Journal of Infection and Public Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Evaluation of enzyme linked immunosorbent assay for stool antigen detection for the diagnosis of cryptosporidiosis among HIV negative immunocompromised patients in a tertiary care hospital of northern India

Ujjala Ghoshal*, Vidhi Jain, Asmita Dey, Prabhat Ranjan

Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form 8 May 2017
Accepted 9 June 2017

Keywords:
Cryptosporidium
Kinyoun
ELISA
Coproantigen

ABSTRACT

The diagnosis of cryptosporidiosis among HIV positive patients has been the focus of many studies worldwide. However, there is a paucity of data on HIV negative immunocompromised patients like post-renal transplant recipients and those with haematological malignancies. Stool microscopy, the conventional method of diagnosis, is fraught with difficulties like cumbersome sample processing and subjective interpretation. Enzyme linked immunosorbent assay (ELISA), on the other hand, is quicker and easier. The present study was conducted in a tertiary care and super speciality hospital of north India. Stool specimens from HIV negative immunocompromised patients were subjected to both modified acid fast staining for oocysts of *Cryptosporidium* and ELISA for detection of *Cryptosporidium* copro-antigen, over a period of six years. Of the 637 specimens evaluated, 97 (15.23%) samples were positive for *Cryptosporidium* by both techniques; 25 (3.92%) specimens were positive by ELISA and negative by microscopy, 14 (2.20%) specimens were positive by microscopy but negative by ELISA, while 501 (78.65%) specimens were negative for *Cryptosporidium* by both techniques. Significant correlation was observed as a measure of agreement (Kappa test value 0.795) between modified ZN stained microscopy and ELISA for the detection of *Cryptosporidium* oocysts. The sensitivity, specificity, positive and negative predictive value of ELISA, keeping stool microscopy as gold standard were 87.38%, 95.25%, 87.39% and 97.28% respectively. We conclude that ELISA may be used as a reliable substitute for microscopy in setups where the case load is higher or expertise in special staining techniques is not available. The cost of the kit can be justified if the sample load is sufficiently high or if immunocompromised patients form a significant patient population.

Introduction

Cryptosporidium, an apicomplexan parasite, has emerged as a human opportunistic parasite causing diarrheal illness worldwide. The first human cases of infection with *Cryptosporidium* were documented by two separate groups in 1976 [1,2]. While infection is mostly self limited in immunocompetent individuals, this parasite can cause unrelenting and fatal illness among immunocompromised patients unless treated properly.

The association between *Cryptosporidium* and diarrhoea in HIV positive patients brought cryptosporidiosis to the forefront as a ubiquitous human pathogen. It was identified as a cause of diarrheal disease outbreaks and is now considered an established cause of childhood diarrhoea and malnutrition. A recent study encompassing seven sites from Asia and Africa found *Cryptosporidium* to be the second most common cause of diarrhoea in children less than five years of age [3].

The *Cryptosporidium* oocyst is fully sporulated, thus highly infectious at the time of shedding in stool. Modified acid fast staining is the current gold standard for the diagnosis of *Cryptosporidium* but its results are far from perfect. The biggest limitation is its subjectivity. Oocysts of *Cryptosporidium* appear characteristically round or slightly ovoid, 4–6 μm in size and acid fast [4]. Fully sporulated forms with four sporozoites take up the modified ZN stain variably, appearing partially acid fast. Empty excysted oocysts are not acid fast. Few structures can be confused with oocysts including yeast

* Corresponding author.
E-mail addresses: ujjalaghoshal@yahoo.co.in, ujjala@sgpgi.ac.in (U. Ghoshal).

http://dx.doi.org/10.1016/j.jiph.2017.06.007

Table 1
HIV-negative immunocompromised patients harbouring *Cryptosporidium*.

Illnesses/diseases	Number of patients with *Cryptosporidium* infection ($N = 80$) among total number of patients ($N = 360$)	Number of specimens with *Cryptosporidium* infection ($N = 136$) among total number of specimens ($N = 637$)
Immunological conditions (patients on steroid therapy for systemic lupus erythematosus, systemic sclerosis, Wegener's granulomatosis)	5	7
Patients with chronic diarrhoea (disease, ulcerative colitis, inflammatory bowel diseases, others with undiagnosed reason for chronic diarrhoea)	24	31
Endocrinology (patients with chronic diarrhoea)	3	5
Patient on radiotherapy	1	1
Renal transplant recipients	42	86
Patients with haematological malignancies	5	6
Total	80	136

like cells, fungal spores, bacterial spores and fat globules [5]. Given the intermittent shedding of the parasitic oocysts, multiple stool specimens need to be screened before being able to declare a true negative result. The sensitivity of microscopy varies from 37% to 90.75% for the detection of *Cryptosporidium* [6–10].

In order to overcome the limitations of microscopy, techniques based on *Cryptosporidium* copro-antigen detection in stool have increasingly become popular. Antigen detection in stool by ELISA is one such technique. It offers higher sensitivity, specificity and higher diagnostic index than stool microscopy [11]. Applied in peripheral settings where experienced microscopists are scarce, it reduces the subjectivity of the diagnostic procedure and increases reliability of the results.

Worldwide, most studies have focussed on cryptosporidiosis in HIV positive patients with or without healthy controls. However, there is a paucity of data on HIV negative immunocompromised patients like post-renal transplant recipients and those with haematological malignancies. Keeping this aspect in mind, the study was conducted in a tertiary care and super speciality hospital of India.

Materials and methods

Stool samples from HIV negative immunocompromised patients suffering from diarrhoea, presenting to a tertiary care centre, were collected during the 6 year period 1st July 2009–30th June 2015.

Stool samples were collected in clean, wide-mouthed, screw capped disposable containers and processed as soon as possible, preferably within one hour for stained microscopy. A portion of stool was kept at 4 °C for *Cryptosporidium* antigen detection. ELISA was performed within a week, as per the manufacturer's instructions.

Stool microscopy

Modified Kinyoun technique

It was performed as per standard method with a few modifications like increasing staining time for 20 min, as reported by our centre in earlier study [12]. Briefly, a thin smear was prepared on a clean glass slide using a concentrated stool specimen. It was air dried and then fixed in methanol for 2–3 min. The slide was then flooded with Kinyoun's stain for 20 min. After washing with water, it was again flooded with 10% acid-alcohol for 2 min. After washing again with water, it was counter stained with 3% malachite green for 1 min. It was washed with water and dried before viewing under 10× eye piece and 100× oil immersion objective of the light microscope, bringing total magnification to 1000. Oocysts of *Cryptosporidium* passed in stool were seen as spherical reddish-pink partially stained structures measuring approx 4–6 μm in diameter.

Cryptosporidium antigen detection in stool using commercial ELISA kit

A portion of unconcentrated stool specimen was subjected to sandwich ELISA according to the manufacturer's instructions for the FDA approved, commercially available ELISA kit "*Cryptosporidium* Stool antigen detection microwell ELISA" (IVD Research Inc., CA, USA).

Results

During the study period, a total of 637 specimens were evaluated for *Cryptosporidium* by both microscopy and ELISA. Modified Kinyoun stain and ELISA were positive in 111/637 (17.43%) and 122/637 (19.15%) specimens respectively. The *Cryptosporidium* infection rate for HIV-negative post-renal transplant patients was 11.67% (42/360) and haematological malignancy patients was 1.38% (5/360) respectively. Some other HIV-negative immunocompromised patients also harboured *Cryptosporidium* which are summarized in Table 1.

Comparative evaluation

Of the 637 specimens evaluated, 97 (15.23%) samples were positive for *Cryptosporidium* by both techniques; 25 (3.92%) specimens were positive by ELISA and negative by microscopy, 14 (2.20%) specimens were positive by microscopy but negative by ELISA, while 501 (78.65%) specimens were negative for *Cryptosporidium* by both techniques.

Details of test results among immunocompromised patients shown in Table 2.

Statistical analysis revealed the significant correlation (Kappa test value: 0.795) between modified ZN stained microscopy and ELISA for the detection of *Cryptospordium* oocysts. The sensitivity,

Table 2
Results of microscopy and ELISA expressed as a 2 × 2 table.

Detection technique	Microscopy +ve	Microscopy −ve	Total
ELISA +ve	97	25	122
ELISA −ve	14	501	515
Total	111	526	637
Statistical analysis	Kappa test value (measure of agreement) = 0.795; ($^{*}p < 0.001$)		

* Significant values, i.e. <0.05.

Please cite this article in press as: Ghoshal U, et al. Evaluation of enzyme linked immunosorbent assay for stool antigen detection for the diagnosis of cryptosporidiosis among HIV negative immunocompromised patients in a tertiary care hospital of northern India. J Infect Public Health (2017), http://dx.doi.org/10.1016/j.jiph.2017.06.007