Identification And Laboratory Diagnosis Of Typical And Atypical Oocyst Of *Cryptosporidium*

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Abstract:

Cryptosporidiosis is usually diagnosed by recognition of oocyst in faecal smears stained by Ziehl-Neelsen, Safranin-methylene-blue, auramine-carbolfuchsine, Succurose-phenol methods.

The atypical oocyst is very fragile and quickly collapses when suspended in solution of high osmotic pressure or in lipid solvent. They are not easily be stained by methods used to stain typical oocysts, but their appearance in sucrose-phenol is characteristic their stability in this solution, though much less than that of typical oocysts is sufficient for them to be recognized and for cases to be diagnosed by microscopy. General findings in patients who excreted atypical oocysts were no different from those who excreted typical oocysts.

الخلاصة :

ان مرض الكربتوسبوريديا يتم تشخيصه عادة بالتعرف على المكيسات في مسحة البراز المصبوغة بالطرق التالية ، زئيل نيلسن ، السفرانين مع المثيل الازرق ، ثم صبغة الاورامين كاربولفوكسين.

ان النوع الشاذ للمكيسات يكون هش وسريع الانكماش عند وضعه في محلول ذو ضغط تنافذي عالي او في مذيبات الدهون ، ولا يمكن صبغه بسهولة بالطرق المستخدمة لصبغ المكيس المثالي ، ولكن ظهوره في محلول السكروز-فينول يكون كافياً لتشخيصه.

ان استقرارية تواجد المكيسات الشاذة في المحاليل المختلفة هو اقل كثيراً من استقرارية تواجد المكيسات الطبيعية وهذه كافية لتمييز هذا النوع في حالة التشخيص بالفحص المجهري.

ان العلامات العامة في المرضى الذين يفرزون المكيسات الشاذة لا تختلف عنها في المرضى الذين يفرزون النوع الطبيعي للمكيسات.

Introduction:

Cryptosporidium parvum is a case of gastroenteritis (1,2). Laboratory diagnosis is usually made by recognizing the characteristic oocysts in stained fecal smears. The method most commonly used are the modified Ziehl-Neelsen, the Kinyoun, the auramine-phenol, and the Safranin-methylene blue technique (3,4,5), they are generally though to be suitable for reliably detecting oocysts (1,2,6).

We have recently seen 12 cases Cryptosporidiosis in which the only oocysts excreted behaved differently from those usually seen, these oocysts would be missed if the above mentioned techniques were used. Here we provide a description of these atypical oocysts, compare them with typical oocysts and describe a simple method for their detection (7).

Materials and Methods :

Diarrhic stool from children who were seen of paediatric hospital. Specimens were usually tested within 2 hours and the experiments described here done. The characteristics of feces found to contain typical oocysts varied from very liquid to hard and fully formed, although the majority of samples were loose.

Staining methods: Oocysts in fecal smears were stained by the modified Ziehl-Neelsen(3), auramine-phenol(8) and safranin-methylene blue method (5) and sucrose-phenol (s-p) method.

500 gm sucrose
6.5 gm phenol
320 ml D. Water

Cryptosporedium oocysts suspended in s-p solution and examined by phase contrast microscopy (4, 6, 9) this method is routinely used to detect oocysts in fecal samples.

Results:

Detection of atypical oocysts on Cryptosporidium cases. Unlike typical oocysts, in smears of fresh feces stained with safranin-methylene blue, there is a small numbers of rounded bodied the size, shape and structure of Cryptosporidium oocysts , however, these bodies did not retain safranin.

Similar results were obtained with other staining method in this respect the bodies resembled typical oocysts from old samples of feces of known cases of Cryptosporidiosis, although they stained well when fresh.

The safranin-methylene blue method is more reliable than acid fast stains for detecting typical oocysts in some instances, typical oocysts in fresh material could not be stained by Ziehl-Neelsen methods, although safranin-methylene blue was satisfactory (5, 10).

In most specimens, typical oocysts gradually lost their ability to retain carbol-fuchsin over a period of weeks.

The characteristic microscopic appearance of unstained Cryptosporidial oocysts suspended in sucrose-phenol method has been noted (9, 11).

They are buoyant and are seen against the underside of the coverslip as pink refractile bodies; yeasts appear green and are found with faecal debris in lower planes of focus. Atypical oocysts resemble typical ones in this respect but are osmotically fragile, they burst after 4-6 min in sucrose-phenol solution leaving barely greenish ghosts; typical oocysts are stable for 3 hours or more. Atypical oocysts are more spherical than typical forms. Their pink refractility and buoyancy are strongly resembled that of typical oocyst.

Discussion:

There is only two species, Cryptosporidium parvum and C. muris, but this species has not been isolated from infected humans, and its oocysts is larger and more ovoid than those of C. parvum (12). Our results indicate that the bodies we describe here are closely related to the oocyst of C. parvum(13).

Atypical oocysts differ from typical ones is not staining by used methods. Osmotic fragility and weak monoclonal reaction. This suggests they have deficient oocyst walls. Thin-walled oocysts have been detected in the gut of experimentally infected mice.

Excretion of low numbers of only atypical oocysts during an outbreak suggests they are infective and not just old or degraded typical oocysts. However, their unusual properties make detection defficult (14).

Sucrose-phenol microscopy permits testing of large samples and the buoyancy of oocysts separates them from yeasts and so on. The tendency of oocysts to float up against the coverslip effectively separates them from yeasts and most artifacts, and this occurrence permits direct examination of relatively thick smear.

S-P microscopy method is at least sensitive than safranin-methylene blue for detecting typical oocysts. Our results further indicate that s-p microscopy can detect atypical and typical oocysts with equal sensitivity, as long as core is taken to screen quickly.

Organisims of a similar size and shape like (yeasts, Blastocystis hominis and Chilomastix mesnilli) have a distinct green refractility and are not confused with the pink oocysts of Cryptosporidium.

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