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Experimental Article

Comparison of Kinyoun's acid-fast and immunofluorescent methods detected an unprecedented occurrence of Cryptosporidium in the Eastern Region of Saudi Arabia

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الملخص

أهداف البحث: يهدف البحث لمقارنة الحساسية والنوعية التشخيصية المتبعة حاليا في المملكة العربية السعودية، وهي صبغة كنيون المعدلة المقاومة للحمض مع طريقة التألق المناعي المباشر للكشف عن وجود الكريبتوسبوريديوم.

طرق البحث: تمت مقارنة الطريقة التقليديه وهي صبغة كنيون المعدلة المقاومة للحمض مع طريقة التألق المناعي المباشر للكشف عن البيض المتكيس للكريبتوسبوريديوم في ١٠٠ عينة براز من المرضى الذين تمت معاينتهم في مجمع الملك فهدالطبي العسكري بالظهران في شهري إبريل ومايو من عام٢٠١٢م.

النتائج: أعطى الفحص بصبغة كنيون المعدلة ٤٩% كنتيجة إيجابية، فيما أعطى الفحص بتقنية التألق المناعي المباشر ٦٦% كنتيجة إيجابية عن البيض المتكيس للكريبتوسبوريديوم. كانت حسابات الحساسية والنوعية لصبغة كنيون بالمقارنة مع تقنية التألق المناعي المباشر٦٦.٦٧% و ٨٨.٢٤% على التوالي، وكانت القيم التنبؤية الإيجابية والسلبية لصبغة كنيون بالمقارنة مع تقنية التألق المناعي المباشر ٩١.٦٧% و ٥٧.٩٦% على التوالي.

الاستنتاجات: تبين أن تقنية التألق المناعي كانت أسهل في الأداء، وأعطت نتائج أكثر حساسية ونوعية لاكتشاف بويضات الكريبتوسبوريديوم الكيسية بالمقارنة مع الطريقة التقليدية التي تتم باستخدام صبغه كنيون المعدلة المقاومة للحمض.

وبذلك تكون النتيجة التي تم التوصل إليها في هذه الدراسة وهي ٦٦% أعلى من الدراسات والمسوحات التي أجريت سابقاً. ولكي لا يبقى هذا المرض دون تشخيص عند كثير من حالات الإسهال ننصح بأن يضاف فحص الكريبتوسبوريديوم ضمن الفحوصات الروتينية التي تعمل للمرضى المصابين بالإسهال في المختبرات الطبية التشخيصية.

الكلمات المفتاحية: الفحص بصبغة كنيون المعدلة المقاومة للحمض; الفحص بطريقة التألق المناعي; كريبتوسبوريديوم; المملكة العربية السعودية

Abstract

Objectives: The aim of this study was to compare the diagnostic sensitivity and specificity of the modified Kinyoun's acid-fast test used widely in Saudi Arabia compared to the direct immunofluorscent assay (DFA) for monitoring the occurrence of *Cryptosporidium* spp.

Methods: We compared the conventional, modified Kinyoun's acid-fast with the Merifluor direct immunofluorscent assay for the detection of Cryptosporidium oocysts in 100 stool samples, among patients who reported to King Fahad Military Medical Complex during April–May, 2012.

Results: The modified Kinyoun's method and the DFA revealed 49 and 66 Cryptosporidium oocyst positives, respectively. Sensitivity and specificity of acid-fast when compared to DFA, were 66.67% (95% CI: 53.99% –77.79%) and 88.24% (95% CI: 72.53%–96.63%), respectively, (Kappa = 0.487 and the 95% confidence interval was 0.328–0.645). The positive and negative

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predictive values of the same method were 91.67% (95% CI: 80.00%−97.63% and 57.69% (95% CI: 43.21% −71.27%), respectively.

Conclusion: The DFA was found to be simple to perform and has been demonstrated to have a higher sensitivity than traditional staining procedures. The current positivity rate of 66% of Cryptosporidium in this study is higher than indicated before by several studies. This disease remains underdiagnosed in current routine laboratory procedures. It is recommended that tests for Cryptosporidium be done as part of a general diarrhea screen during standard stool tests in diagnostic laboratories.

Keywords: Cryptosporidium; Immunofluorescent; Kinyoun's acid-fast; Kingdom of Saudi Arabia

Introduction

Cryptosporidium is a genus of coccidian parasites associated with gastrointestinal diseases in all vertebrates, including humans.[1] Although many species of *Cryptosporidium* have been reported in humans, *C. hominis* and *C. parvum* are responsible for the majority of human infections.[2,3] *C. parvum* is found in humans as well as in a number of other animals, whereas *C. hominis* apparently infects only humans.[4,5] In developing countries *Cryptosporidium* infections occur mostly in children under five years of age,[6–8] although outbreaks do occur worldwide in all age groups.[9] Immunocompromised people are most likely to be affected more seriously.[10]

Cryptosporidium is usually diagnosed by microscopic detection of oocysts in stool specimens. Although various methods have been described for the detection of oocysts, the diagnosis of *C. parvum* is usually made by using modified acid-fast,[11] or immunofluorescence staining[12,13] on concentrated or unconcentrated fecal smears. The acid-fast stain is not universally recommended for the detection of *C. parvum* oocysts in fecal samples largely because of previous data that suggested a low specificity and sensitivity.[14,15]

Antigen detection by immunoassays has become widely used for the diagnosis of cryptosporidiosis. Detection of antigens on the oocyst wall of the organism in stool specimens, using direct fluorescent-antibody for diagnosis of cryptosporidiosis, has been reported several times. Good sensitivities and specificities have been reported for some of these tests in several comparative studies.[16–21] These techniques increase the sensitivity compared to routine light microscopy and are easy to perform.[13,22–24]

The immunofluorescent technique has been demonstrated to have a higher sensitivity than traditional staining procedures and is often considered as a gold standard for evaluating other diagnostic techniques.[21,22,25,26]

The Merifluor *Cryptosporidium*/*Giardia* Direct Immunofluorescence Assay (DFA) uses the principle of direct immunofluorescence. The combined fluorescein isothiocyanate-labeled monoclonal antibodies are directed against the cell wall antigens of *G. lamblia* cysts and *Cryptosporidium parvum* oocysts.

Cryptosporidium remains underdiagnosed under the current routine laboratory procedures, based mainly on the detection of oocysts in fresh unconcentrated fecal smears stained with modified Ziehl−Neelsen (mZN). However, the use of appropriate sensitive methods can improve case detection of *Cryptosporidium*.

Most previous studies on the epidemiology of intestinal parasites in Saudi Arabia did not include *Cryptosporidium*[27,28] and most of the available data on the occurrence of the disease were hospital based. *Cryptosporidium parvum* and *C. hominis* remain the two most frequent species detected[29] with various ranges of prevalence, mostly among children below two years of age,[30–32] and immunocompromised patients.[33] The *Cryptosporidium* oocyst was detected in 20 (32%) of the symptomatic children, but only nine (4.7%) of the asymptomatic children in Jeddah, Saudi Arabia.[34]

Typically, *Cryptosporidium* testing is currently not included in the routine examination of stool for ova and parasites. The aim of this study was to compare the diagnostic sensitivity and specificity of the mZN test used widely in Saudi Arabia compared to the DFA for monitoring the occurrence of *Cryptosporidium* spp.

Materials and Methods

Specimen collection

One hundred stool specimens were collected in Para-pak /Para-Pak 10% SAF (Sodium Acetate acetic acid) from individuals of various ages who reported to the outpatient clinic of King Fahad Military Medical Complex between April-May, 2012 presenting with abdominal symptoms, mainly diarrhea. All specimens were anonymized and processed separately.

Modified Kinyoun's acid-fast technique for Cryptosporidium

The modified Kinyoun's acid-fast stain was performed as previously described.[35] In brief, fecal smears were dried on a slide warmer at 60 °C, before being fixed with absolute methanol for 30 s, and stained with Kinyoun's carbol fuchsin for one minute. The preparations were de-stained with acid alcohol for 2 min, then counterstained with Malachite green for another 2 min. Slides were then rinsed with distilled water, dried on a slide warmer at 60 °C for about 5 min, mounted with a cover slip using Cytoseal 280 mounting medium, and examined under 100× oil objectives for the detection of *Cryptosporidium* oocyst.

Direct immunofluorescence assay

The MeriFluor *Cryptosporidium*/*Giardia* direct immunofluorescence detection kit procedure was used (Meridian Diagnostics, Inc., Cincinnati, Ohio) according to the manufacturer's directions. In brief, a drop of fecal material was smeared over the entire slide well and dried at room temperature. Positive and negative controls were applied whenever the procedure was performed. One drop of a detection