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Original Article

Duodenal villous morphology assessed using magnification narrow band imaging correlates well with histology in patients with suspected malabsorption syndrome

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Background and Aim: Narrow band imaging with magnification enables detailed assessment of duodenal villi and may be useful in predicting the presence of villous atrophy or normal villi. We aimed to assess the morphology of duodenal villi using magnification narrow band imaging and correlate it with histology findings in patients with clinically suspected malabsorption syndrome.

Methods: Patients with clinical suspicion of malabsorption presenting at a tertiary care center were prospectively recruited in this diagnostic intervention study. Patients underwent upper gastrointestinal endoscopy using magnification narrow band imaging. The villous morphology in the second part of the duodenum was assessed independently by two endoscopists and the presence of normal or atrophic villi was recorded. Biopsy specimen was obtained from the same area and was examined by two pathologists together. The sensitivity and specificity of magnification narrow band imaging in detecting the presence of duodenal villous atrophy was calculated and compared to the histology.

Results: One hundred patients with clinically suspected malabsorption were included in this study. Sixteen patients had histologically confirmed villous atrophy. The sensitivity and specificity of narrow band imaging in predicting villous atrophy was 87.5% and 95.2%, respectively, for one endoscopist. The corresponding figures for the second endoscopist were 81.3% and 92.9%, respectively. The interobserver agreement was very good with a kappa value of 0.87.

Conclusion: Magnification narrow band imaging performed very well in predicting duodenal villous morphology. This may help in carrying out targeted biopsies and avoiding unnecessary biopsies in patients with suspected malabsorption.

Key words: duodenum, histology, malabsorption syndrome, morphology, narrow band imaging

INTRODUCTION

DUODENAL MUCOSAL LESIONS are a frequent cause of malabsorption and, in the majority of these patients, conventional white light endoscopy (WLE) does not show any mucosal abnormality.[1] Hence, biopsy of abnormal-appearing areas on WLE would miss the diagnosis in many patients. Advances in endoscopic technology have facilitated visualization of the mucosal surface in greater detail and enabled the detection of lesions not apparent on conventional WLE.[2] Magnification narrow band imaging (NBI) endoscopy allows clear visualization of duodenal villi and assessment of their morphology.[3] This technique helps identify villous atrophy prior to biopsy as well as recognize patchy lesions that may help obtain a targeted biopsy, thereby increasing the diagnostic yield of the biopsy specimen.[1,4,5]

The current practice of obtaining random duodenal mucosal biopsies from normal-appearing areas in all patients with suspected malabsorption is not optimal as the histology may be normal in a large number of patients which adds to the cost of treatment. NBI, by virtue of being able to identify normal microsurface and microvascularity may enable us to avoid unnecessary biopsies from normal-appearing areas.[3,6] We aimed to assess the sensitivity and specificity of NBI compared to histology in the diagnostic evaluation of patients suspected to have disorders causing malabsorption.

METHODS

PATIENTS WITH CLINICAL suspicion of malabsorption planned for upper gastrointestinal endoscopy (UGIE) along with duodenal biopsy were prospectively

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recruited for the present study at our hospital between 2009 and 2012 after obtaining informed consent. Malabsorption was suspected in patients who had any or a combination of these symptoms: (i) chronic diarrhea; (ii) anemia; (iii) weight loss; and (iv) positive celiac serology. These selection criteria were kept quite broad to include most patients with suspicion of malabsorption syndrome in a clinical setting. Exclusion criteria included patients <15 years of age or those with an already established cause of malabsorption or presence of bleeding diathesis that precluded duodenal biopsy or failure to obtain informed consent for participation in the study. The study was approved by the institute review board and ethics committee.

UGIE was carried out using the NBI magnification endoscope manufactured by Olympus (EVIS EXERA, GIF H-180) which has a digital zoom facility to magnify images to at least 50-fold. NBI imaging uses two wavelengths of light and this is achieved by placing an optical filter at the light source which allows these two wavelengths of light to pass through. The images obtained were displayed on a high-definition television monitor. Any debris, food particles or mucus seen on the mucosal surface were cleaned with water before examination. The mucosa in the second part of the duodenum was carefully assessed initially using white light without magnification for the presence of features such as scalloped mucosal folds, mucosal nodularity, atrophic mucosal folds and mosaic pattern.[7–9] Subsequently, magnification NBI endoscopy was carried out and morphology of villi in the entire second portion of the duodenum was determined. This included assessment of the length of villi compared to the width, and their shape. In normal villi, the length is much greater than the width which gives it a finger or leaf shape. In atrophic villi, the length is reduced which gives it a shortened, stubbed or gyriform shape. In total villous atrophy, the villi are absent. Presence of numerous finger- or leaf-shaped villi denote a normal pattern whereas shortened or stubbed villi, villi in gyriform configuration or complete absence of villi suggests villous atrophy.[1,10] The last feature is indicative of total villous atrophy. Evaluation for the presence of patchy areas of atrophy interspersed with normal areas was also done. Images of duodenal mucosa during white light and magnification NBI endoscopy were obtained. In normal-appearing mucosa, at least four random images were taken from a different area of the second part of the duodenum (D2). When abnormalities were seen, images were taken from both abnormal and normal areas. Additional time required to carry out NBI examination was recorded.

A minimum of three duodenal mucosal biopsy specimens was taken from the D2 in all study subjects. When NBI examination in the entire D2 was normal, random mucosal biopsies were taken from duodenal mucosa. In patients with abnormality, biopsies were taken from the abnormal area on NBI. Sedation was given to patients on request and 2–3 mg i.v. midazolam was used for this purpose. All endoscopies were carried out by one of two endoscopists (AKD, KGS) with adequate prior experience in carrying out UGIE using magnification NBI. For any single patient, the doctor carrying out the endoscopy provided his grading of villous morphology after the procedure while the second endoscopist provided his own independent grading based on the images saved on the computer. In this way, duodenal villous morphology assessment for each patient was done by both endoscopists independently.

The duodenal mucosal biopsy specimens were fixed in buffered formalin for 12 h and then routinely processed with paraffin. Multiple serial sections of 5 μm thickness (minimum three levels) were stained with hematoxylin and eosin (H&E). The duodenal biopsy specimens were examined by two pathologists who were blinded to the clinical details of the patient. A villous crypt ratio of >3:1 was considered normal; a ratio of 1–3:1 denoted the presence of mild to moderate atrophy and a ratio <1:1 signified severe villous atrophy.[11] In addition to villous morphology, crypt architecture and presence of other lesions and parasites were also assessed. The clinical details, endoscopic findings and biopsy report for each patient was recorded on a standard proforma. The performance of magnification NBI endoscopy for detecting duodenal villous atrophy was compared with the histology finding, which is considered the gold standard. The agreement between the two endoscopists in detecting the presence or absence of villous atrophy was also determined.

Statistical analysis

Categorical data were presented as proportions whereas continuous data were presented as mean with standard deviation, or median with range. Sensitivity, specificity, positive and negative predictive values of magnification NBI endoscopy to detect duodenal villous atrophy were calculated separately for the two endoscopists. The agreement between them on the presence or absence of villous atrophy was determined using the kappa test. A κ-value of >0.8 signified excellent agreement. Statistical analysis was done using SPSS for Windows v.13 (SPSS Inc., Chicago, IL, USA)

RESULTS

A TOTAL OF 100 participants were included in the present study. Their mean age was 37.5 + 12.4 years and 42% were female. Diarrhea was present in 66% of the patients, 46% had significant weight loss, 41% abdominal pain and 9% anemia. Serological test for anti-tissue trans-

Table 1 Performance of magnification NBI endoscopy in detecting duodenal villous atrophy (first endoscopist)

Histology	Magnification NBI		Sensitivity	Specificity	Positive predictive value	Negative predictive value
	Villous atrophy present	Villous atrophy absent				
Villous atrophy present	14	2	87.5%	95.2%	77.6%	97.6%
Villous atrophy absent	4	80				

NBI, narrow band imaging.

Table 2 Performance of magnification NBI endoscopy in detecting duodenal villous atrophy (second endoscopist)

Histology	Magnification NBI		Sensitivity	Specificity	Positive predictive value	Negative predictive value
	Villous atrophy present	Villous atrophy absent				
Villous atrophy present	13	3	81.3%	92.9%	68.4%	96.3%
Villous atrophy absent	6	78				

NBI, narrow band imaging.

glutaminase antibodies (anti-tTG) was positive in 11% of subjects. The additional time taken to examine D2 using magnification NBI ranged from 3 to 7 min with a median time of 4 min. There were no procedure-related complications among the study subjects.

The performance of magnification NBI endoscopy in detecting duodenal villous atrophy with histology, considered as the gold standard, is shown for each of the two endoscopists in Tables 1 and 2, respectively. Of the 100 patients, 16 had atrophy of duodenal villi on histology and 14 of them were detected by one endoscopist (sensitivity 87.5%) using magnification NBI whereas the other endoscopist identified 13 (sensitivity 81.3%) of them. Only one patient had severe atrophy of villi on histology which was correctly identified by both endoscopists as a case of severe atrophy. All the subjects where magnification NBI was normal and histology showed duodenal villous atrophy had only mild villous atrophy on histology. The test performed well in identifying subjects with normal villi and the specificity was >90% for both endoscopists. Magnification NBI also had excellent negative predictive values with figures of 97.6% and 96.3% for the two endoscopists, respectively. The kappa-value for interobserver agreement was 0.87 which signifies a very good agreement between the two endoscopists in terms of identifying the villous pattern using magnification NBI. The findings on NBI and the corresponding histology images are shown for one patient each with normal duodenal villi, mild villous atrophy and severe villous atrophy in Figures 1, 2 and 3 respectively.

Among the 16 patients with villous atrophy on histology, one patient had scalloped duodenal mucosal fold and another

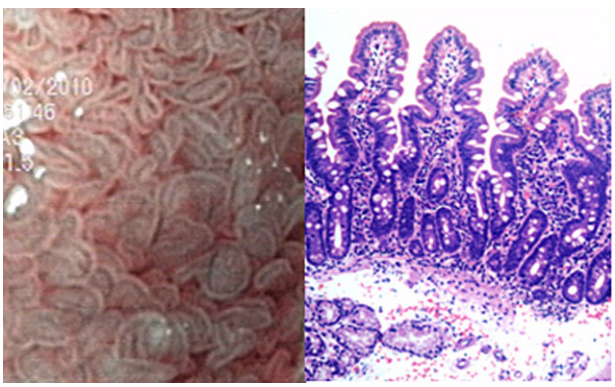

Figure 1 Appearance of normal duodenal villi on magnification narrow band imaging (Left) and histology (Right, 20× magnification).

patient had atrophic duodenal mucosal fold on white light examination which were detected by both endoscopists. Duodenal white light examination was reported as normal in the remaining ninety-eight patients by both endoscopists. Thus, white light endoscopic examination had a poor sensitivity of only 12.5% in identifying the presence of underlying villous abnormality.

The final diagnosis in 15 of the patients with villous atrophy was celiac disease in seven, tropical sprue in three, giardiasis in two, strongyloidiasis in one, AIDS enteropathy in one and Crohn's disease in one, whereas the diagnosis could not be established in one patient with villous atrophy. Table 3 shows the performance of NBI among the 15 subjects with malabsorption and duodenal villous atrophy on

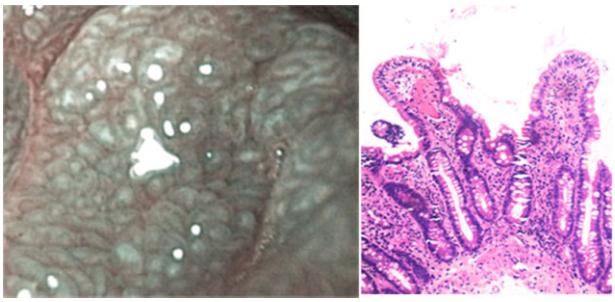

Figure 2 Appearance of mild duodenal villous atrophy on magnification narrow band imaging (Left) and histology (Right, 20× magnification).

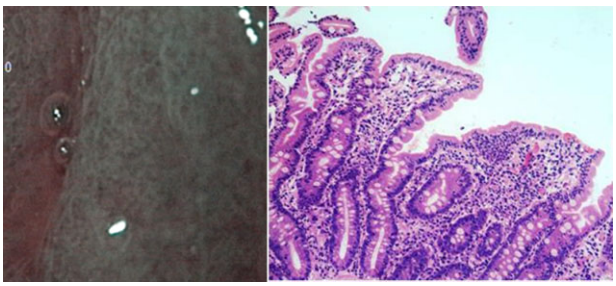

Figure 3 Appearance of severe duodenal villous atrophy on magnification narrow band imaging (Left) and histology (Right, 20× magnification).

Table 3 Performance of NBI in patients with malabsorption syndrome with duodenal villous atrophy

Diagnosis	No. patients	Duodenal villous atrophy detected by NBI (Endoscopist 1)	Duodenal villous atrophy detected by NBI (Endoscopist 2)
Celiac disease	7	6	5
Tropical sprue	3	3	3
Giardiasis	2	2	2
Strongyloidiasis	1	1	1
AIDS enteropathy	1	1	1
Crohn's disease	1	1	1
Total	15	14 (93.3%)	13 (86.7%)

NBI, narrow band imaging.

histology. Among the 84 patients with normal duodenal villi, 49 were diagnosed as having functional bowel disease (11 of them satisfied the ROME III criteria for irritable bowel syndrome [IBS]) and six had a positive tTG test but no histological features of celiac disease. In the same group, six patients had iron deficiency anemia, four had anemia as a result of vitamin B12 deficiency, four had inflammatory bowel disease, three had intestinal tuberculosis and three had parasitic infestation (*Giardia* in two and *Strongyloides* in one). Diagnosis in the remaining subjects was not clear. The final diagnosis in the six patients where the second endoscopist reported villous atrophy but histology was normal was iron deficiency anemia in two, functional bowel disease in two, vitamin B12 deficiency in one and colonic tuberculosis in one.

DISCUSSION

THE GOOD PERFORMANCE of magnification NBI endoscopy in assessing duodenal villous morphology among our patients suggests that this technique has the potential to play an important role in the evaluation of patients with suspected malabsorption. With a sensitivity of >80%, the likelihood of missing a lesion is low. In addition, an impressive specificity (close to 95%) and negative predictive value (>96%) suggests that a number of unnecessary biopsies can be avoided which will reduce the cost, time and complications associated with the procedure. The excellent interobserver agreement and absence of complications further supports the clinical application of this procedure.

Malabsorption is a global problem seen by clinicians all over the world. The actual etiology may vary depending on the stage of development of the nation and its geographical location.[12] For instance, celiac disease is the dominant cause of malabsorption in many developed nations, whereas gastrointestinal infections and tropical sprue are commonly seen in the underdeveloped and developing nations.[12,13] Efforts have been made by researchers in the last 15 years to enhance the capability of endoscopes to view the duodenal mucosa in detail and assess the morphology of villi for predicting the presence or absence of villous atrophy as well as patchy lesions.[2] In 1997, Siegel *et al*. showed that magnification endoscopy with chromoendoscopy has a sensitivity of 94% in detecting villous atrophy.[14] Another study using high-resolution magnification endoscopy in 191 patients yielded similar results.[15] A classification of villous atrophy on magnification endoscopy was proposed in 2004 where Z1 denotes normal villi, whereas Z2 to Z4 denote the presence of villous atrophy with increasing severity.[10] Establishing the presence of atrophy is of prime importance and requires a biopsy to be done. Grading the degree of atrophy may be more challenging using magnification NBI but clinically less useful as all of these patients would warrant a biopsy irrespective of the degree of atrophy. Therefore, the main focus of the present study was to evaluate the ability of magnification NBI to detect the presence or absence of duodenal villous atrophy.

This is the largest study on application of magnification NBI in detecting duodenal villous atrophy. The study was done in a group of patients where prior diagnosis was not known which makes the setting similar to an actual clinical situation. A previous study on 21 subjects that included three patients with celiac disease and 18 normal subjects showed this technique to have a sensitivity of 93.3% and a specificity of 97.8%.[3] A host of other advanced endoscopic methods have been used to assess duodenal villous morphology which include optimal band imaging, enhanced magnification endoscopy after instilling 3% acetic acid and optical coherence tomography.[1,13,16] All these studies have reported a very high sensitivity of 95–100% in detecting villous atrophy. Recently, attempts have been made to identify not only the villous morphology but also the histology of duodenal mucosa in real time using techniques such as confocal endomicroscopy and endocytoscopy.[17–19] In fact, a study from Australia showed that confocal endomicroscopy outperformed histology in diagnosing celiac disease.[17]

Prior to the development of advanced endoscopic techniques, presence of findings such as reduced duodenal folds, scalloped folds and grooves in duodenal mucosa on normal white light endoscopy received significant attention.[20] While these findings had a good specificity, their poor sensitivity was soon realized as a number of cases of celiac disease had normal duodenal mucosa during conventional endoscopy.[9,21] The sensitivity of white light examination in our series was only 12.7% which also highlights this limitation. This perhaps was the main trigger for research in improving endoscopic techniques to detect duodenal mucosal abnormality, as mentioned earlier.

Although our study shows the utility of magnification NBI in examining duodenal mucosa, it has a few limitations. We did not identify any case of patchy lesion. This may be due to the fact that only seven out of our 16 patients with villous atrophy had celiac disease. Even though the sensitivity of magnification NBI was >80% in our study, it was lower than the 90–100% reported in other series. Most of these studies had a great proportion of patients with moderate to severe villous atrophy which is easier to detect, unlike our study where most patients had mild villous atrophy. Interpreted differently, our study shows that NBI performs reasonably well even in detecting the presence of mild villous atrophy. The role of magnification endoscopy alone versus magnification NBI in detecting duodenal villous atrophy was not assessed within this study in order to minimize procedure time. Another minor limitation is that images obtained by NBI are not as bright as white light images but this did not significantly hamper assessment of duodenal villous morphology. The duodenal villi are easier to appreciate during NBI as a result of the increased contrast and may require less examination time than magnification endoscopy, but this remains to be proved.

In conclusion, magnification endoscopy with NBI is a promising technique for assessment of duodenal mucosal details and optimizing mucosal biopsy in patients with suspected malabsorption.

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CONFLICT OF INTERESTS

AUTHORS DECLARE NO conflict of interests for this article.

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