitivity of the bulb as compared to a (gold standard) second part = 100%, while the specificity of the bulb = 94.7%

Table 1: *Marsh's classification of small-intestinal lesions

Stage 0	Preinfiltrative mucosa
Stage I	Increase in the number of intraepithelial lymphocytes (IELs) to more than 30 per 100 enterocytes.
Stage II	Crypt hyperplasia. In addition to the increased IELs, there is an increase in crypt depth without a reduction in villus height.
Stage III	Villous atrophy; A partial, B subtotal, C total. This is the classical celiac lesion.

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Table 2: duodenal histology in the studied patients according to Marsh classification

Histology	1st part No. / total (%)	2nd part No. / total (%)
Normal	16/44 (36.3%)	17/44 (38.6%)
Marsh I	6/44 (13.6%)	8/44 (18.1%)
Marsh II	12/44 (27.2%)	10/44 (22.7%)
Marsh III	8/44 (18.1%)	7/44 (15.9%)

Table 3: Histological changes of duodenal bulb in the studied groups according to Marsh classification

	Marsh's classification							
Sex	Stage 0	Stage I	Stage II	Stage III A	Stage III B	Stage III C	Duodenitis	total
Females	9	1	5	4	2	1	1	23
Males	7	5	7	1	0	0	1	21

Table 4 : Histological changes of the second part of duodenum in the studied groups

	Marsh's classification							
Sex	Stage 0	Stage I	Stage II	Stage III A	Stage III B	Stage III C	Duodenitis	total
Females	10	2	4	3	2	1	1	23
Males	7	6	6	1	0	0	1	21

Table 5: Histological changes of the bulb versus second part of duodenum in four patients

Sex / No	Histological changes	
	Duodenal bulb	Second part
Female	Stage II	Stage I
	Stage II	Stage 0
3	Stage III A	Stage II
Male / 1	Stage II	Stage I

Table 6 : histological lesions as detected in the duodenal bulb versus second part of the duodenum

	Second part		
The Bulb	**positive**	**negative**	**Total**
positive	**25**	**1**	**26**
negative	**0**	**18**	**18**
Total	**25**	**19**	**44**

Sensitivity of the bulb = 100%

Specificity of the bulb = 94.7%

Discussion

In spite of the introduction of sensitive and specific serological testing, the diagnosis of celiac disease (CD) is still based on the recognition of characteristic histological changes on duodenal biopsies followed by clinical and histological improvement on a gluten free diet, however, the major draw backs are the need to perform upper GI endoscopy, to which there may an aversion especially by asymptomatic patients, the difficulty of obtaining adequate and properly oriented tissue samples and occurrence of patchy mucosal that can be missed by the biopsy. In recent years it has turned out that the development of (CD) lesion in small bowel is a dynamic process, which may present in various histologic forms. At one end the spectrum is a mucosa with normal architecture and an increase in Intra Epithelial Lymphocytes (IELS), at the other end is the classical flat mucosa.

Histological features supporting the diagnosis of (CD) are architectural changes of villi / and or crypts, an increase in Lamina propria cell density, and an increase in (IELs) counts. Exact histological findings are required for diagnostic purposes and for monitoring of (CD) patients. This has become possible by using Marsh classification[6].

Almost all of the studied patients with suspected celiac disease showed similar mucosal changes in biopsies, taken from either duodenal bulb or second part of duodenum (90.9%) except in four patients. This was consistent with very few papers published on this issue[7]. Other studies have also concluded no difference in biopsy specimen quality from different locations of the duodenum in respect to mucosal architecture in celiac disease[8].

A number of 25(IELs)/100 epithelia cells (mean + 2SD) is taken as the upper limit of the normal range for duodenal mucosa. An increase in (IELs) is mandatory for the histological diagnosis of celiac disease. Published-work continue to cite an upper limit of 30 or more lymphcytes/100 epithelial cells as an increased number which is mandatory for histological staging of (CD)[9]. In

this study a number of 30 and more lymphocytes was considered as an abnormal. 6(13.6%) of the studied patient where in stage 1, whose mucosal changes showed only Lymphocytosis with normal villous architecture, and since intraepithelial Lymophocytosis in an otherwise normal biopsies is somewhat non specific, but in nearly 10% of cases can be the initial presentation of (CD). Therefore these patients need to be monitored and investigated for (CD)[10].

Endoscopic features of (CD) include scalloping of folds, reduced or absent duodenal folds, mucosal fissures or grooves and mosaic appearance to the mucosa. However, the signs cannot be relied upon for the detection of (CD) because they are neither sensitive nor specific[11-18]. In this study, the only endoscopic signs, absent duodenal and scalloped folds were seen in three patients, all were females and all showed stage III B histological features.

The mucosal lesion in (CD) is patchy. Magliocca FM, et al in their study reported the presence of total villous atrophy in one biopsy while other duodenal sample taken from different, duodenal portions were normal or showed mild lymphocytes and plasma cell infiltration of lamina propria[19]. In this study, we have obtained four biopsy samples from each first and second part of duodenum in order to identify patchy mucosal atrophy and therefore a correct diagnosis in (CD).

In this study, the sensitivity and specificity of duodenal bulb biopsies were 100% and 94.7 respectively. This means that duodenal bulb biopsies are reliable for the diagnosis of histological features of celiac disease.

In conclusion, this study has demonstrated that mucosal specimens taken from duodenal bulb and second part are strongly correlated histologically, therefore, we suggest that in the diagnosis and follow up of celiac disease, mucosal specimens may be taken from the duodenal bulb especially for those patients in whom mucosal samples from the second part of the duodenum would be difficult.

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الخزعات التشخيصية لبصلة الاثنى عشري في الداء البطني

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ملخص البحث :

هدف الدراسة: تقييم خزعات بصلة الاثني عشري بما يخص التغيرات النسيجية في تشخيص الداء البطني مقارنةً بالخزعات من الجزء الثاني منه.

طريقة الدراسة: تم الحصول على خزعات بواسطة المنظار الداخلي من 44 مريضاً (23 مريضاً من الإناث و 21 من الذكور) يُشَك سريرياً بإصابتهم بالداء البطني, وأخذت اربع خزعات من كل من بصلة الاثنى عشري والجزء الثاني منه.ووصفت التغيرات النسيجية بحسب تصنيف مارش.

النتائج: كانت التغيرات النسيجية متشابهة في الخزعات المحصلة من بصلة الاثنى مقارنةً بالجزء الثاني منه في جميع المرضى عدا اربعة منهم.

الخلاصة: إن النماذج المخاطية المأخوذة من بصلة الاثنى عشري ومن الجزء الثاني منه مرتبطة إرتباطاً وثيقاً من الناحية النسيجية.