Çukurova Üniversitesi Tıp Fakültesi Dergisi
Journal of Cukurova University Faculty of Medicine

Araştırma Makalesi / Research Article

Prevalence of Cryptosporidium Parvum and Isospora Belli Infections Among Children Presenting with Diarrhoea at Queen Elizabeth Central Hospital

Queen Elizabeth Central Hastanesine Diyare ile Başvuran Çocuklarda Cryptosporidium Parvum and Isospora Belli Enfeksiyonları Prevalansı

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Çukurova Üniversitesi Tıp Fakültesi Dergisi (Journal of Cukurova University Faculty of Medicine) 2012; 37(3): 133-138

ABSTRACT

Purpose: Diarrhoea in children is a common problem in low resourced countries including Malawi. It is caused by many intestinal parasites including *Cryptosporidium parvum* and *Isospora belli.* Both infections are prevalent in severely immunocompromised individuals and may cause severe life-threatening diarrhoea. The main aim of the study was to assess the prevalence of *Cryptosporidium parvum* and *Isospora belli* infections among children presenting with diarrhoea admitted to QECH - paediatrics wards and methods used for diagnosis. Some specific objectives were to determine the prevalence of *Cryptosporidium parvum* and *Isospora belli* infections among human immunodeficiency virus (HIV) seropositive and HIV seronegative children with diarrhoea, to evaluate diagnostic methods used at QECH to identify intestinal parasites and to determine diagnostic methods that can be recommended for implementation.

Methods: Faecal samples were collected from all children presenting with diarrhoea upon admission or already hospitalised were recruited excluding all children without or with acute diarrhoea (less than three days). Four smears were prepared from each sample: 2 for Ziehl Neelsen staining and the other 2 for Auramine-phenol staining. The data was entered into Microsoft Excel and analysed using Epi-Info.

Results: A total of 25 children with diarrhoea under the age of five years; 10 were human immunodeficiency virus -seropositive, 7 were HIV-seronegative and 8 of them their status was unknown. *Cryptosporidium parvum* was detected in 4 children (16%) at 4.5% and 36.1% confidence intervals and none had *Isospora belli at* 0.0% and 86.3% confidence intervals. Out of those 4 who had *Cryptosporidium parvum*, 3 children were HIV-seropositive and 1 child was HIV-seronegative at a frequency of 30% and 14.3% respectively. No infection was seen in children whose HIV-serostatus was unknown.

Conclusion: Modified Ziehl Neelsen technique and auramine-phenol staining can be used to examine stool at QECH for C.parvum and I.belli identification. Although I.belli is prevalent in our environment, it has not yet been detected in children and or evolved as an endemic pathogen and so far remains as an opportunistic pathogen in adults only. There is need to repeat the study in rainy season and include gold standard method.

Key words: Cryptosporidium, Isospora, Prevalence, Malawi, Queen Elizabeth Central Hospital.

ÖZET

Amaç: Diyare çocuklarda Malawi'yide içeren gelir düzeyi düşük ülkelerde sık görülen problemlerden biridir. Diyare, Cryptosporidium parvum and Isospora belli gibi pek çok intestinal parazitlere bağlı olarak ortaya çıkar. Her iki enfeksiyon Her iki enfeksiyon immune direnci düşük olgularda yaşamı tehdit eden diyareye neden olabilir. Çalışmanın temel amacı QECH başvuran diyaresi olan çocuklar arasında enfeksiyonların bu enfeksiyonların sıklığını araştırmaktır Ayrıca Cryptosporidium parvum ve Isospora belliile enfekte olan insane immuneksikliği virusu (HIV) seropozitif ve HIV seronegatif diyareli çocuklarda hastalığın sıklığı yanı sıra tanı yöntemlerinin değerlendirilmesi amaçlanmıştır.

Yöntem: Gaita örnekleri akut diyare (3 günden az) ve diyare olmayan cocuklar hariç kliniğimize kabul edilen ve yatmakta olan diyareli çocuklardan alınmıştır. Her bir örnekten dört yayma hazırlanmıştır: İki tanesi Ziehl,Neelsen boyaması diğer ikisi ise Auramine-Fenol boyaması ile incelenmiştir. Elde edilen veriller Microsoft Excel programına kaydedilip, Epi-Info programı kullanılarak analiz edilmiştir.

Bulgular: Beş yaşın altında diyareli 25 çocuk çalışmaya dahil edildi. Bunlardan 10 tanesi HIV seropozitif, 7 tanesi HIV seronegatif 8 tanesinin ise durumu bilinmiyordu. 4 çocukta (16%) cryptosporidium parvum güven aralıkları %4.5 ve %36.1 arası ve hiçbir çocukta Isospora belli tespit edilemedi %0.0 ve %86.3 güven aralıklarında. Cryptosporidium parvum tespit edilen 4 çocuğun 3 tanesi HIV-seropozitif frekansı %30, 1 tanesi ise HIV-seronegatifti frekansı ise %14.3 idi. HIV durumu bilinmeyen çocukların hiç birinde enfeksiyon tespit edilmedi.

Sonuç: Modifiye edilmiş Ziehl-Neelsen tekniği ve Auramine-Fenol boyaması QECH'e gelen gaita örneklerinin C.parvum and I.belli tespitinde kullanılabilir. Her nekadar, I.belli bizim bölgemizde yaygın olsada çocuklarda tespit edilmemiştir. Ayrıca bu organizmaların endemik patojen olarak evrimleşmedikleri ve halen yetişkinlerde fırsatçı endemik patojenler olarak hayatlarını sürdürdükleri tespit edilmiştir. Bu çalışmanın özellikle altın standart olarak kabul edilen metodlarla yağmurlu sezonda tekrarlanması gerektiği düşünülmektedir.

Anahtar Kelimeler: Isospora, Prevalence, Malawi, Queen Elizabeth Central Hospital.

INTRODUCTION

Diarrhoea is a common problem in tropical countries and is caused by many intestinal parasites including *Cryptosporidium parvum* and *Isospora belli*. In third world countries 95% of the HIV infected patients develop episodes of diarrhoea during the period of their illness[2].

Cryptosporidium parvum and *Isospora belli* are transmitted by insects and infection is usually acquired by ingesting mature oocysts[3]. Cryptosporidia are common animal protozoa. *Cryptosporidium parvum*, causes diarrhoea in human beings. Infection is usually water-borne (contaminated water) or acquired from animals (zoonosis). Large numbers of oocysts are often present in faeces; they are partially acid-fast. The infection usually responds to symptomatic treatment, with fluid replacement if necessary[4].

Cryptosporidiosis is a highly infectious illness with multiple modes of transmission through contaminated drinking water, person-to-person and zoonosis (through ingestion of animal faeces[5]. It has a serologic prevalence as high as 30-60% in industrialized countries[6,7] and 95% in some tropical and developing countries[5]. Cryptosporidia are etiologic agents in 10-35% of cases of AIDS-associated diarrhoea, and cryptosporidiosis results in a significant morbidity and mortality in children with AIDS world-wide[8,9].

Cryptosporidium parvm and *Isospora belli* causes an acute self-limiting diarrhoea illness in immunocompetent individuals but may become chronic and may lead to severe and life threatening conditions, especially in children, and individuals who are immune compromised for whatever reason (HIV infected, iatrogenically immune suppressed or severely malnourished). Children present with profuse watery diarrhoea without blood and other symptoms include crampy abdominal pains, vomiting and low grade fever. Dehydration can easily occur due to excessive fluid losses[3].

A study conducted in south India showed that *Cryptosporidium parvum* was found to be prevalent among children and HIV-seropositive adults, indicating its endemicity in this region. *Cryptosporidium* was detected in three groups, i.e. children (8.7%), HIV- seropositive adults (6.85%), and HIV- seronegative adults (1%) and *Isospora* were detected among HIV-seropositive individuals at a frequency of 16%[1].

A study conducted in 2003 in Malawi showed that *Cryptosporidium parvum* (11%) and *Isospora belli* (12%) infections are a significant cause of diarrhoea among HIV/AIDS patients at QECH and that examination of stool specimen for parasites among patients with diarrhoea may provide data for a more appropriate management of cryptosporidiosis and isosporiosis in HIV/AIDS patients. The study also provided support for the UNAIDS Recommendation for co-trimoxazole as a prophylaxis for *Isospora belli* infection in HIV-infected patients[2,7].

In Malawi, diarrhoea is a major public health problem among children under the age of 5 years. Although at present, knowledge of the aetiology and information on the prevalence of the intestinal protozoa is a prerequisite for the institution of control/prevention measures and specific treatment. *C.parvum* and *I.belli* have been reported in adults who are HIV-seropositive in medical wards of QECH. However, there are no prospective studies on the prevalence of *C.parvum* and *I.belli* among Malawian children even though they have been reported to cause diarrhoea among children in developing countries.

The present study was conducted to assess the prevalence of C.parvum and I.belli infections among children presenting with diarrhoea, and to determine the prevalence of *C.parvum* and *I.belli* infections among HIV-seropositive and HIV-seronegative children with diarrhoea and the methods used for diagnosing these intestinal protozoa.

The findings of the study may help to reduce mortality due to cryptosporidiosis and isosporiosis since the prevalence of these infections is known

in children presenting with diarrhoea and also the findings will be used to introduce laboratory tests for *Cryptosporidium parvum* and *Isospora belli* at Queen Elizabeth Central Hospital.

METHODS

All children presenting with diarrhoea (defined as "loose to watery stool, three times a day for more than three days) were enrolled in the study. All children without diarrhoea or diarrhoea less than three times a day were excluded from the study.

A patient demographic form which comprised name, age, sex, nutrition status, HIV status, history of diarrhoea, source of water and urban or rural place of residence was completed from information available in patients' files including HIV status since it is mandatory for every patient in the wards to have their HIV status known. Mothers/guardians were advised on how to collect stool samples and they were required just to collect a single specimen.

After specimen collection, smears were made from each sample on the same day and stained with Auramine-phenol (Appendix E) and modified Ziehl Neelsen (ZN) (Appendix F). The ZN slides were examined for *Cryptosporidium parvum* and *Isospora belli* oocysts using light microscopy while Aurmanine-stained slides were examined using fluorescent microscopy. Three investigators examined each slide in the first place. In case of disagreement a new slide of the stool sample was prepared and examined; the results were verified by supervisors.

Study numbers (codes) were used for participants' names and the data collected was entered into Microsoft excel and cleaned, then was analyzed using Epi info. Percentage estimate (frequency) was used to find the proportion of children infected at 95% confidence interval.

RESULTS

From 44 children with diarrhoea that were recruited in the study, 25 submitted stool sample. Eight children were discharged before submitting the specimen and some recovered from diarrhoea while 9 withdrew from the study because of personal (parents/guardians) reasons and 2 children died in the process. Of the remaining, 25 diarrhoea cases, 10 were HIV-seropositive representing 40% while 7 (28%) children were HIV-seronegative and 8 (32%) children their status was not known.

In stools of 4 (16%) children with diarrhoea *Cryptosporidium parvum* oocysts were seen in both methods Ziehl-Neelsen and Auramine-phenol staining at 4.5% and 36.1% confidence intervals. Out of 4, 3 (30%) children with cryptosporidiosis were HIV seropositive and 1 (14.3%) child was HIV negative. None of the stool samples showed *Isospora Belli* oocysts (Tables 1).

Table 1: Baseline and clinical/laboratory characteristics of 25 patients enrolled in the study

Characteristic	Diarrhoea (ratio)	Percentage	Confidence Interval
Number of children Submitted stool sample	25	100%	
Number of male/female Children	(15/10)		
Number with urban/rural Residency	(18/7)		
Nutrition status (Malnourished/Well-Nourished	(12/13)		
Number of HIV Positive children	10	40%	
Number of HIV Negative children	7	28%	
Number of HIV Unknown children	8	32%	
Total number *C.parvum* Infections	4	16%	4.5%, 36.1%
HIV positive	3	30%	
HIV negative	1	14.3%	
HIV unknown	0	0.0%	
Total number *I.belli* Infections	0	0.0%	
HIV positive	0	0.0%	
HIV negative	0	0.0%	
HIV unknown	0	0.0%	

Stool examination of *Cryptosporidium parvum* and *Isospora belli* is not usually done at Queen Elizabeth Central Hospital. The methods used to diagnose *Cryptosporidium parvum* and *Isospora belli* which can be implemented include modified Ziehl Neelsen technique and auramine-phenol staining.

DISCUSSION

Diarrhoea is a common complication among young children living in low resourced countries and it induces weight loss and eventually may lead to persistent diarrhoea malnutrition syndrome. No studies were carried out in Malawi on diarrhoea caused by intestinal protozoa until 2003 when a study was conducted to assess the importance of *C. parvum* and *I. belli* infections as a cause of diarrhoea among patients admitted to the Medical Wards of Queen Elizabeth Central Hospital (QECH; 2) and thus shedded more light on the occurrence of these intestinal protozoa in the environment and affecting adults. In this previous study, in 26 of 121 (22%) of the patients with diarrhoea an infection with *C. parvum* or *I. belli* was found. *Cryptosporidium parvum* was detected in 13 (11%) of them and 14 (12%) had an *Isospora belli* infection; a mixed infection was found in one patient. The infections were only seen in HIV positive patients[2].

In our study 16% of children with diarrhoea were infected with *C. parvum*, which is higher than the adult prevalence[2]. However, much lower prevalence rates were reported among young children with diarrhoea in India: 8.7%[1]. In HIV seropositive children the prevalence of *C. parvum* was 30% and in HIV seronegative cases the prevalence of *C. parvum* was found in 14.3%. The 16% of *C. parvum* gastro-enteritis in children that was found in this study is in accordance with the range found in other studies[10] *Isospora belli* oocysts were not detected in the stool samples of

the children. There are no reports on isosporiasis in children except for a study from Delhi, India; where only 7 cases of isosporiasis were detected over a period of one decade, out of which two were HIV-infected[1]. This finding suggest that, although *I. belli* is prevalent in our environment, it has not yet been detected in children and or evolved as an endemic pathogen and so far remains as an opportunistic pathogen in adults only. This is in contrast to *C. parvum*, which not only occurs frequently as an opportunistic pathogen but, confirming the previous QECH based study can be called an endemic disease.

Stool examination of *Cryptosporidium parvum* and *Isospora belli* is not usually done at Queen Elizabeth Central Hospital hence it was impossible to evaluate diagnostic methods used to diagnose intestinal parasites at QECH. The diagnosis is entertained on clinical presentations only and requires confirmation, as in immune compromised children (11) it takes a more pernicious course. The methods used to diagnose *Cryptosporidium parvum* and *Isospora belli* which can be implemented include modified Ziehl Neelsen technique and auramine-phenol staining but there is need to have a gold (confirmatory) method to calculate specificity and sensitivity of the above mentioned methods (modified Ziehl Neelsen and auramine-phenol). The stains used in these methods are cheap; the procedures are easy to perform and are not time consuming.

Our study had certain limitations: Since it was a hospital-based study mild infections might not have been examined, and the examination of single faecal samples from each child could have resulted in lower prevalence rates. The study period was very short (2 weeks) and also the study was done during the dry season which made it impossible to reach the estimated sample size. Community-based longitudinal studies are required to assess the actual disease burden caused by these intestinal protozoa and elucidate their epidemiology. Rapid diagnostic tests (RDTS) were not done (due to reduced funding) which might have led to miss some infections. The RDTS are more sensitive than the microscopy methods (Ziehl Neelsen and Auramine-Phenol staining). The HIV status of some children was not known because the paediatric VCT centre run out of reagents for testing HIV at some point during our study.

CONCLUSION

In this study, *Cryptosporidium parvum* was found to be prevalent among children with diarrhoea admitted to paediatric wards of Queen Elizabeth Central Hospital; indicating its endemicity in this population. *Isospora belli* appears to be infrequent intestinal protozoa in children. And examination of stool sample for intestinal protozoa among children with diarrhoea provides data for a more appropriate management, which can be done in QECH as it is necessary, easy and cheap. The sample size was not enough to give significant results and a gold standard method is required. Examination of stool specimen for parasites among patients with diarrhoea may provide data for a more appropriate management of cryptosporidiosis in young children. But stool examination for *C. parvum* is not done at QECH and therefore, the following need to be done:

The hospital should implement and introduce modified Ziehl Neelsen and Auramine phenol staining techniques for examination of stool samples for *C. Parvum* and *I.belli.*

Areas for further studies;

Repeat the same study using gold standard methods (immunofluorescent assays with monoclonal antibodies) because they are very sensitive such that no infection (intestinal protozoa) can be missed.

Repeat the same study during rainy season when they are a lot of diarrhoea cases with a larger sample size.

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geliş tarihi/received :16.05.2012
kabul tarihi/accepted:12.06.2012