Technical Notes on High-Frequency Ultrasound Duodenography and Colonography Imaging of Giardial Lesions

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Abstract

Background: Worldwide G. lamblia is the third most common agent of diarrheal disease with over 300 million cases annually. Simple technical notes for clinicians are presented on use of high-frequency ultrasound imaging for duodenography and colonography in patients with *Giardia lamblia* infection. **Methods:** Ultrasound images were obtained from 100 consecutive patients with symptomatic giardiasis and 40 healthy controls. High-frequency annular array transducer of 7.5 MHz was used to obtain B-mode ultrasound grayscale and color images of the duodenum and colon with and without water contrast. The diagnosis of *G. lamblia* was based on clinical presentation, serial stool microscopy, and finding of flagellates in duodenal aspirates. **Results:** We demonstrated normal duodenum and colon echoanatomy in control subjects. In patients with giardiasis, the lesions of the duodenum and colon were associated with increased dimensions and wall thickness compared to healthy controls ($P < 0.05$). The ultrasound features of giardial lesions were characterized by increased wall echogenicity, flattening or loss of duodenal folds and/or colonic haustration, hyperechoic floating foci demonstrating chaotic motility, increased perilesional tissue echogenicity, and altered colonic peristalsis. **Conclusion:** In conclusion, high-frequency B-mode ultrasound imaging with and without water contrast demonstrated the details of duodenal and colonic echoanatomy in normal subjects and patients with giardiasis. The technique could be applied in the clinical setting of rural practitioners.

Keywords: Diarrhea, gastrointestinal tract, parasites, tropical diseases, waterborne diseases

Introduction

Giardiasis is the most common protozoal infection in many developing countries, due to open sewers in city streets and contamination of drinking water with sewage. The use of human and animal fecal material as fertilizer is an important source of infection. In Nigeria, a major source of the intestinal parasite infection is the use of fecal material as fertilizer for growing some vegetables such as garden egg, also known as eggplant, *Solanum aethiopicum*. In Igboland, Nigeria, garden egg is eaten raw to welcome guests at traditional ceremony, and is a very good source of dietary fiber, potassium, manganese, copper, and thiamin (Vitamin B1). In developed countries such as the United States, giardiasis is the most common parasitic infection,[1] and is implicated in most cases of traveler's diarrhea. Giardiasis is a major cause of endemic and epidemic diarrhea worldwide. The microscopic feature of *Giardia lamblia* demonstrates a binucleated flagellated protozoan parasite. *G. lamblia* is transmitted by fecal-oral route through ingestion of food or water contaminated with cysts. Humans are the major reservoir of infection. Giardiasis could also be sexually transmitted.[2] The groups at high risk include young children, immune-compromised patients, and those without prior exposure. The microscopic diagnosis of giardiasis is based on finding cysts or trophozoites in wet preparations of feces, and both life cycle stages have characteristic appearances.[3] Millions of *G. lamblia* cysts could be shed in stool by a single patient each day. Diagnosis is based on microscopic detection of *G. lamblia* cysts in fecal smears of three pooled individual specimens,[4] and supplemented by antigen detection in stool

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samples using immunofluorescence.[5,6] In light infection, the patient could be asymptomatic, but heavy infection would be marked by severe chronic diarrhea, but not dysentery. In light infection, trophozoites are found in the duodenum (82.5%) but rarely in the colon (0.4%).[7] Pathology could show an entirely normal light microscopic appearance of the duodenal mucosa in 96.3% and mild villous shortening and mild inflammation of the lamina propria in 3.7%.[7] However, in moderate-to-severe infection, trophozoites appear in colonic and ileal biopsies.[8,9] Confocal light microscopy reveals evidence of cytoskeletal rearrangement with exposure to live *G. lamblia* trophozoites, which induce localized condensation of F-actin and loss of peri-junctional alpha-actinin in human colonic and duodenal monolayers and reduce transepithelial electrical resistance.[10]

There is increasing interest to use ultrasound imaging for the diagnosis of nonparasitic and parasitic diseases such as *Echinococcus*, schistosomiasis, and amebiasis.[11,12] However, there are only a few reports on the use of imaging modalities to evaluate abdominal lesions associated with *G. lamblia*.[13-15] It has been demonstrated that there are intestinal cytoskeletal changes in giardiasis which manifest in wall thickening, increased diameter, unraveling of duodenal folds and colonic haustrations, and colonic wall motion abnormality.[15] It was also demonstrated that live trophozoites could be indirectly visualized as hyperechoic floating foci (HFF) on fatty food substrates.[15] Similar observations have been made by others, who reported the use of ultrasound in abdominal lesions caused by *Clonorchis sinensis*.[16] These ultrasonic features in giardiasis may be distinct from those in amebiasis.[15,17] We performed sonographic examination of the duodenum and colon using a 7.5 MHz annular array transducer and investigated the usefulness of this technique in characterizing the normal echoanatomy of the duodenum and colon and its application to the diagnosis of giardial infection. In the present study, we demonstrate important technical notes of a simplified approach to imaging for rural physicians with limited technical skills in diagnostic ultrasound imaging. Simple water-contrast sonographic examination of the duodenum and colon using a 7.5 MHz annular array transducer, by the bedside of the patient, provides information which until now requires extensive studies including esophagogastroduodenoscopy and colonoscopy tests. We have extended our previous studies[18] on giardial lesions to demonstrate the application of the present technique for the rural physician.

Methodological Procedures

Subjects

There were 140 subjects recruited for the study. The study group included 100 consecutive patients (male = 55; female = 45; mean ± SD age = 40 ± 17 years) who had symptomatic giardiasis. For comparison, sonograms obtained from 40 healthy controls (male = 20; female = 20; mean ± SD age = 38 ± 13 years), who had no history or parasites in stool, were used. The clinical symptoms, history of disease onset, and duration were collected using standardized questionnaire designed to determine the qualitative and quantitative characteristics of symptoms, and duration of illness. The reported intensity and periodicity of abdominal pain were noted. According to the onset and duration of illness at presentation, the patient population was categorized as (1) acute – within 1 month or less, (2) subacute – within the past 12 months or less, and (3) chronic – after 12 months. The epidemiological exposure to contaminated food or water was noted for each subject. The exclusion criteria included history of alcoholism, ulcerative lesions, major gastrointestinal disease such as polyposis, collagen vascular diseases, pancreatic diseases, liver diseases, Crohn's disease, tropical sprue, colon cancer, and diverticulitis. The protocol was approved by the Institutional Human Research Ethics Review Board.

Stool analysis

Diagnosis was based on laboratory microscopic detection of *G. lamblia* cysts or motile flagellates in fecal smears of three pooled individual specimens or duodenal aspirates. *G. lamblia* and other parasites were examined by direct microscopy of wet preparations of stool sample.[20] At least two parasitologists examined the stool samples and discrepancies resolved by repeated tests examined by a third parasitologist not involved in the initial evaluation. The quantitative estimation of *G. lamblia* infection in stool sample was based on count per high field (phf) of view.

Ultrasound examination

The abdominal ultrasound examination was performed using B-mode and color-flow Doppler ultrasonography with 2.5 MHz and 7.5 MHz annular array transducers of a duplex color-flow Doppler ultrasound system (Agilent HP/Philips SONOS 5500, Philips Medical Systems, Cambridge, MA, USA). The examination began with deeply located abdominal structures using a 2.5 MHz probe. The detailed examination of duodenal walls and folds was performed using a 7.5 MHz probe beginning in the right hypochondriac (1) and epigastric (2) regions [Figure 1]. This was followed by examination of the colonic walls and haustra of the ascending colon starting from the McBurney's point that lies one-third of the distance laterally on a line drawn from the umbilicus to the right anterior superior iliac spine. The examination proceeds upward to the right lumbar, (4) right hypochondriac (1), and turning clockwise to the epigastric (2), left hypochondriac (3), left lumbar (6), and left iliac (9) regions [Figure 1]. The sonographic examinations preceded in a blinded manner to results of stool analysis. All ultrasound examinations were performed with and without water contrast after overnight fasting (for at least 16 h) using standard scanning procedure. Water-contrast imaging was performed by having adult subjects take at least 1 L of water prior to examination. Subject examination was performed in the supine horizontal, left posterior oblique, and left lateral decubitus positions using the intercostal and subcostal approaches. The internal organs including liver, gallbladder, spleen, pancreas, duodenum, colon, and kidneys were routinely evaluated in all subjects. Color-flow Doppler sonography was used to examine the localization of lesions in relation to

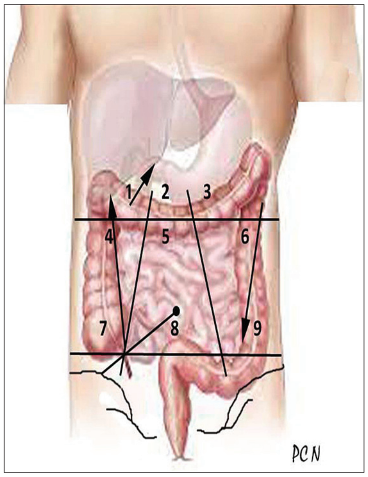

Figure 1: Protocol of abdominal scanning of the ascending and descending colons

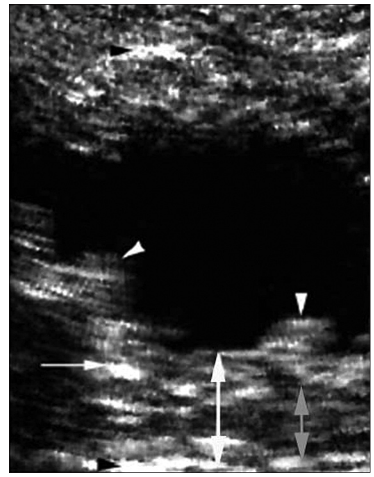

Figure 2: Sample sonogram of normal duodenum

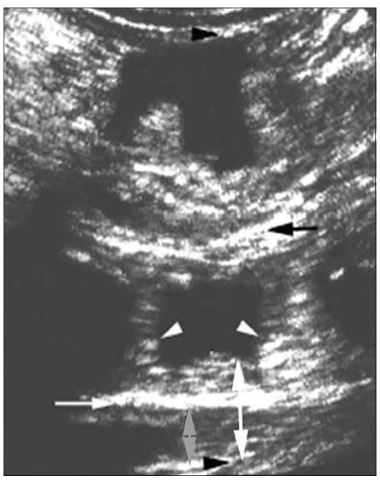

Figure 3: Sample sonogram of normal ascending colon

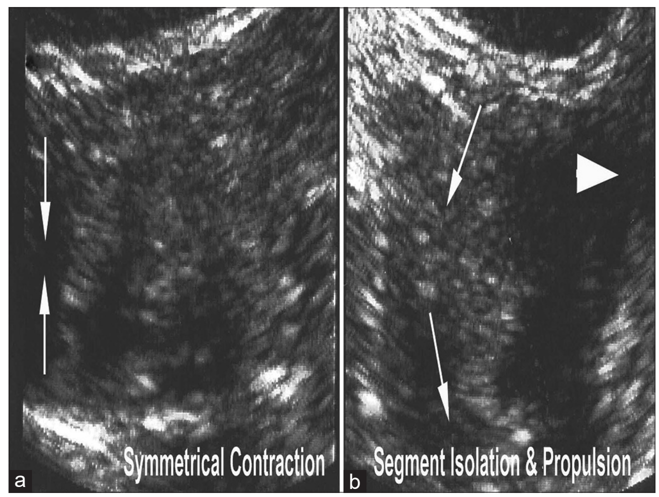

Figure 4: (a and b) Sample sonograms of normal colonic peristalsis showing symmetric contraction (a) and relaxation (b) of the walls

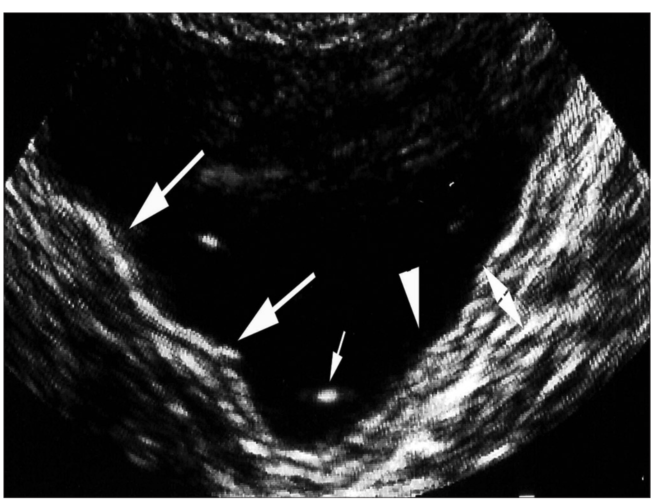

Figure 5: Sample sonograms of the duodenal wall in patients with acute giardiasis

vessels. All ultrasound studies including measurements and grading of echogenicity were performed by a single trained sonographer using built-in software. Measurements were taken between peristaltic waves. The measurement end points are shown in Figures 2 and 3. The measurements of wall thickness in the duodenum (with water contrast) [Figure 2; within double white arrow ends] were measured between two mucosal folds of Kerckring[18] [Figure 2; white arrow heads], from the surface of the moderately echogenic mucosa, through the hyperechoic submucosa [Figure 2; white arrow] and hypoechoic muscularis [Figure 2; within double gray arrow ends] to the hyperechoic serosa layer [Figure 2; bottom black arrow head]. The wall thickness of the colon with water contrast [Figure 3; within double white arrow ends] was measured between two haustra [Figure 3; white arrow heads], from the surface of the moderately echogenic mucosa [Figure 3; top arrow of the double gray arrows], through the hyperechoic submucosa [Figure 3; white arrow], and hypoechoic muscularis [Figure 3, within double gray arrow ends] layers, to the hyperechoic serosa layer [Figure 3; bottom black arrow head], and diameter measurement taken from near wall serosa [Figure 3; top black arrow head] to far wall

Figure 6: Sample sonogram of the duodenum in chronic giardiasis

Figure 8: Sample sonogram of the colon in chronic giardiasis

serosa [Figure 3; bottom black arrow head]. The measurement cursor line was aligned perpendicular to the echogenic *teniae coli libera* [Figure 3; black arrow] which runs midway between the near and far wall serosae in long-axis view of the ascending and descending colons. Giardial lesions were characterized by location, wall thickness, echogenicity of wall tissue, cross-sectional diameter, presence or absence of flattening or loss of duodenal folds and/or colonic haustration, presence or absence of hyperechoic floating foci (HFF) demonstrating chaotic motility, presence or absence of perilesional tissue echogenicity, and type of colonic peristalsis. Chaotic motility was defined as sonographically observed rapid floatation movements in all directions by hyperechoic foci, between peristaltic waves.

Statistical analysis

Statistical analysis was performed using *t*-test statistics and analysis of variance (ANOVA) for group comparison with the software package (StatSoft, Statistica, Tulsa, OK, USA). Mean values were reported as mean ± SD. Level of statistical significance was set at $P < 0.05$.

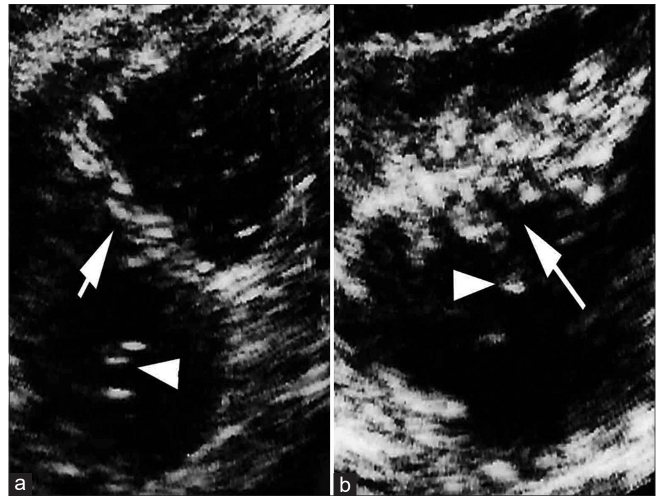

Figure 7: (a and b) Sample sonogram of the colon in acute giardiasis in the ascending (a) and descending (b)

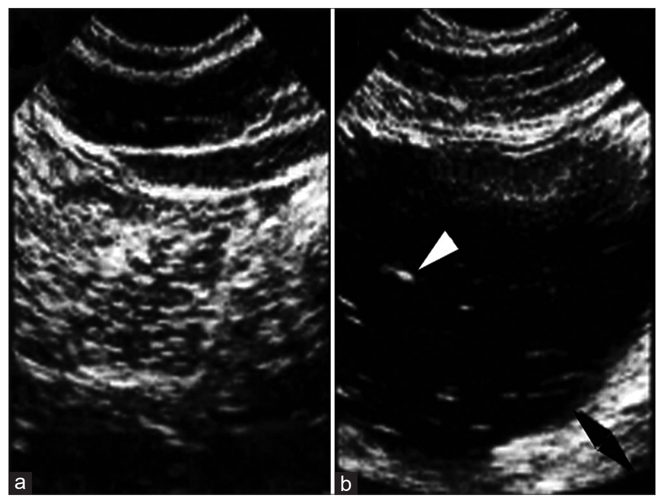

Figure 9: (a and b) Sample sonograms of the duodenum without (a) and with (b) water contrast

Results

Almost all patients complained about abdominal pain, particularly in the right upper quadrant, flatulence, rumbling, and perceived weight loss.

Measurements of dimensions of the duodenum and colon were taken at maximal dilation between peristalses using images taken with water contrast. Table 1 summarizes the intestinal wall dimensions in control subjects and patients with giardiasis.

Sonographic findings in normal duodenum

The wall thickness of the duodenum was 3.5 ± 2.2 mm. As is described in Figure 1, the duodenal wall was visualized as alternate bands of moderately echogenic mucosa with hyperechoic core submucosa which are thrown into folds of Kerckring,[18] arranged circularly, a middle hypoechoic muscularis layer and an outer hyperechoic serosa layer.

Table 1: Dimensions of intestinal walls in control subjects and patients with giardiasis

Intestine	Subjects	Wall thickness (mm)	Cross-sectional diameter (mm)	*P* value of comparison to controls
Duodenum	Controls	3.5±2.2		<0.05
	Giardiasis	6.0±2.5		
Ascending colon	Controls	3.9±1.4	32.0±13.3	<0.05
	Giardiasis	6.3±3.6	39.8±19.6	
Descending colon	Controls	3.8±0.8	30.7±8.5	<0.05
	Giardiasis	6.4±6.0	38.2±19.7	

Sonographic finding in normal colon

The ascending colon diameter was 32.0 ± 13.3 mm and wall thickness was 3.9 ± 1.4 mm. The descending colon diameter was 30.7 ± 8.5 mm and wall thickness was 3.8 ± 0.8 mm. Figure 3 shows the colonic wall comprising alternate hypoechoic and hyperechoic bands corresponding to the histological layers. The latter comprises a moderately echogenic mucosa, a hyperechoic core submucosa, a hypoechoic muscularis layer, and an outer hyperechoic serosa. The outer layer of longitudinal muscle in the colon demonstrated a relatively hyperechoic *teniae coli libera*. Local movements of the colon aid the absorption of water and help to form feces by providing a kneading action. The peristaltic movements are brought about by contractions of segments of circular muscles and the adjacent portions of the *teniae coli*. The latter movements are termed segmentations; they produce folds of the wall known as haustra [Figure 3; white arrow heads]. The haustra are isoechoic with the mucosa. The homogenous haustra have pyramidal shape with regular contours, and are spaced at regular intervals.

Normal colonic peristalsis

Colonic peristalsis was observed sonographically in control subjects using real-time images taken with water contrast. During peristalsis, there were circumferential symmetrical contraction rings [Figure 4a; white arrows] formed between adjacent haustra, and sequential segment isolation from the rest of the gastrointestinal tract (GIT) followed by antegrade propulsion of content [Figure 4b; white arrows]. Contractions of the smooth muscle in the colonic walls produced a rise in intraluminal pressure within the isolated chamber.[18,19,21] This was followed by relaxation of one of the two rings enclosing a segment, and resulted in peristaltic propulsion of the colonic inclusions [Figure 4b; white big arrow head].[22] The walls maintain symmetric contours during contraction [Figure 4a; white arrows] and relaxation [Figure 4b; white arrows].

Sonography of Giardiasis in Symptomatic Patients

Duodenum

In patients, the duodenal wall thickness (6.0 ± 2.5 mm) was greater than that in healthy controls ($P < 0.05$). All patients (100 cases) had duodenal inclusions of HFF [Figure 5; white small arrow]. The echoanatomy was characterized in acute (23 cases), and subacute (35 cases) giardiasis, as flattened duodenal folds, with undulating surface of "blunted saw tooth" appearance [Figure 5; white arrows]; at other sites, there was total "unfolding" [Figure 5; white arrow head] due to loss of normal cytoskeletal structure. There was altered wall echogenicity [Figure 5; with water contrast]. The muscosa, core submucosa, and muscularis layers [Figure 5; white double arrow heads], were almost as hyperechoic as the serosa layer, separated by thin hypoechoic borders. The chronic phase (42 cases) showed that all the duodenal wall layers were significantly hypertrophied and equally hyperechoic with poor distinction between the inner mucosa [Figure 6; black small arrow head] from the middle muscularis [Figure 6; black arrow] and from the outer serosa layer [Figure 6; black big arrow head]. There was compression of the surrounding tissue with increased echogenicity separated by periduodenal wall edema [Figure 6; white arrow].

Colon

In symptomatic giardiasis, there are increased intestinal dimensions. The diameter (39.8 ± 19.6 mm) and wall thickness (6.3 ± 3.6 mm) of the ascending colon were greater than that in healthy controls ($P < 0.05$). Similarly, the diameter (38.2 ± 19.7 mm) and wall thickness (6.4 ± 6.0 mm) of the descending colon were greater than that in healthy controls ($P < 0.05$). In acute giardiasis (23 cases), HFF [Figure 7a; white arrow head, with water contrast] were seen in both the ascending [Figure 7a] and descending colons [Figure 7b]. The haustra were flattened [Figure 7b; big white arrow], and at different locations replaced by intestinal septations [Figure 7a; white small arrow]. Similar findings were present in the subacute (35 cases) phase. In the chronic phase (14 cases), significant clinical complications may develop. There were increased wall thickening, increased echogenicity, and loss of haustration in both the ascending and descending colons [Figure 8; white small arrow]. The colonic wall tri-band structure was replaced by a single hyperechoic band [Figure 8; white small arrow].

Differential Diagnosis

The major differential diagnosis for giardiasis was with the ciliated protozoal infection balantidiasis that could potentially give rise to HFF. Balantidiasis is caused by a ciliated protozoan – *Balantidium coli*, and was excluded based on clinical, epidemiologic, and laboratory findings. There was absence of history of balantidial dysentery in any of our patients. It has been suggested that amebiasis may cause thickening of the submucosal layer due to hypervascularity of the bowel wall[17] but contrasts with wall thickening observed in giardiasis.

Follow-Up of Sonographic Characteristics

We identified four cardinal sonographic signs for diagnosis of giardial intestinal lesions which included: (A) wall thickening, (B) increased wall echogenicity, (C) flattening or loss of duodenal folds or colonic haustration, and (D) presence of hyperechoic floating foci. Ultrasound scanning of patients was performed and repeated stool analysis was done within 1 month of initial treatment with a 10-day course of metronidazole (500 mg three times daily). At 6 months after the initial examination, follow-up studies were performed if required. Posttreatment follow-up studies revealed that there were significant reduction in lesion dimensions, decreased wall echogenicity, and reduction in compression of perilesional surrounding tissue. The echoanatomy improved in ultrasound scan images and correlated with clinical improvement with reduced intensity and frequency of abdominal discomfort and other symptoms. There was a negative stool test or reduced count of trophozoites phf. The presence of hyperechoic floating foci in the duodenum could be monitored with high-frequency ultrasound without [Figure 9a] and with [Figure 9b] water-contrast imaging.

Discussion

The present study demonstrated detailed echoanatomy of the duodenum and colon using high-frequency B-mode ultrasound with water-contrast imaging in healthy subjects and patients with giardiasis. A comparison of sonograms in controls and patients with giardiasis, demonstrated significant differences that could be categorized as: (a) changes in echoanatomy of the intestinal walls, (b) imaging features associated with changes in cytoskeletal rearrangement attributable to the presence of trophozoites of *G. lamblia* in the intestine, and (c) abnormality of colonic peristalsis.

The changes outlined above could be easily detected by rural physicians using a bedside ultrasound imaging system. The characterization of the severity of the lesions could easily define measures to be taken either within the local clinical setting as outpatients, inpatients, or for referral to more established centers with specialists for further management. Further studies are required to determine criteria for selection of patients that may benefit for intravenous injection treatment, for example, with metronidazole compared to those to use per oral tablets. The duration of treatment also needs to be considered in severe infection. Failure to select treatment modalities that could eliminate the parasites could lead to treatment failures and drug resistance.

Ethical constraints prevented us from performing routine intestinal biopsies in the present study, however, published literature provides histologic evidence in symptomatic cases of giardiasis that suggests shortening and thickening of the villi and increased cellular content of the lamina propria in variable degrees.[7,23] In addition to these changes, focal acute inflammation in the crypts and sometimes in the villi is considered to be highly characteristic. The inflammatory cells are mainly polymorphonuclear leukocytes and occasionally eosinophils. Other pathological features include epithelial changes such as vacuolization and compression in association with focal acute inflammation.[23] The sonographic features of increased wall thickness and echogenicity and flattening of duodenal folds and/or colonic haustration may well be explained by the effects of exposure to live *G. lamblia* trophozoites on intestinal cytoskeleton.[10] Furthermore, focal inflammatory processes may cause edematous wall thickening which may not withstand shear stress exacted by intestinal contents and may thus expand leading to increased dimensions and unraveling of duodenal folds and colonic haustrations.[15,18,19] The compression of surrounding tissues may cause perilesional tissue ischemia and edema with resulting space-occupying effects. The sonographically observed wall changes in giardiasis appear to be characteristic and differ from the marked thickening of the bowel wall submucosa in amebic colitis.[15,17,19]

The sonographically observed HFF have been associated with giardial infection.[15,18,19] It is plausible that HFF derive from the enabling condition of fluid retention and malabsorption in giardiasis. The retention could aid the floatation of food particulate substances, especially of fatty origin, since steatorrhea (fatty stool) is a frequent manifestation of giardiasis.[23] The fatty echogenic substrates become “floaters” for millions of flagellated trophozoites of *G. lamblia*, with chaotic motility, observable in real-time sonographic imaging. Even though, the observations may not be peculiar to giardial infection, since others have associated floating echogenic foci in the gallbladder with active *C. sinensis* infection.[16]

Abnormal intestinal motility observed sonographically as asymmetric colonic contractions are of interest and may well explain the clinical symptoms of abnormal colonic peristalsis associated with giardiasis. The latter may have potential therapeutic implications, for example, would ultrasound findings aid the selection of patients that may benefit from stool softeners without a risk of obstruction? The latter question remains to be answered in future studies.

In Nigeria, the therapy given to most patients with giardiasis is based on the use of metronidazole. Metronidazole, though effective, has significant toxicity and possible cancerogenic effects, especially with long-term use. Drug resistance is also a major concern with the use of metronidazole. Alternatives may include the use of nitazoxanide and tizoxanide, which were found to be more effective against flagellated organisms *in vitro*,[24] and have been approved for clinical use. The present study has a relatively small sample size, and lacks histopathological correlation of observed lesions. Future studies should be undertaken with larger sample population by a number of trained sonographers. Regardless of these drawbacks, ultrasound was informative in the diagnosis of giardiasis and remained to be evaluated for sensitivity and specificity in comparison to other conditions.[25]

In conclusion, there is a good clinical utility for high-frequency B-mode ultrasound imaging of the intestine in giardiasis. The use of water-contrast imaging demonstrated the echoanatomy of the duodenum and colon in healthy subjects as well as pathological changes associated with *G. lamblia* infection. However, further studies in larger sample population are required to evaluate the sensitivity and specificity of ultrasound imaging in correlation to histopathology of observed lesions. High-frequency ultrasound imaging is a simple non-invasive technique that could be applied by the beside for diagnosis and follow-up of patients with giardiasis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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