Acute diarrhea associated with *Dientamoeba fragilis* and *Clostridioides difficile* coinfection: A laboratory diagnostic challenge

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DOI: https://doi.org/10.5281/zenodo.10074779

Summary

Acute infectious diarrhea is a common health problem among adults. It is mainly associated with bacterial or viral infections. Clinical and laboratory investigation can be challenging. It is possible that instead of one infectious agent, other agents, not easily recognized, can be also responsible for acute diarrhea manifestation. Herein, a case of community-acquired acute diarrhea due to a rare coinfection with an intestinal protozoan (*Dientamoeba fragilis*) and an anaerobic bacterium (*Clostridioides difficile*) coinfection in an elderly patient is described.

Key words
Dientamoeba fragilis, Clostridioides difficile, coinfection, community-acquired diarrhea

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Introduction

Acute infectious diarrhea lasts fewer than two weeks[1] and it is considered a common health issue among adult population in the community.[2] Acute diarrhea is mainly of bacterial or viral etiology, with the latter being the most common one;[3] parasites are more often implicated in chronic diarrhea.[4] Comorbidities, foodborne illness, and traveling are mostly associated with cases of bacterial acute diarrhea.[2] A thorough patient history facilitates risk factor evaluation.[2] Signs and physical findings such as those of inflammatory diarrhea and/or severe dehydration can yield data necessary to direct testing and treatment.[2] Laboratory workup needs to be reserved for patients presenting with severe dehydration, persistent fever, and blood in the feces; for those that are immunocompromised; for cases with suspicion of hospital-acquired infection; and for suspect outbreaks.[2] In case of acute infectious diarrhea, the major aim of treatment is to prevent and treat dehydration.[1] In contrast to viral gastroenteritis that is usually self-limiting,[5] acute diarrheal illness caused by bacterial and parasitic agents requires pharmacological therapy. Antimicrobial agents are only effective against bacterial infections, such as shigellosis, campylobacteriosis and *Clostridioides difficile* infection, and against intestinal protozoan infections.[2]

Case Description

An elderly patient was admitted to a hospital in the city of Athens, Greece, with a four-day history of severe diarrhea and weakness but not vomiting. On arrival to the emergency department, the patient had a low-grade fever and was hemodynamically stable. Physical examination was unremarkable except for mild abdominal distension and increased bowel sounds; no palpable masses were found. Computed tomography of the abdomen did not show any abnormal findings. Significant labs included mild leukocytosis with neutrophilia of 9,765/mm^3 and a raised C-reactive protein of 233 mg/dL; low serum potassium levels of 3.1 mEq/L with elevated serum sodium levels of 150 mEq/L; increased blood urea nitrogen levels of 142 mg/dL and increased serum creatinine levels of 2.0 mg/dL; and low serum albumin levels of 2.9 g/dL. Admission testing came back negative for SARS-CoV-2. The patient, who lived alone, had not engaged in any social gatherings during the CoVID-19 lockdown. Neither previous hospitalization nor antibiotic exposure within the prior three months was reported. Nevertheless, the patient had been experiencing annoying abdominal discomfort for quite some time; laxatives had been used when the patient was unsatisfied with bowel habits.

As part of the routine laboratory evaluation, a fresh stool sample was obtained from the patient to be promptly tested for enteric pathogens. Fecal microscopy did not identify any intestinal parasites, although some structures resembled single-cell parasitic forms. However, rapid antigen testing was negative for *Entamoeba histolytica, Giardia* and *Cryptosporidium*. Fecal occult blood test was positive. *C. difficile* was detected in a lateral flow device; patient's sample was positive for the constitutive antigen glutamate dehydrogenase and for toxin A but negative for toxin B. Bacterial stool culture was negative for the most common enteropathogens. The plausible explanation of patient's diarrhea was an infection with a diarrheagenic strain of *C. difficile*, acquired outside of hospitals. Further questioning revealed that the patient had used lactulose syrup 48 hours before symptom onset. Oral vancomycin (125 mg four times daily) was started.

After three days of treatment for *C. difficile* infection, the patient was still suffering from severe diarrhea with no signs of clinical improvement. In the requested stool examination, the observed amebalike structures seemed to thrive. Therefore, a specialist in parasitology was contacted. On microscopic examination of patient's stool wet mounts, the expert recognized several ameboid trophozoites of *Dientamoeba fragilis* (Figure 1a.). Permanent staining (Figure 1b.) confirmed the presence of *D. fragilis* trophozoites in patient's feces.

As this protozoan parasite can cause gastrointestinal disease in humans, oral metronidazole (500 mg thrice daily) considered the drug of choice for *D. fragilis* infection was added to treatment regimen. Upon the completion of treatment, patient's diarrhea resolved and lab test results returned to normal; no parasites were detected in repeated stool examinations.

Comments

The possibility of *C. difficile* infection was initially considered. It has been recently argued that colonization and subsequent infection with *C. difficile* could be linked to gastrointestinal disturbances, regardless of whether they are associated with antibiotic exposure or with other risk factors.[6] Moreover, it has now been in dispute that recent laxative use, itself causing diarrheal disturbances, influences the likelihood of *C. difficile*-infection diagnosis.[7,8] The elderly patient with diarrhea was treated for community-acquired *C. difficile* infection on clinical grounds such as inflammation, dehydration accompanied by electrolyte and

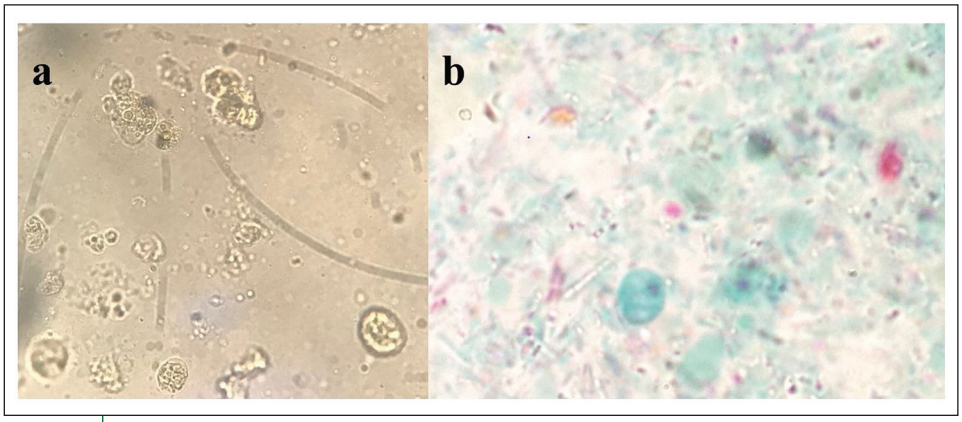

Figure 1 **a.** *Dientamoeba fragilis* trophozoites in a wet mount prepared from the patient's fecal sample (x400). **b.** *Dientamoeba fragilis* trophozoites in a fecal smear permanently stained (x1000). Trichrome stain facilitated the identification of the two characteristic *D. fragilis* nuclei.

renal function abnormalities, and protein loss. Fecal occult blood test is often positive in case of *C. difficile* infection. This clinical picture in the geriatric patent with new-onset diarrhea was suggestive of *C. difficile*-associated diarrhea.[9] The patient's strain of *C. difficile* was toxigenic. It is possible that carriage of toxin-producing *C. difficile* strain progresses to state of disease, especially after diarrheal events.[6] However, this strain was found to produce toxin A alone; immunoassay results had been double-checked. It is now believed that toxin A can evoke gastrointestinal symptoms independently of toxin B.[10] The pathogenic potential of toxin A, traditionally described as 'enterotoxin,'[9] is still debated. Compared to toxin B, traditionally described as 'cytotoxin,' toxin A is considered weaker[9] and its ability to cause severe damage and clinical symptoms is currently doubted.[10] Thus, when the reviewed patient's condition showed no improvement, other possibilities needed to be scrutinized.

On further workup, *D. fragilis* was found in the feces of the elderly patient. This came as a surprise. This protozoan is an unflagellated trichomonad parasite considered to have lost its flagella through reverse evolution to adapt to life in the gut, thus adopting an ameba-like appearance.[11] As the patient did not have any close relatives, no support could be offered to the widely suggested mode of *D. fragilis* transmission from children with *Enterobius vermicularis* infection to close family members.[12] Moreover, this protozoan parasite has been very rarely stumbled across in Greece. Until now, there have been only three documented cases infected with *D. fragilis* in the country, that is, two international travelers[13] and a locally infected farmer[14] presenting with gastrointestinal symptoms. Traveling abroad has not be taken into consideration due to patient's advanced age and CoVID-19 travel ban. However, before enforcement of movement restrictions in Greece during lockdown, the patient had stayed some time at a farmhouse in patient's native place each year. Interestingly, this small village was located in the principally rural region (Western Greece), in which the aforementioned *D. fragilis*-infected farmer lived (Vassalou E, personal communication). In the case of the elderly patient, long-standing, paucisymptomatic *D. fragilis* infection of potential zoonotic origin could not thus be excluded. This is in line with patient's long-term experience of alternating episodes of constipation and diarrhea. Nevertheless, in addition to minor symptoms reminiscent of those seen in irritable bowel syndrome, *D. fragilis* has been also incriminated *per se* in provoking diarrhea[11] but its pathogenicity has not yet been fully understood.[11]

Confronting the results, a treatment working against the two pathogens, *D. fragilis* and *C. difficile*, was opted: metronidazole has been used to manage anaerobic bacterial infections and protozoal infections;[15] metronidazole and oral vancomycin have been clinically used to treat *C. difficile* infection.[7]

Both *C. difficile* and *D. fragilis* infections may have similar clinical manifestations.[9,11] It can be difficult, however, to distinguish between them if present at the same time, as they seldom co-exist in a patient.

Laxative-induced, non-antibiotic gastrointestinal disturbances leading to microbial dysbiosis might have served as a trigger[6,8] for the concurrent manifestation of *D. fragilis* and *C. difficile* infections in the elderly patient. Thus, the patient, who was colonized by a toxin A-producing *C. difficile* strain, could have de-

veloped symptoms.[6,10] One might also hypothesize that toxin A generation contributed to further disruption of gut microbiota homeostasis[10] resulting in the thriving of *D. fragilis*, long since harbored in the patient's intestine. This could have enabled intensification of the once paucisymptomatic *D. fragilis* infection aggravating patient's diarrhea.

In contrast to possibly overdiagnosed *C. difficile* infection,[7] infection with *D. fragilis* has been largely overlooked. In acute infectious diarrhea, identifying the causative agent(s) is not always easy. Sometimes, instead of one potential agent becoming immediately apparent, there might have also been others going unnoticed, especially if they are rare and/or emerging as in the case of *D. fragilis*.[12,13] In developed countries with a low intestinal parasitic burden, such as Greece, enteric parasites are often neglected, while expertise in parasitology is lacking. Hence, clinicians and clinical pathologists need to update their knowledge on parasites, in order to have in mind the involvement of parasites in cases of infectious diarrhea and detect their presence.

Περίληψη

Οξεία διάρροια με συλλοίμωξη *Dientamoeba fragilis* και *Clostridioides difficile*: Μία εργαστηριακή διαγνωστική πρόκληση

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Η οξεία διάρροια λοιμώδους αιτιολογίας είναι σύνηθες πρόβλημα υγείας στους ενήλικες. Σχετίζεται κυρίως με λοιμώξεις από βακτήρια και ιούς. Η κλινική και εργαστηριακή διερεύνηση μπορεί να αποτελέσει πρόκληση. Είναι δυνατό, αντί για ένα λοιμογόνο παράγοντα, να υπάρχουν και άλλοι που δεν εντοπίζονται εύκολα αλλά μπορεί να ευθύνονται και αυτοί για την εκδήλωση της οξείας διάρροιας. Περιγράφεται μια περίπτωση οξείας διάρροιας στην κοινότητα από σπάνια συλλοίμωξη με πρωτόζωο του εντέρου (*Dientamoeba fragilis*) και αναερόβιο βακτήρο (*Clostridioides difficile*) σε ηλικιωμένο ασθενή.

Λέξεις κλειδιά
Dientamoeba fragilis, Clostridioides difficile, συλλοίμωξη, διάρροια της κοινότητας

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