Study of enteric parasites in HIV infected patients with diarrhoea

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Abstract:
Introduction: Enteroparasites are related to gastrointestinal alterations among patients with HIV/AIDS, some causing severe manifestations. Cryptosporidium spp are major cause of diarrhea in developing countries mainly affecting HIV infected individuals with low CD4 lymphocyte counts. As many of these infections are amenable to treatment, an early and accurate diagnosis is important. In present study we have tested the diagnostic performance of ELISA for cryptosporidium antigen detection considering the modified Kinyoun acid fast staining as a gold standard.

Materials and methods: This study enrolled 220 HIV infected patients presenting with diarrhea. Stool samples were examined microscopically by saline and iodine mount. Modified Kinyoun acid fast staining technique was used to detect coccidian parasites. Whole procedure was repeated after concentration of the samples which were not showing any parasite by direct microscopic methods. Commercially available Cryptosporidium antigen detection ELISA was performed for Cryptosporidium Ag detection on formalinised 37 stool specimens of patients showing Cryptosporidium oocysts by microscopy and 40 stool samples not showing any parasite by microscopy.

Results: Sensitivity and specificity of Cryptosporidium antigen detection by ELISA were 91.9% & 95% respectively. Positive predictive value & negative predictive value of Cryptosporidium antigen detection ELISA were 94.4% & 92.7% respectively.

Conclusion: Cryptosporidium antigen detection ELISA test is useful for screening large number of stool specimens in a short time period which provides good alternative for conventional staining methods. However, in resource limited setup with availability of expert microbiologist, microscopy still holds importance.

Key words: Cryptosporidium spp, ELISA, Antigen detection

Introduction:
Enteric opportunistic parasitic infections are noted as commonest among coccidian parasitosis. The modified acid-fast or the Kinyoun acid fast stain are most desirable for detection of oocyst of coccidian parasites. With either stain, the oocyst of Cryptosporidium species stain red. Performance of ELISA has been evaluated in HIV positive and HIV negative patients by various authors. They have shown varied sensitivity from 60% to 92.6% and specificity between 90 to 100%. Hence, we evaluated the Cryptosporidium antigen detection ELISA in fecal samples of HIV infected patients with...
diarrhoea to know its diagnostic use for screening purpose.

**Materials & methods:**
The present cross-sectional type of study approved by institutional ethical committee comprises 220 HIV positive patients presented with diarrhoea, attending the tertiary care hospital during period of one year from January 2012 to December 2012. Diarrhoea defined as 2 or more fluid or 3 or more soft stools per day. Stool samples were collected from these patients and processed to examine for various enteric parasites. Macroscopic and microscopic examination of stool samples was performed. Microscopic examination included saline & iodine mount. Modified Kinyoun acid fast staining was done by modified Kinyoun cold technique. Microscopic examination was repeated after concentration for samples which were not showing any parasite by direct microscopic methods. Concentration of the stool was done by formal ether sedimentation method.

From each stool sample, 1gm (Approximately pea size) of stool stored in 5% of formalin solution in screw-cap container for ELISA test at 2°C-8°C to detect *Cryptosporidium* antigen. Commercially available *Cryptosporidium* antigen (stool) detection ELISA was performed for *Cryptosporidium* Antigen detection on formalinised stool specimens. ELISA was performed as per instructions of a kit (DRG International, Inc) on following groups of HIV positive patients with diarrhoea:

1) 37 stool samples in which *Cryptosporidium* oocyst was detected by microscopy with modified Kinyoun acid fast staining.
2) 40 samples not showing any enteric parasite by microscopy with saline mount, iodine mount, modified Kinyoun acid fast staining.

Interpretation of ELISA was done as per instructions in the kit. Considering microscopy as a gold standard, results of ELISA for *Cryptosporidium* antigen detection were evaluated.

**Observation and results:**
Comparative analysis of ELISA for *Cryptosporidium* antigen detection with microscopy

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<tr>
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<th>ELISA Positive</th>
<th>ELISA Negative</th>
<th>Total</th>
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<tbody>
<tr>
<td>Microscopy Positive</td>
<td>34</td>
<td>03</td>
<td>37</td>
</tr>
<tr>
<td>Microscopy Negative</td>
<td>02</td>
<td>38</td>
<td>40</td>
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<tr>
<td>Total</td>
<td>36</td>
<td>41</td>
<td>77</td>
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Oocyst of Cryptosporidium spp was detected in 37 patients by microscopy in study group. Out of these 37 stool specimens, 34 were positive and 3 were negative by antigen detection ELISA. Out of 40 samples not showing Cryptosporidium oocyst by microscopy, 38 patients were negative by Cryptosporidium antigen detection ELISA. Two samples negative by microscopy were positive by Cryptosporidium antigen detection ELISA. Thus, considering modified Kinyoun acid fast staining as gold standard, sensitivity and specificity of Cryptosporidium antigen by ELISA were 91.9% & 95% respectively. Positive predictive value & negative predictive value of Cryptosporidium antigen ELISA were 94.4% & 92.7% respectively.

Discussion:
In present study, Cryptosporidium spp was detected in 37 stool samples of HIV positive patients presenting with diarrhoea by modified Kinyoun acid fast staining method. Out of these 37, results of 34 specimens were correlated with Cryptosporidium antigen detection ELISA. Considering microscopy as a gold standard, three samples which were detected by microscopy, were found to be negative by Cryptosporidium antigen detection ELISA. Ungar et al\textsuperscript{12} carried out the study to evaluate enzyme-linked immunosorbent assay (ELISA) to detect Cryptosporidium antigens in human feces on frozen stools from patients with Cryptosporidiosis. In their study, 11 samples could be detected by microscopy but not by ELISA. They found that, in samples with fewer than five oocysts per 0.01 ml of concentrated stool, the ELISA fails to detect antigen because the antigen is inaccessible or not recognized by the detecting polyclonal antibodies. They suggested other reason for this could be infection by some distinct Cryptosporidium isolates like C. baileyi or antigenic variation in geographical area. They also suggested that inability to detect Cryptosporidium antigen by ELISA may be due to samples contain amount of free antigen below the sensitivity level of the assay.\textsuperscript{12}

Forty samples not showing any parasite were subjected to Cryptosporidium antigen detection by ELISA. Out of 40 stool samples not showing Cryptosporidium by microscopy, results of 38 samples were matched with Cryptosporidium Antigen detection ELISA. Two samples negative by microscopy showed positive result by Cryptosporidium antigen detection ELISA. The reason for this could be patient not actively excreting oocyst at the time of specimen collection. Ajjampur et al\textsuperscript{2} also suggested that there is infrequent shedding of oocysts of Cryptosporidium.

Considering modified Kinyoun acid fast staining as gold standard, detection of Cryptosporidium antigen by ELISA showed sensitivity, specificity 91.9% & 95% respectively which is correlated with other studies.\textsuperscript{6,12,13,14} Positive predictive value & negative predictive value of ELISA test were 94.4% & 92.7% respectively, which were comparable to that of study by Tuli et al\textsuperscript{6}. Marquesl et al\textsuperscript{15} evaluated diagnostic performance of Cryptosporidium immunoenzymatic assay. They suggested that although the cost of the enzyme-linked immunosorbant assay is higher than that of modified ZN stain, this method allows Cryptosporidial diagnosis, even when the parasite’s integrity is compromised leading to inconclusive microscopic diagnosis. They concluded that ELISA is useful assay for ruling out cryptosporidiosis in immunocompramised patients. Present study suggests that, microscopy by modified Kinyoun acid fast staining is more efficacious than performing ELISA in terms of time, equipment and cost for routine
diagnosis. But for microscopic examination, skill is needed especially with special staining techniques. It also needs expert microbiologist for recognition and morphological differentiation of coccidian parasites. On the other hand, ELISA for Cryptosporidium antigen detection is less subjective for interpretation of the results. Also it has advantage over the microscopy in specimens with inconclusive microscopic findings. ELISA of Cryptosporidium antigen detection could be done on preserved stool samples with easy sample preparation. Hence the technique is convenient for screening of large number of specimens in short time period especially for large scale epidemiological surveys. In present study Cryptosporidium antigen detection ELISA was performed on limited number of stool samples due to cost constrains. Hence further evaluation of this ELISA with large number of samples needs to be done to confirm the obtained results.

Conclusion:
Cryptosporidium antigen detection ELISA test is useful assay with good sensitivity and specificity for screening of large number of fecal samples. Also it is less subjective. Microscopy by modified Kinyoun acid fast staining still holds importance due to its low cost especially in resource limited setups but where expert microbiologist is available.

References
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